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Control measures for BRSV

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Introduction

Bovine respiratory disease is one of the most common and economically important disease complexes in young cattle worldwide (Griffin, 2014) and is estimated to cause agricultural losses of ~580M Euro per year only in the European Union (Nicholas & Ayling, 2003). The etiology involves several pathogens and the clinical outcomes are influenced by environmental and host factors. Bovine respiratory syncytial virus (BRSV) is continually considered as a major viral player (Assie et al., 2009; Stott et al., 1980), however, the diagnosis is not straightforward and infections are probably frequently underdiagnosed. This virus can cause severe disease as single pathogen, but co-infections are often detected (O'Neill et al., 2014). Viral infections are most likely preceding bacterial colonisation of the lung in the majority of cases of bacterial pneumonia (Babiuk et al., 1988), and a considerable number of healthy animals harbour potentially pathogenic bacteria in the upper and lower airways, which multiply during favourable circumstances (Angen et al., 2009; Holman et al., 2015). Expectedly, prevention of BRSV would lead to reduction of antibiotic use, increased economic revenues and reduced animal suffering, especially in intensive beef cattle rearing.

With the common final goal to exclude any consequences of BRSV infections from cattle production systems, different preventive measures against BRSV have different objectives. These range from reducing the clinical expression of the infection and associated production losses, up to, at long term, attempting to eradicate the virus from a herd, region or country. Whereas continuous vaccination is a conviction by necessity for many, due to the characteristics of current production systems and vaccines, others aim to use vaccination as a temporary tool to prevent virus circulation and protect sensitive animals until the risk for infection has been reduced. Yet another category prefers to relay on control programs based on strict biosecurity combined with disease monitoring, without vaccination. Based on virological and epidemiological characteristics of BRSV, as well as the type of cattle that needs to be protected, different control measures can be implemented, some of which will be reviewed in this presentation. .

Virus characteristics important for control

To control BRSV it is important to have a good understanding of some of the virus characteristics. BRSV is an enveloped single-stranded RNA virus, classified as a *Pneumovirus* in the *Paramyxoviridae* family and *Mononegavirales* order (International Committee on Taxonomy of Viruses). Though antigenic subgroups have been identified (A, AB, B and untyped), there is only one serotype and antibody cross reactivity occurs with human, caprine and ovine RS viruses (Stott & Taylor, 1985; Langedijk et al., 1997). The virus RNA genome contains 10 genes that code for 11 proteins and gene sequence data divide BRSV in phylogenetic subgroups, with isolates from different time periods and geographical origin (Valarcher et al., 2000). However, compared with other RNA viruses (even human (H)RSV), BRSV demonstrates only limited genetic variation and this favours the effect of cattle vaccination. Particularly constant is the gene coding for the surface fusion protein, which is very important for infection of host cells and which induces protective immune responses (Taylor et al., 1992).

Mostly due to the lipid envelope of BRSV, which is formed by the host cell membrane during virus budding, the virus is considered fragile outside the host and is very sensitive to detergents. However, HRSV remains infective several hours on protective clothing and can thereafter be re-isolated *in vitro* (Hall et al., 1980; Kingston, 1968) and BRSV may survive storage for 300 days in cell culture media in the fridge (Tjornehoj, 2000). The survival of RSV might be longer if measured by indirect infection of naïve individuals *in vivo*. Human RSV diagnosis peaks during periods with low outdoor ultraviolet B radiation, at mean daily temperatures below 6°C and above 24°C and when relative humidity is near 40% (Welliver, 2007). The impact of humidity on virus survival is complex: in air with low humidity, respiratory droplets evaporate fast and small droplets and aerosol nuclei settle slowly, increasing the risk of aerosol spread. In contrast, in air with high humidity, the droplets evaporate slowly and settle faster with a greater mass, which would increase the immediate indirect transmission (Paynter, 2015). Nevertheless, as shown for HRSV, once the respiratory droplets have dried, virus remains infective longer on surfaces in dry air (Kingston, 1968).

Bovine RSV is endemic in most parts of the world (Valarcher & Taylor, 2007). The seroprevalence is in general high (~30-100%) but is varying depending on the type and age of animals sampled. It is highest in areas with high cattle density (Elvander, 1996; Beaudeau et al., 2010a), in big herds (Norström et al., 2000) and in areas where animal exchanges are common (Elvander, 1996; Widgren & Frössling, 2010). Moreover, it is increasing with the age of

animals (Hägglund et al., 2005). Infections follow typically a seasonal pattern, with highest disease incidence between autumn and spring in temperate climate zones, and lower disease incidence during summer (O'Neill et al., 2014; Stott et al., 1980). It appears that the virus continues to circulate between animals and herds also during periods with less disease (Klem et al., 2013; Hägglund et al., 2005). Nevertheless, it has been suggested that BRSV persistently infect animals between outbreaks and that it may be re-activated, based on repeated outbreaks in herds and antibody titre increases in absence of re-infections (Van der Poel et al., 1993; Van der Poel et al., 1997). Furthermore, viral messenger RNA has been detected in lymph nodes of animals 71 days after infection, indicating active production of virus proteins (Valarcher et al., 2001). It remains to be investigated if persistent virus may be re-excreted and to clarify the importance of this phenomenon in virus epidemiology.

The routes of BRSV transmission are not completely understood. The high infectivity of this virus is illustrated by its spread between herds when introduced in BRSV-free areas (Elvander, 1996; Inaba et al., 1972). However, some areas were also able to return free from BRSV circulation following epidemics, possibly with the aid of limited animal exchanges, low density of cattle herds (Elvander, 1996; Ohlson et al., 2013) and strong, actively acquired herd immunity (including in calves). As an example, an area of northern Sweden with low BRSV seroprevalence in 1990 (Elvander, 1996) suffered from severe, rapidly spreading epidemics involving animals of all ages at a 10-years interval (mid 1990s and 2005) and BRSV-circulation seems again absent, at least since 2009 (Ohlson et al., 2013).

An efficient way of virus introduction in herds is introduction of infectious animals and direct transmission between animals by respiratory droplets (Elvander, 1996; Hall & Douglas, 1981a; Uttenthal et al., 1996). Whereas human RSV does not spread by air from an infected infant within a room (Hall & Douglas, 1981a), bovine RSV can spread by air between interconnected rooms, from several infected calves to sentinel animals (Mars et al., 1999). This is consistent with the rapid within-herd spread commonly observed in bovine outbreaks, and with the aerosol spread used by Influenza A, another enveloped RNA virus of similar morphology and size (Paynter, 2015). In contrast to the possible indoor spread of BRSV by air, several authors concluded that airborne spread between herds is not of great importance. This was based on the observation of a mixed pattern of herds with and without BRSV-specific antibodies in pooled milk samples from primiparous cows (Ohlson et al., 2010a) and failure to demonstrate spatial autocorrelation between herds classified as previously infected (Ohlson et al., 2010b). The distance between studied herds was not reported in this paper, but was less than 100 km. The diverging data on aerosol spread between human and bovine RSV within a building may be due to the quantity of virus included in the studies (*i.e.* the number of virus shedders). When BRSV is introduced in a herd with many naïve animals, the virus amplification will increase compared to in a partially immune herd (shown for foot-and-mouth disease virus, FMDV) (Cox & Barnett, 2009), resulting in increased infection dose of individuals, which will shorten the incubation time (shown for HRSV) (Hall et al., 1981b), impact the disease severity (Assie et al., 2009; Kimman et al., 1988), the virus shedding (shown for HRSV) (Hall et al., 1981b) and further transmission of the virus. However, such severe BRSV outbreaks are easy to identify for the application of hygienic measures before visiting another herd.

Indirect transmission by contaminated fomites, such as human hands, clothes or material, is generally considered to be very important in the transmission of BRSV. Herds closed with regard to animal introduction are commonly infected (Widgren & Frössling, 2010) and outbreaks sometimes follow visits from animal professionals (personal observation). Moreover, HRSV can be re-isolated from hands that have been in contact with contaminated fomites (Hall et al., 1980) and human infections can be reduced by hand hygiene (Hall, 2000; Jefferson et al., 2009). The specific degree of hygienic measures required to stop BRSV introduction in naïve herds is not known. With a high number of susceptible animals, the risk of successful virus introduction is higher than in herds with high herd-immunity. Indeed, the probability for indirect transmission depends both on viral concentrations on fomites and other exposure factors (L'Huillier et al., 2015), as well as the number of contacts between contaminated material and sensitive individuals. Thus, big herds in densely cattle-populated, BRSV-endemic areas, which contain many susceptible animals (*i.e.* calves) and in which professionals work in several farms a day due to short distances between herds (Widgren & Frössling, 2010; Nöremark et al., 2013) will suffer a great risk for virus introduction. Influenza A was demonstrated to transmit indirectly by personnel from infected to sentinel pigs despite hand hygiene and change of clothes. The authors suspected transmission to occur by clothes worn underneath coveralls or areas of the skin of personnel that were not washed (Allerson et al., 2013). It is likely that BRSV is similarly very contagious, sometimes resulting in outbreaks in herds without animal introduction, neither visitors, excepted the milk tanker or other professionals who were not in contact with the animals (personal observations). This brings suspicions to a possible role of small wild animals in transmission between nearby situated farms. Undeniably, both birds and rodents are likely to be in contact with cattle secretions in the feeding area and BRSV infections are commonly diagnosed in neighboring farms (Elvander, 1996; Hägglund et al., 2005; Hägglund et al., 2007). Only a small proportion of sampled wild ruminants (roe, elk), cats and dogs showed anti-RSV activity in sera, indicating that the role of these animals in virus transmission to cattle is not major (Van der Poel et al., 1995) (Valarcher & Hägglund, unpublished data).

Following BRSV circulation in herds located in areas where the infection is endemic, disease is generally observed in calves between one and six months of age, probably because most older animals are immune and clinically protected (Van der Poel et al., 1993), although these were in some cases shown to shed virus following re-infection (Baker et al.,

1986; Kimman et al., 1987). On the other hand, in non-endemic areas, in immunologically naïve herds or groups of animals, the morbidity is often close to 100%, involving cattle of any age, whereas the mortality is usually low, but may reach 20-30% (Bryson et al., 1978a; Schreiber et al., 2000). Though the primary infection may be subclinical, it often results in clinical signs ranging from increased coughing, lacrimation and nasal discharge to pyrexia, tachypnea, abdominal dyspnea and abnormal breathing sounds. Subcutaneous emphysema, anorexia and reluctance to lie down are more occasionally observed, and the latter is associated with poor prognosis. Death is generally preceded by strong depression, severe abdominal dyspnoea, bradypnea, and cyanosis (unpublished observations including outbreaks described in Valarcher et al., 2000).

The major target cells for virus replication are epithelial cells in the respiratory tract and pneumocytes (Viuff et al., 2002; Viuff et al., 1996) and the clinical disease is additionally partly caused by detrimental effects of inflammatory responses. Direct immunopathological mechanisms have also been suggested to be involved in the pathogenesis of BRSV, such as excessive immunoglobulin (Ig)G_1-enhanced complement binding and mast cell activation (Jolly et al., 2004; Kimman et al., 1989b). Importantly, bronchiolar obstruction by mucus and cell debris results in atelectasis and emphysema and, in parallel, mucociliary damage and virus-induced alterations of pulmonary immune responses lead to secondary infections that can prolong and aggravate the disease (Bryson et al., 1978b). Consequently, necropsy in late stages of respiratory disease often reveal bacterial infections when BRSV is no longer detectable, which illustrates the importance of early diagnosis in BRSV outbreaks (Verhoeff & van Nieuwstadt, 1984a; Larsen et al., 1999).

Though excessive immune responses may contribute to the pathology induced following an established BRSV infection, fast and strong local BRSV-specific immunity is crucial to prevent BRSV entry and establishment. Rapidly induced BRSV-specific IgA of high magnitude, detected in the lumen of the nose and the tracheobronchial tree, was repeatedly associated with near complete protection against severe experimental BRSV challenge (Kimman et al., 1989c; Blodörn et al., 2014) and was generally absent in calves that died or were euthanised during severe natural infections (Kimman et al., 1989a). Cytotoxic T lymphocytes (CTL) in the respiratory lymph nodes and lung probably also play a pivotal role, by clearing the infection through induction of apoptosis of infected cells (Blodörn et al., 2014; Taylor et al., 1995; West et al., 1999). Though the quantity of virus-neutralising antibody in sera is commonly used as indicator of RSV protection in humans, this parameter did not always correlate to protection against experimental infection in calves (Blodörn et al., 2014; Hägglund et al., 2004; Makabi-Panzu et al., 2014). To target control measures to virus shedding populations, in order to stop virus transmission, research needs now to focus on the identification of other, more precise parameters predictive of protection against natural infection, such as innate, natural defense molecules in airways and certain patterns of combined immune parameters.

Despite a consistent lack of correlation with protection, systemic total (neutralising and non-neutralising) BRSV-specific antibody (serum IgG) is the parameter most often used to estimate protection in field settings. Old cows with high levels of actively acquired serum IgG appear to be clinically protected, but these have probably also good local immune memory. Nevertheless, high levels of systemic IgG may be protective on its own, since calves with high levels of maternally derived antibody (MDA) are at least partly protected against natural infection (Kimman et al., 1987; Westenbrink et al., 1989). The protectiveness of systemic MDA depends on its quantity and quality, as well as the infection dose to be overcome (Hall et al., 1981b). The reason that low to moderate levels are mostly not protective (Baker et al., 1986; Kimman et al., 1987) is probably that these antibodies do not prevent infection at the virus entry site, but rather acts in the tissue when the infection is established (*e.g.* by virus neutralization, complement activation - Kimman et al., 1989d- and opsonisation). Indeed, very little or no MDA can be detected in the lumen of the airways. .

Though maternal lymphocytes may be transferred to the calf circulation from non-frozen colostrum and can have positive effects on both specific and general immune responses (Donovan DC et al., 2007; Reber et al., 2005), the BRSV naïve calf lacks preexisting cellular (CTL) memory cells in the lung for rapid clearance of its first infection. However, such cells will actively be primed for rapid expansion following reinfection. B lymphocytes on the other hand are commonly less activated than T lymphocytes in the young calf: even low to moderate levels of MDA inhibit active antibody responses against BRSV infection, partly by masking B cell epitopes on the virus, which are needed to initiate plasma cell development and antibody production. A consequence of this is that the MDA decline despite infection and calves are only partly protected from reinfection (Blodörn et al., 2015). These animals will not have local IgA, nor virus-neutralising IgG at the initial stage of their second BRSV infection.

The duration of active immune responses in animals that do not have MDA while infected will depend on the magnitude of the initial responses, the effector and memory cell- and antibody half-life (26.6 days for IgG_1), the possible induction of long-lived, antibody producing plasma cells in bone marrow and persistence of antigen in lymph nodes, which possibly generate continuous stimulation of immunity. In animals infected while seronegative, systemic IgG lasts at least 4 months and local IgA up to 3.5 months or longer (Kimman et al., 1987; Schrijver et al., 1996; Uttenthal et al., 2000). Furthermore, such animals were shown to be protected against a homologous strain after 3 months (Tjornehoj, 2000). At least in some adult individuals, IgG antibodies may persist in serum and milk for more than three years after natural infection, without new reinfections (Klem et al., 2014). However, it is not clear how many infections or vaccinations are required to get such a long lasting immunity.

When identifying target populations and periods during which animals need to be protected, it is essential to take into account the type of production, its BRSV incidence and disease pattern. As stated above, calves are clinically the most affected in dairy herds within BRSV-endemic areas (Van der Poel et al., 1993; Kimman et al., 1988), whereas disease is additionally frequently seen in adults in areas with low prevalence (Elvander, 1996; Inaba et al., 1972; Hakhverdyan et al., 2005). Highly producing first and second parity cows seem to develop the most severe clinical signs among adults (Elvander, 1996; Hakhverdyan et al., 2005) and BRSV infections result in affected milk yield as well as bulk tank milk somatic cell counts (Beaudeau et al., 2010b). Over a six-month period, seroconversions were detected in 40% of Norwegian dairy herds with BRSV-antibody seronegative young stock at first sampling (n = 54). Moreover, Canadian dairy calves with respiratory disease under three months of age had lower titers of BRSV- and bovine herpesvirus type 1-specific antibody at 1-7 days of age compared to healthy controls (Windeyer et al., 2013). In Irish dairy and suckler herds, BRSV was detected by real-time PCR in 11.6% of respiratory outbreaks (n = 1364), and was commonly detected together with bovine coronavirus, BCoV, the most frequently detected virus (22.9% of outbreaks) (O'Neill et al., 2014). In Dutch veal calf production units, which commingled young calves from dairy herds an all-in all-out manner, calves with low levels of antibodies to BRSV and BCoV at arrival at 2-4 weeks of age had higher risk for respiratory disease during the three first weeks (Pardon et al., 2015). Furthermore, BRSV was detected in 7 of 24 respiratory outbreaks occurring at 3-5 (or more) weeks after arrival and seroconversions to BRSV occurred in 6 of 15 herds studied (Pardon et al., 2011). On the other hand, in a Swedish bull testing station that received 4-8-month-old calves from about 100 different suckler herds only during summer, and with a preceding quarantine in the original herd at pasture, BRSV infections occurred only every second year out of six studied, and was associated to a severe disease outbreak once (Hägglund et al., 2007). This contrasts a French study including 51 conventional fattening operations and nearly 700 beef calves, in which most unvaccinated calves seroconverted to BRSV within six weeks after arrival. The management of these herds was not reported. The risk of respiratory disease during the first six weeks was lower in pens with a large number of calves that were seropositive to BRSV and *Mannheimia haemolytica* at arrival at 6-10 months of age, and higher in pens with more seroconversions to these pathogens (Assie et al., 2009). Different control measures are applicable in the different type of productions described above.

Control by biosecurity

Biosecurity alone can likely be used to control virus spread and serve as single tool for eradication in areas where animal exchange between BRSV-free herds is possible. However, until the epidemiological cycle of the virus is clarified (*e.g.* the importance of carrier cattle and different indirect transmission routes) the biosecurity has certainly to be very broad and strict to safely stop the virus at all instances (*i.e.* for zero risk of virus introduction). Alternatively, farmers can agree to risk some virus introduction that perhaps results in disease in adult animals.

There are several advantages with control by biosecurity without vaccination: the introduction of several pathogens can be avoided (including highly contagious, if the biosecurity is strict). Furthermore, vaccine costs, the risk of pathogen spread with contaminated vaccines and immune-induced pathology are omitted and serological surveillance of virus spread is feasible.

Possible drawbacks may be that, following rare virus introduction, the entire herd might be naïve and both morbidity and mortality will be higher than in a partially immune herd. Losses may concern valuable, high-producing adult animals, in addition to young stock (Elvander, 1996). As mentioned above, the virus amplification will be maximised compared to in a partially immune herd, resulting in faster and more severe disease and better transmission. Moreover, calves in free herds will not have MDA against the concerned pathogens and might risk disease if sold to infected production units (Pardon et al., 2015). Nevertheless, such calves would respond well to vaccination.

The implementation of high level of biosecurity might also imply isolation of farmers from social events. In some areas of Europe, cattle auctions and exhibitions, as well as occasions of calf commingling for further transport are frequent and socially very important for the entire society, and especially for the farmer community. Visits between neighbor farmers are also central (Nöremark et al., 2013). To avoid isolation due to cumbersome visitor shower and material disinfection, a better understanding of the epidemiology of BRSV could help to design specific biosecurities. Though some data indicate that not providing boots to visitors increases the risk of BRSV infection (Ohlson et al., 2010b), BRSV incidence should be further studied in big herds that use visitor protection wear and hand hygiene, that are closed with regard to animal introduction and that are located in areas with high cattle density and BRSV seroprevalence. If such visitor hygiene appears insufficient to protect from virus circulation under these conditions, other means should be studied further.

Whereas dairy and suckler beef herds additionally may be able to control the direct virus introduction (*e.g.* through quarantines at pasture or animal introduction only from BRSV-free herds), this is difficult in herds that continuously commingle a large number of animals from many origins. However, the number of introductions can maybe be reduced, *e.g.* by using separations in transports and quarantines, with a limited number of animals from a limited number of herds per section, as well as separated ventilation, material and strict staff hygiene. To reduce the severity of BRSV disease in sensitive or partly immune animals, the infection dose can be reduced and the immune system can be

optimized, with or without vaccination. The infection dose was shown to be the most important factor for HRSV shedding in experimentally infected adult volunteers (Hall et al., 1981b). This dose can be reduced, *e.g.* by: decreasing the animal density, age sectioning in small animal groups (Kimman et al., 1988), early isolation of sick animals that are high virus shedders, good ventilation and hygiene of buckets and nipples. The immune system can be optimized, *e.g.* by: sufficient and prompt administration of good quality colostrum, sufficient nutrition already early in life, dry bedding, analgesia during surgical intervention and decreased transport times.

Several actors are working for increased biosecurity to reach BRSV control at regional or national levels without vaccination (Klem et al., 2013; Ohlson et al., 2013). The proposed major mean to reach this is by classifying herds as BRSV free or not, based on antibody detection in pooled milk of five first parity cows followed by bulk tank milk in dairy herds, to make aware the animal owner of the herd susceptibility to BRSV. A high level of biosecurity will be ensured, animal exchange is suggested to occur between BRSV-free herds, herd-specific protection wear will be used by visitors and an alert system will be established between farmers and animal professionals (Ohlson, 2010c). This strategy is based on the following assumptions:.

- that direct animal and indirect human transmission of virus are the main routes of transmission and that routes that are difficult to control by biosecurity is not common (airborne, milk collection, or small wild animals). This has been suggested based on the fact that not providing boots to visitors increased the risk of BRSV infection (Ohlson et al., 2010b) and that some herds were defined as BRSV-free in areas where other herds had previously been infected (Klem et al., 2013; Ohlson et al., 2010a).

- that virus persistence is not of major importance for virus spread. This was assumed based on the fact that BRSV self-clearance is possible within herds and in an area (Ohlson et al., 2013) and that virus circulation occurs during the summer period (Klem et al., 2013).

As highlighted above, it remains to investigate if BRSV can be avoided by practically applicable biosecurity measures alone, at long term in large herds in highly cattle populated areas, at a large scale. Since the duration of antibody vary in calves and adults, and probably after primary and succeeding infections, it could be advantageous to study BRSV in these herds by monitoring the same, initially seronegative individuals, at monthly intervals. New seronegative sentinels should be searched and monitored once individual seroconversion has been detected. Indeed, repeated herd classification and seroconversion based on antibody detection in different individuals at each sampling imply some uncertainties for data interpretation.

Control by vaccination

In some production systems that cannot only rely on biosecurity for BRSV control, vaccination is an option. The vaccination strategy will be different in different production systems and according to what is tried to be achieved. In order to control clinical signs to avoid losses and treatments, vaccines can be used that reduce clinical signs but allow continuous virus shedding. However, as shown at the global level for rinderpest virus (Roeder et al., 2013) and in some European countries for FMDV (Sutmoller et al., 2003), vaccination could also be used as a temporary tool in highly endemic areas or herds, to stop virus spread. This necessitates vaccines that protect against virus shedding following infection, rapidly after vaccination and during a reasonably long period of time. It also requires knowledge about which animal age groups are major virus shedders, to tailor vaccination schemes to become cost effective. In fact, though some previously infected animals shed BRSV following reinfection, the role of subclinically infected adults in BRSV transmission as a whole is not clear (Van der Poel et al., 1993).

Using the currently available BRSV vaccines, the effect of vaccination and the vaccine approach needed depend on the category of animal to be vaccinated. In BRSV-seronegative animals, such as calves from BRSV-free herds and calves or young stock in which the MDA or active immunity has waned (*e.g.* before moving to fattening units at 6-8 months of age), commercial parenterally administered vaccines is likely to protect well, at least during a limited period of time (months) and if all animals in the herd are well vaccinated (Assie et al., 2009; West et al., 1999; Ellis et al., 2001; Verhoeff & van Nieuwstadt, 1984b). However, booster doses are needed for long lasting immunity. Moreover, some virus shedding following infection (West et al., 1999) may result in continuing circulation in the cattle population, which requires constant control before risk periods.

Calves designated to commingling in veal calf units (in the Netherlands at 2-4 weeks), in fattening units (in Sweden at 2-8 weeks) or in heifer contract rearing units (in UK at 2-6 months), need to be immunised at a young age, before shipping from the dairy or suckler beef herd. These animals might encounter BRSV at the very early stage of commingling, or even before or during the transport, and thus rapid immunity is of importance.

Maternal immunization has the advantage to potentially protect the very young animal, as shown by passive transfer of BRSV-specific antibodies by colostrum from vaccinated mothers (Dudek et al., 2014; Ellis et al., 1996). However, while susceptible mothers become actively protected, the protection of calves is passive, and would probably only be

partial, at least at the scale of a population. The effect is dependent on the intake and absorption of sufficient quantities of good quality colostrum and failure of passive transfer is still very common (Pardon et al., 2015). Moreover, as mentioned above, by being absent from airway lumen, MDA does not fully protect from virus entry, and clearance of virus is suboptimal by lack of cellular immune memory.

Direct immunisation of young calves is potentially more effective, if the inhibiting effect of BRSV-specific MDA can be overcome. These antibodies inhibit particularly well the humoral responses to parenterally administrated vaccines and, consequently, calves are not optimally protected when the BRSV-specific MDA decline to below protective levels (Schreiber et al., 2000; Hägglund et al., 2004; Larsen et al., 2001; Ellis et al., 2014). Nevertheless, cellular priming mediates reduction of virus shedding (Mawhinney et al., 2005; van der Sluijs et al., 2010) and can also reduce the clinical signs of disease as well as lung pathology (Blodörn et al., 2014). Due to varying levels of MDA at vaccination and differing abilities to generate immune responses, immunity induced by early vaccination will be insufficient at varying ages and several boosts must be performed in a calf group to ensure protection of all individuals. This is time consuming and expensive.

Intranasal (i.n.) administration of attenuated live BRSV vaccines showed better efficacy in calves with MDA and induced good protection against severe challenge 5 and 14 weeks after vaccination (Blodörn et al., 2014; Vangeel et al., 2007; Ellis et al., 2013). However, some issues remain, such as the duration of protective immunity (Ellis et al., 2010) and shedding of vaccine virus during long periods (Timsit et al., 2009) that theoretically implies risks for *in vivo* passage and reversion to virulence. However, by using a vaccine virus with a deletion of a gene that is coding for a surface protein of BRSV (small hydrophobic protein, SH), vaccine virus shedding was almost abolished in calves with MDA and transmission to seronegative sentinel pen-mates did not occur (Blodörn et al., 2014). This vaccine induced near complete clinical and virological protection against severe challenge, five weeks after a single i.n. administration to 4-9 weeks old conventional calves with MDA (Blodörn et al., 2014). By combining i.n. priming by this vaccine, with a heterologous, intramuscular, subunit vaccine boost (based on internal HRSV proteins that induced BRSV-specific T cells), the immunity seemed both more diverse and stronger after an additional seven weeks, compared to following a homologous i.n. boost with the attenuated live vaccine (Makabi-Panzu et al., 2014). Consequently, a heterologous prime-boost strategy can possibly be used to broaden and prolong BRSV-specific immunity in young calves and bring us towards better BRSV control.

Ideally, like the aforementioned experimental vaccines, future vaccines should have DIVA characteristics (Differentiation of Infected from Vaccinated Animals) to allow serological studies of their own efficacy and duration of immunity in the field, as wells serological monitoring of virus spread in vaccinated populations. Indeed, since DIVA vaccine-induced antibodies are distinguishable from those induced by vaccination and subsequent natural infection, the duration of vaccine-induced immune responses may be studied without using costly facilities for animal isolation in order to exclude natural infections. Furthermore, since poorly protected animals seem to develop significantly more antibodies to the DIVA protein than do protected animals upon virus encounter (shown for bluetongue virus) (Anderson et al., 2014), the matching between vaccine and field strains would get obvious at population level. Finally, vaccine-induced adverse clinical signs can be separated from disease caused by natural infection simultaneous to vaccination, sero-epidemiological studies would become feasible in vaccinated areas, and BRSV diagnosis in vaccinated animals with clinical signs becomes possible by serology.

In conclusion, better knowledge on BRSV epidemiology is needed to design applicable biosecurity measures and to tailor vaccination to stop virus circulation in a cost-effective manner. Improved biosecurity measures, adapted to different type of productions, could be used alone, or be combined with vaccines in order to decrease the disease impact, especially in naïve herds with high risk for virus introduction. A DIVA approach will enable serological monitoring to preserve a BRSV free status despite vaccination. By developing vaccines that induce long duration of protection in the presence of MDA, BRSV can be better controlled. A market with BRSV-free, but immune, animals would at any scale improve cattle health. Since vaccine outcomes partly depend on virus infection dose, vaccination should not replace management improvements, but need to be used as a complement to these, to obtain maximum effect.

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Are Clostridia (especially *C. botulinum*) linked to chronic health problems in dairy herds?

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In recent years, unusual herd health problems in dairy cattle have been reported by veterinarians and farmers across Germany. According to reports, *Clostridium (C.) botulinum* and/or its toxins and/or antibodies were detected in affected animals. The authors named this previously unknown form of botulism "chronic/visceral botulism", caused by toxicoinfection. Non-specific clinical symptoms such as ill thrift, decreased milk yield and increased morbidity and mortality have been described on a herd level and have resulted in the abandonment of entire dairy herds in extreme cases. Individual animals have been recorded with a range of clinical signs including indigestion, laminitis, ataxia, retracted abdomen, forced respiration and death.

The following case-control study was carried across 31 Bavarian dairy farms (case group n = 21; control group n = 10) and included 1720 dairy cows, of which 284 (case and control cows) were examined further. After passing a standardized clinical examination, the animals were sampled for bacteriological, parasitological, serological and hematological examination. Questionnaires were used for additional data collection and covered specific fields within each farming enterprise.

Analysis of fecal and rumen fluid by real-time PCR, revealed that Clostridia Species were present on 74.2% of the dairy farms evaluated. There was no difference in the frequency of detection between the case and the control group or between the case and control cows on each farm. The distribution of Clostridia was found to be 71.4% *C. perfringens,* 24.7% *C. novyi*, 2.6% *C. botulinum* and 1.3% *C. haemolyticum.* Serum antibodies against BoNT-C1 and -D were detected in 78.2% of the farms with no statistically significant difference between the case and control groups. There was a statistically significant difference between the cows within the case group (84.6% - control cows vs 70.2% - case cows).

There was no difference between case and control group regarding the poor husbandry conditions however, the cows in the case group showed significantly poorer hygiene, increased detection of skin lesions on bony prominences of the proximal body and an increase in the prevalence and severity of lameness. No correlation was found between any of these factors and the frequency of detection of *Clostridium* Species.

Leptospirosis associated with emergent congenital jaundice syndrome in bovine aborted fetuses

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During the second semester of 2014, Southern Belgium faced an unusual increase of congenital jaundice cases in bovine aborted fetuses. Thus, 119 cases of icteric fetuses from 113 farms were sent to our laboratories in the second half of 2014. Diagnostic investigation led to positive serological results in dams (microscopic agglutination test) for various *Leptospira* serogroups (mainly *Grippotyphosa* and *Australis*).

In order to confirm the link between *Leptospira* spp. and this emergent syndrome, samples of aborted fetuses from fall/winter 2014-2015 were submitted to an adjusted PCR method for the detection of pathogenic *Leptospira*. For each fetus, spleen and placenta, if present, were analyzed. Aborted fetuses were classified into 4 groups:.

- icteric fetuses (n = 74),

- non icteric fetuses coming from farms concerned with at least one case of icteric abortion (n = 37),

- non icteric fetuses coming from farms free of icteric cases (n = 39),

- non icteric fetuses showing remarkable lesions such as hemorrhages and coming from farms free of icteric cases (n = 39).

PCR results were: 51 positives cases (69%) in group 1, no positive case in group 2, 1 positive case in group 3, no positive case in group 3.

These results show that detection of pathogenic *Leptospira* is strongly correlated with the icteric picture. They also indicate that these abortions caused by *Leptospira* spp. are effectively sporadic and not contagious at a herd level, suggesting a limited excretion of bacteria by the infected cows.

Infection of a dairy herd by *Mycoplasma* (ex *Eperythrozoon*) *wenyonii*: first description in France

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In November 2014, a dairy herd (50 lactating cows, 8,500 kg/cow/year) was suddenly affected on two occasions (4 and 15 animals, 20 days apart) by a disease characterized by hyperthermia (40.5ºC- 41ºC), tachypnoea with no anomaly at auscultation, a drop in milk production (50 to 80% for the affected animals), oedema of teats, joint swelling at the hock with rigidity upon movement.

Blood smears showed an erythro-infection compatible with *Mycoplasma wenyonii* on various samples, and justified the initial therapeutic treatment: antibiotic (oxytetracyclin, 10 mg/kg for 3 days) and an inflammatory substance (meloxicam, 0.4 mg/kg, one shot).

Complementary exams (n = 5) revealed an anaemia (Hb = 92 g/L, HCT = 27.2%, RBC = $5.32 \cdot 10^{12}$/L), neutrophilia, (2/5), lymphopenia (5/5) and an inversion of the formula (5/5). The following biochemical parameters (n = 3) were normal: GGT, total bilirubin, urea, creatinin, Ca, Mg, P, ASAT). A high fibrinogen rate (7.16 g/L), total protein (84.4 g/L), of which albumins (36.1 g/L) can also be observed.

Clinical signs disappear rapidly after the establishment of the treatment, and milk production comes back to a level a little under the original level after about 10 days. Controls on the production done before and after the episode show an average loss of 12% in the affected animals [from -3 to -21%]; non-affected animals also suffered a loss of 5% on average (subclinical infection?). Affected animals are statistically older ($n = 19$, $p < 0.05$). Concerning the somatic cell counts, the affected animals show non-significant variations.

The vector-borne transmission of this disease has been made possible at this time of year by the mild climate (between the two occasions, the daily temperatures were always superior to 7ºC). A quite large population of diptera has been observed and some examples captured close to the stable.

This first description in France shows the importance of a surveillance of atypical clinical phenomena, as well as the need for investigations means when emerging, but also in the epidemiological follow-up of such a case.

The epidemiology of resistant *Escherichia coli* in cattle

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E. coli was first described by Theodor Escherich in 1885. A diverse range of strains of *E. coli* can occur in the intestine of cattle and these include primarily commensals, as well as bovine pathogens and isolates which can cause disease in man. To understand the epidemiology of resistance in *E. coli* in cattle, it is necessary to understand the epidemiology of this complex flora of *E. coli* strains which can occur in the intestine of cattle. Pathogenic strains of *E. coli* usually possess specific virulence factors and the genes encoding these factors can be transmissible between *E. coli* strains either by means of plasmids or on bacteriophages. Plasmids and other mobile genetic elements (MGEs) are of course also a major means for dissemination of antimicrobial resistance genes both between strains of *E. coli* and to other bacteria (for example to *Salmonella*).

The complexity of the *E. coli* flora in the intestine and in faeces means that a number of different *E. coli* colonies must often be investigated to characterise the diverse flora which may be present. Although a range of types of *E. coli* can occur in the intestine and faeces of cattle, those which are more frequently recovered have been considered better colonisers of the intestinal tract (Hinton, 1985). In most cattle, it has been found that a few types tend to occur as major components of the intestinal flora with a range of other types occurring in lesser numbers. The same feature is observed for groups of animals where a few O-serotypes are isolated from most animals with an increasing number identified from fewer and fewer animals. Hinton examined ten *E. coli* isolates from faecal swabs of cattle of various ages and (using serotyping to differentiate between them), detected a range of 0-8 different O-serotypes per animal, whilst the average number was 1.83-3.15, with the lower figure obtained for adults and the higher figure for calves. A notable exception where this strain diversity may not be observed is in neonatal animals with clinical disease caused by enterotoxigenic *E. coli* (ETEC). Strains of ETEC responsible for the disease can represent the dominant or sole colony present in faeces (Butler & Clarke, 1994).

Hinton et al. described how the *E. coli* faecal flora appears to become less complex as animals grow older, with the average number of O-serogroups lower in adults than it is in calves (Hinton et al., 1982). Hinton also reported that *E. coli* from calves tend to be more resistant than those from adults and surmised that the multiply-resistant strains of *E. coli* which colonised calves in the first week of life were the result of historical and current exposure to antimicrobials (Hinton, 1986). Current scanning surveillance in England and Wales of *E. coli* from cattle reveals that this pattern still holds true (UK VARRS Report, 2013) and it is also frequently reported in other studies. The *E. coli* flora of calves was found to be unstable, with dominant types of *E. coli* persisting sometimes for only a short time before being replaced (Hinton, 1986).

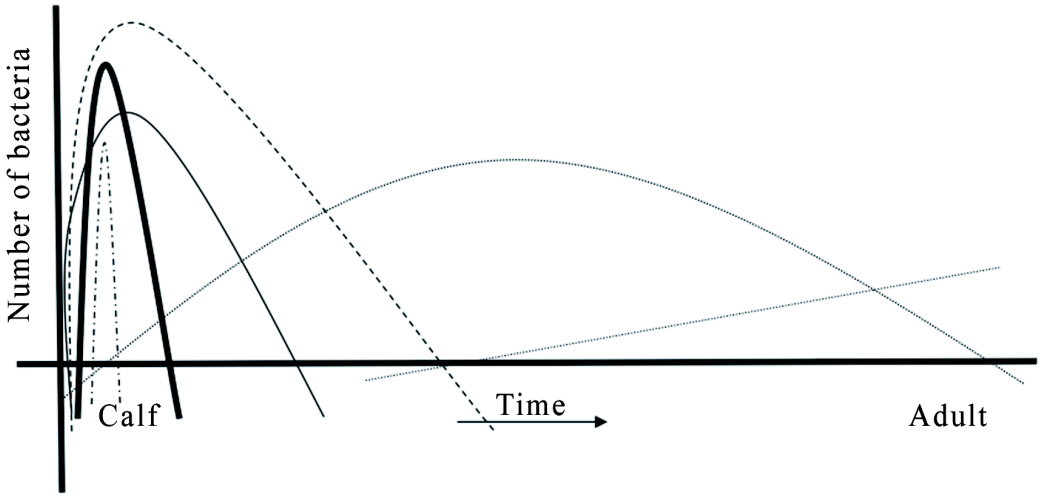

Figure 1: Schematic diagram of successive waves of colonisation of the intestine of calves with different strains of *E. coli* (each line represents a different strain of *E. coli*)

Various methods have been used to differentiate between different strains of *E. coli*. Formerly, methods such as serotyping in conjunction with biotyping were used, the latter looking at various phenotypic characteristics such as the ability to ferment various carbohydrates, or metabolise amino acids, the possession of enterotoxins or the production of

colicin. These methods were replaced by pulsed field gel electrophoresis (PFGE) which in turn has now often been replaced by other genetic methods of strain differentiation, including multi-locus sequence typing (where several house-keeping genes are sequenced and differences between the sequences used to differentiate between strains) and whole genome sequencing. Earlier work sometimes found different "biotypes" of the same O-serotype colonising calves, as well as clonal expansion of single clones and the acquisition of different resistances by what was presumed to be the same clone (Hinton et al., 1982). Superimposed on the strain diversity of *E. coli*, it is necessary to consider the transfer of resistance genes by plasmids and other mobile genetic elements. Although this paper focuses on *E. coli*, other bacteria present in the intestinal flora or environment may also provide opportunities for exchange of genetic material.

Hinton (1986) reported a very interesting observation in pigs, which is likely to have relevance to other species including cattle, in some circumstances. In pigs, after weaning tetracycline resistance in the faecal flora can increase dramatically, because of the dominance of the faecal flora by a single serotype ("Abbotstown") which happens to be tetracycline resistant (Hinton, 1986).

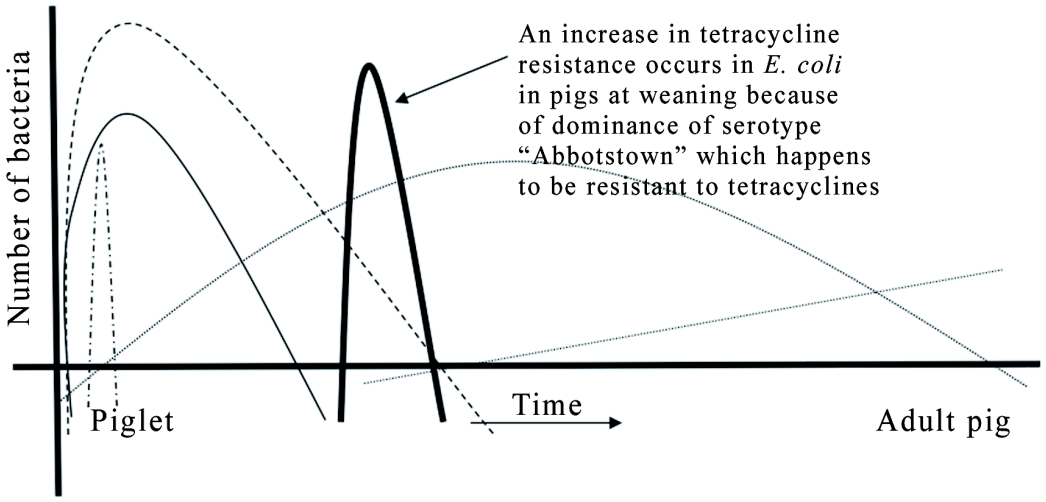

Figure 2: *E. coli* serotype "Abbotstown" in pigs at weaning (Each line represents a different strain of *E. coli* "Abbotstown" is schematically represented in bold)

New strains of *E. coli* may be acquired from food, the environment or other animals. Bettelheim et al. differentiated *E. coli* strains by serotype and biotype and found that strains isolated from babies born in a London hospital could also be present in their mothers' faeces. Gothefors et al. (1976) reported the transfer of strains occurred from the babies to their mothers, presumably following contact with nappies contaminated with faeces. Similar investigations in cattle have revealed that a proportion of strains isolated from young calves may also be identified in the faeces of their mothers (Hinton et al., 1985) and cows may be a source of *E. coli* for their calves. Thus there are parallels observed between the epidemiology of *E. coli* man and animals.

The factors responsible for the instability of the *E. coli* faecal flora in young animals and its progressive simplification as animals grow older have been considered to include:

- the diversity of the *E. coli* population in the environment,
- dietary effects,
- *E. coli* strain characteristics,
- immune effects,
- interactions with other bacterial species in the intestinal microflora (Hinton, 1985).

Commensal and pathogenic *E. coli*

Commensal *E. coli* are those which in most circumstances inhabit the gastro-intestinal tract without causing any disease. However, in certain circumstances any *E. coli* can opportunistically cause disease, for example in intestinal trauma a strain could contribute to peritonitis or in neonatal calves with insufficient colostral immunity it could cause neonatal septicaemia. Even where antimicrobial resistance occurs in strains of *E. coli* which are regarded as commensals with limited potential to cause disease, they constitute a reservoir of resistance genes which may be transferable to other strains of *E. coli* or to other organisms (for example *Salmonella*) which may have greater relevance for animal or public health. Resistance in commensal *E. coli* is therefore also considered of significance.

Pathogenic *E. coli* affecting cattle and those *E. coli* strains carried by cattle which are zoonotic can be divided into several categories or pathotypes -

Enterotoxigenic *E. coli* (ETEC). These strains possess specific adhesins and enterotoxins.

Verotoxigenic *E. coli* (VTEC). These strains produce verotoxin (Shiga toxin). A subset of VTEC isolates may be termed enterohaemorrhagic *E. coli* (EHEC). These EHEC strains can also form attaching and effacing lesions.

Enteropathogenic *E. coli* (EPEC). These strains are able to produce attaching and effacing lesions.

ETEC is a major cause of neonatal calf diarrhoea; EPEC and VTEC (including EHEC) can often be isolated from both diarrhoeic and healthy calves and their role in disease has been suspected; a recent meta-analysis of published data from 1951-2013 is available (Kolenda et al., 2015).

[Types not well- recognised in cattle such as enteroaggregative *E.coli* (EAEC), diffusely adherent *E.coli* (DAEC) and enteroinvasive *E.coli* (EIEC) (Kolenda et al., 2015; Johnson & Nolan, 2009) are not considered in detail further].

Mobile Genetic Elements (MGEs)

In addition to the diversity of strains of *E. coli* inhabiting the bovine intestine, MGEs can transfer resistance genes (and sometimes other characteristics) between strains of *E. coli*. Although a single *E. coli* clone can encode ~ 2000 genes, all *E. coli* have been estimated to collectively encode ~18,000 genes (Touchon et al., 2009). Plasmids are double-stranded, extra-chromosomal circular DNA molecules, capable of autonomous replication, which do not carry genes essential for the growth of their host cell under normal conditions (Carattoli, 2009). Plasmids are usually classified into incompatibility (Inc) groups according to traits concerned with plasmid maintenance in the host cell - incompatible (i.e. related) plasmids cannot be stably maintained in the same host cell. However, bacterial cells may contain several different plasmids which are compatible and which may also co-transfer (transfer together) during bacterial conjugation. In bacterial conjugation, bacteria are linked by a tubular structure which allows a copy of the plasmid DNA to be transferred from a donor bacterial cell to a recipient, which did not formerly possess the plasmid. A recent review (Johnson & Nolan, 2009) listed the plasmid incompatibility groups and recorded those groups in which multiple antimicrobial resistance (MDR) has been reported - plasmids belonging to most Inc groups have been reported to show MDR. This is important because different virulence plasmids are important factors in the production of disease caused by several different *E. coli* pathotypes, including ETEC and enteropathogenic *E. coli* (EPEC), as well as those types less well known in cattle, such as enteroaggregative *E.coli* (EAEC), diffusely adherent *E.coli* (DAEC) and enteroinvasive *E.coli* (EIEC) (Kolenda et al., 2015; Johnson & Nolan, 2009). Therefore it seems that plasmids belonging to most Inc groups have the potential to acquire MDR genes.

In a longitudinal study of a UK dairy farm, it was shown that an IncK plasmid which carried resistance to third generation cephalosporins by encoding the extended-spectrum beta-lactamase enzyme CTX-M-14, was highly promiscuous and able to disseminate widely between unrelated strains of *E. coli* on the farm (Liebana et al., 2006). This longitudinal study represents a general scenario where resistance on a highly promiscuous plasmid which is not restricted by plasmid incompatibility is able to spread between numerous different strains of *E. coli*.

MGEs frequently carry several antimicrobial resistance genes on the same structure (genetic linkage). This genetic linkage has the consequence that use of any one compound to which the MGE confers resistance will result in co-selection of the other resistances which are carried (Figure 3). Linkages of antimicrobial resistance genes to genes conferring advantages relating to intestinal colonisation in calves have also been proposed (de Verdier et al., 2012).

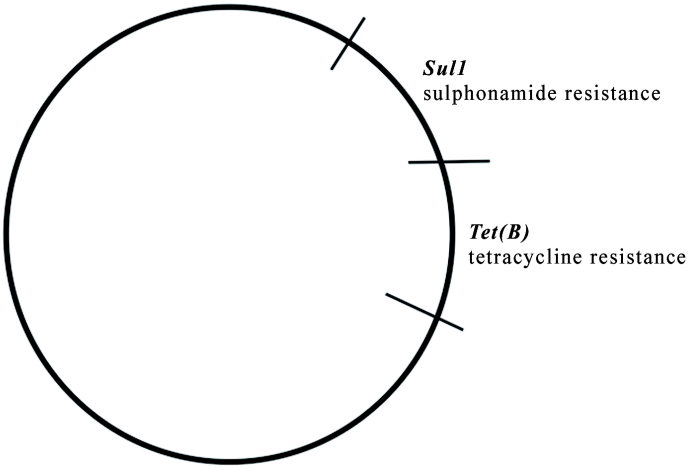

Figure 3: The principle of co-selection

Integrons are frequent sub-components of two types of MGEs - plasmids and transposons and the structure of class 1 integrons (which are very common in *E. coli*) includes the *Sul1* gene conferring sulphonamide resistance. This gene is often accompanied on class 1 integrons in animal *E. coli* isolates by genes conferring resistance to tetracyclines and streptomycin, accounting for the frequent occurrence of resistance to these three compounds as a core or frequent resistance pattern in isolates which show MDR. In accounting for the frequent occurrence of this pattern in *E. coli* isolates we observe today, it is probably important to consider the degree of usage of these compounds in the decades following their introduction into veterinary medicine and the selective pressure this will have exerted in promoting the occurrence of such genetic structures.

In human medicine, an epidemic clonal strain of Extended-Spectrum Beta-Lactamase-producing *E. coli* (sequence type 131) has emerged and spread globally (Rogers et al., 2011). This strain is only infrequently reported from animals but can cause serious disease in man, including predominantly urinary tract infections. This situation represents a further scenario where one particular MDR *E. coli* strain and its related progeny (i.e. a clone) have spread globally. Parallels to the global emergence and dissemination of this strain in human medicine have not yet emerged in *E. coli* in cattle or other food-producing animals.

Genetic linkage can also involve genes which confer virulence traits. Thus, ETEC possess fimbriae enabling them to adhere to the intestinal epithelium (for example F5/ K99) as well as heat- stable and heat-labile enterotoxin genes. These genes are commonly present on plasmids, occurring in a number of O-serotypes of *E. coli* (most commonly O8, O9, O20 and O101) which have all been recorded as ETEC strains in cattle (Butler & Clarke, 1994). Linkage of antimicrobial resistance genes to these ETEC virulence genes on plasmids has been reported. In bovine (and porcine) ETEC Harnett and Gyles reported that it is common to find genes for STI, colonising fimbriae and antimicrobial resistance on a single plasmid (Harnett & Gyles, 1985), resulting in strains acquiring the ETEC virulence plasmid, concurrently acquiring resistance. On farms experiencing problems with neonatal calf diarrhoea, a higher occurrence of resistance in *E. coli* in calves has been demonstrated (de Verdier et al., 2012; Gunn et al., 2003).

Considering verotoxigenic / Shiga toxigenic *E. coli* (VTEC), these organisms can acquire resistance, but VTEC O157 isolates are often relatively sensitive. Human cases of VTEC O157 infection are rarely treated with antibiotics because it is thought that treatment might increase the release of toxins from dying bacterial cells. Although VTEC O157 usually also carries a particular plasmid, most of the virulence genes in this VTEC strain are carried on a phage which is integrated into the bacterial chromosome (Johnson & Nolan, 2009).

Finally, it should be noted that *E. coli* as an inhabitant of the intestine and of the environment has extensive opportunities for exchange of genetic material with a much wider range of bacteria than would say an obligate pathogen of the udder, since the udder is an environment which is normally sterile (Martel & Coudert, 1993).

Antimicrobial usage as a driver of resistance

Antimicrobial usage is generally recognised as a prime driver of antimicrobial resistance in bacteria in the calf intestine (Berge et al., 2005; de Verdier et al., 2012), indeed a general relation between the degree of antimicrobial usage and the degree of emergence or occurrence of antimicrobial resistance is usually evident whether humans or animals are considered, although it is not always easy to demonstrate (Schechner et al., 2013). Although the effects on the intestinal flora of individual calves of short duration treatment may be transient, the cumulative effect of repeated treatment of

different calves probably exerts a long-term selective pressure for the emergence of resistant strains over time. These strains are likely to have made compensatory changes enabling them to compete fully with non-resistant strains which do not carry the burden of acquired resistance genes. The time required to undergo this evolutionary process to ensure no loss of fitness, probably explains the lag in the widespread occurrence of resistance which can often be observed following the introduction of new antimicrobial compounds. Other factors also play a part in determining the occurrence of resistance - the increase in prevalence of tetracycline-resistant strains of *E. coli* which can be observed in pigs at weaning has already been mentioned. The role of the environment in the persistence or introduction of MDR *E. coli* affecting calves on farms and the role of animal/ human or vector movements are other factors.

Considering the resistance to a wide range of beta-lactam compounds (penicillins and cephalosporins) which is conferred by ESBL-production in *E. coli*, it was found that strains of *E. coli* carrying the extended-spectrum beta-lactamase CTX-M-1 were selected and proliferated in pigs to which amoxicillin, ceftiofur or cefquinome had been administered (Cavaco et al., 2008). However, selection and proliferation of AmpC resistant *E. coli* with the AmpC beta-lactamase enzyme CMY2, was not observed in a separate study on dairy cattle administered ceftiofur, but rather a drop in the total Gram-negative enteric bacterial population occurred, which facilitated detection of resistant strains. In this case, horizontal transmission of CMY2 between strains of *E. coli* was not observed (Singer et al., 2008). Daniels et al. reported transfer of plasmids carrying CMY2 between *E. coli* strains in an experimental study of calves whether they had been treated or untreated with ceftiofur and further that the level of ceftiofur use was not associated with the occurrence of resistance in commensal *E. coli* in an observational study on pooled adult cattle faeces obtained from dairy herds. Pereira et al. examined the effects of *in vivo* selection of resistant *E. coli* after ingestion of milk with added drug residues and found that calves fed milk containing residues had significantly greater proportions of *E. coli* resistant to several antibiotics than control calves fed only raw milk.

A recent APHA study (Brunton et al., 2014) investigated the effect of feeding waste milk containing antimicrobial residues (including residues of cefquinome) to a group of 25 calves, whilst a control group of 25 calves received milk replacer containing no antimicrobial residues. The study was performed on a farm known to harbour ESBL-producing *E. coli*. The prevalence of ESBL-producing *E. coli* declined significantly more slowly over time in the group receiving waste milk plus antimicrobial residues than in the group receiving milk replacer.

Observed differences in these studies between calves and adult cattle could of course be related to different routes of antimicrobial administration (oral, intra-mammary, parenteral). A strain of *Bacillus cereus* has also recently been reported from the intestine of an adult bovine which produced a metallo-beta-lactamase able to efficiently hydrolyse ceftiofur (Erickson et al., 2014). It is therefore possible that the other components of the commensal flora of the intestinal tract and their ability to degrade antimicrobials in the intestine may also influence the occurrence of resistance in *E. coli*.

Conclusion and practical considerations

Highly resistant *E. coli* infections in adult cattle, (for example highly resistant *E. coli* causing mastitis in dairy cows), may result from adult cattle being exposed to *E. coli* in the calf environment. Calf strains of *E. coli* tend to be more resistant than *E. coli* strains from adults.

Terminal hygiene and disinfection and all-in; all-out management of calf accommodation are important to try to break the ongoing cycle of transfer of resistant *E. coli* to neonatal calves from their environment.

Minimising resistance in *E. coli* in the calf environment means that calves with neonatal colisepticaemia as a result of sub-optimal colostrum intake or navel infections may have infections which are more readily treatable.

Treatment of bovine *E. coli* infections with antimicrobials should be targeted against those *E. coli* strains which are causing problems, whilst minimising the effects on commensal *E. coli*. The role of non-antimicrobial treatment in (for example) therapy of neonatal calf diarrhoea should not be overlooked (Meganck et al., 2014).

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What's wrong with metronidazole?

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An estimated 2000 bulls are slaughtered, each year in Texas in an effort to control bovine trichomoniasis. The literature shows that a test and removal program for Texas is ill advised because of low test sensitivity and the extent of animal mixing. The results of this control program, thus far, do not look promising. On the other hand, blanket treatments of bull populations have been 100% effective at treating bovine trichomoniasis using antimicrobials from the nitro-imidazole family. However, in the United States, the Federal Drug Administration (FDA) has banned all use of all members of the nitro-imidazole family in food-producing animals because they are reported to be carcinogenic. One of the earliest, documented decisions occurred as long ago as 1977 when the International Agency for Research on Cancer (IARC) found that metronidazole caused cancer.

This presentation reviews the merits of that decision the continuing ban on the use of metronidazole in food-producing animals.

Impact of an alternative protocol to treat bovine respiratory disease using decreased antimicrobial regimen on therapeutic efficacy and antimicrobial consumption

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Objectives

In the context of requested decrease of antimicrobial use in human and veterinary medicine, the aim of our study was to assess the efficacy and the impact on antimicrobial consumption of a decreased antimicrobial regimen administered at early stage of Bovine Respiratory Disease (BRD).

Materials and Methods

195 young bulls (YBs) from 6 commercial farms were randomly assigned to one of the two experiment group E and L, based on the method of detection of illness and treatment regimen. In E, the YB ruminal temperature was continuously followed. YBs presenting an increase in ruminal temperature over 40.2°C persisting more than 12h and presenting no or slight signs of disease were considered as "Early detected" and treated with 2 mg/kg of marbofloxacin, a fluoroquinolone commonly used in the treatment of BRD. In L, YBs presenting moderate or severe signs of illness were considered as "Late detected" and treated with 10 mg/kg of marbofloxacin, corresponding to a classical field situation. In case of relapse, YBs were treated according to the farmers' habits. Number of metaphylactic, marbofloxacin and relapse treatments were recorded for each YB. Treatment efficacy was evaluated based on clinical recovery and proportion of relapse treatments. Treatment incidence (TI) based on used daily doses (UDD) was calculated to assess antimicrobial consumption.

Results and Conclusion

In E, proportions of marbofloxacin and relapse treatments were higher (respectively 45 and 37%) than in L (25 and 7%). 97% and 100% of YBs treated in E and L were cured at the end of the study.

The mean values of sum TI_{UDD} marbo and relapse were 40 and 46 in groups E and L, respectively. Overall, the amount of antibiotics used was slightly decreased in E, even if the proportion of treatments increased. Values of TI_{UDD} were strongly influenced by the presence of a metaphylactic treatment in the herds, TI_{UDD} of metaphylactic treatment representing almost 90% of the total TI_{UDD}. .

Our results suggest that a lower antibiotic dose, associated with an early detection of disease didn't affect treatment efficacy while decreasing drug consumption, evidencing possible antimicrobial use rationalization under field conditions, to limit impact on public health.

Antimicrobial resistance in *Escherichia coli* implicated in calf scours: study in the centre of France between 2011 and 2013

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Diarrhea in newborn calves is a major cause of economic losses in farms. The most frequent pathogens isolated from calves less than 8 days of age are *Escherichia coli*, rotavirus, coronavirus and *Cryptosporidium parvum*, but many risk factors also contribute to clinical expression.

The aim of this study was to update our knowledge about the prevalence of these pathogens in the French department of Allier, and to type and test the isolated *E. coli* for their antimicrobial resistance.

Thus, blood and feces of 125 diarrheic and 61 healthy calves less than 8 days of age have been sampled between January 2012 and January 2013. Major pathogens have been sought and blood levels of immunoglobulin G1 were evaluated by radial immunodiffusion. Questionnaires have also been filled in by farmers and veterinarians implicated in this study. Though the various pathogens have been isolated more frequently in diarrheic calves, other factors were also decisive, such as a failure in passive immunity transfer or a lack of mulching.

Regarding antimicrobial resistance found in *E. coli* isolates, its rate in this departement was generally lower than in the rest of France. However, it is still worrying. Moreover, the use of some molecules such as cephalosporin was found to be associated with a higher level of resistance in *E. coli* isolated from unhealthy calves. Prevention, including good housing conditions and good practices of animal husbandry, mostly around birth, is a key factor to face newborn calf's diarrhea.

Optimization of treatment protocols for clinical mastitis under Belgian field conditions using antimicrobial resistance profiling

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In current dairy farming, bovine clinical mastitis is one of the most prevalent and costly diseases. Ideally, treatment decisions should be culture-based, which is a time consuming technique (of at least 24 hours). Therefore, the aim of this study was to assess the efficacy of standard treatment protocols for clinical mastitis in relation to bacterial cultivation and antimicrobial resistance profiling.

In a 2 year period, 210 milk samples were collected from clinical mastitis in 28 farms in Flanders. Clinical mastitis cases were classified into three major types. Type I was characterized by early post-partum occurrence, a watery appearance and elevated rectal temperature, type II by the presence of flakes and lumps during mid to late lactation and finally type III by a watery appearance and elevated rectal temperature during mid to late gestation. Milk samples from the affected quarters were aseptically collected. Subsequently, a treatment was started depending on the type of mastitis. Fluoroquinolones, Tylosin or Cephalosporins were administrated systemically in case of type I, type II, and type III mastitis, respectively. After cultivation the pathogenic bacteria were identified as *Mycoplasma* spp., *Enterobacteriaciae*, *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp. or *Escherichia* coli as was their sensitivity pattern to 14 different antibiotics by means of the Speed Mam Color® (Virbac Animal Health). The test was performed following the manufacturer's instructions and enabled determination of sensitivity or resistance within 24 hours. When the assay revealed a dismatch between the initial treatment regime and the antimicrobial sensitivity profile, the treatment was changed accordingly.

A variety of bacteria spp. were isolated, including *Staphylococcus* spp. (n = 97), *Streptococcus* spp. (n = 61), *Escherichia coli* (n = 44) and *Mycoplasma* spp. (n = 27). For *Streptococcus* spp. and *Staphylococcus* spp. resistance against Sulphadimidine-Trimethoprim (74%), Tylosine (38%), Cefquinome (18%) and Penicilline (7%) was detected. Based on the antimicrobial resistance profiling, 78 out of 210 cows initially received an ineffective treatment due to antibacterial resistance. Within an individual farm, bacteria belonging to the same species often showed different resistance patterns.

Our findings reinforce the need for culture based therapy to increase the success rates of bacterial treatment and to reduce unjustified antimicrobial drug use.

Update on *Staphylococcus aureus* mastitis

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Introduction

Staphylococcus aureus is Gram-positive, facultatively anaerobic, non-motile, non-sporulating, catalase-positive, coagulase-positive coccus that is generally regarded as a leading cause of contagious mastitis in dairy cattle. In many countries, it primarily causes subclinical intramammary infections (IMI) that often become chronic. Furthermore, these infections are often refractory to routine intramammary antimicrobial therapy. The economic impact of *S. aureus* mastitis is usually manifest through increased milk somatic cell count (SCC) and decreased milk production. However, there are regional and herd differences and in some instances *S. aureus* can be associated with a high incidence of clinical disease.

In the 1960s a significant body of work was published which set the stage for our current understanding of *S. aureus* mastitis and established standards for contagious mastitis control on dairy farms (Davidson, 1961; Wilson & Davidson, 1961; Neave et al., 1966; Dodd et al., 1969; Neave et al., 1969). From this work came the five-point mastitis control program. Implementation of the five-point plan, which was later developed into the NMC 10-point mastitis control plan, has led to a reduction in prevalence of *S. aureus* mastitis on many farms. However, *S. aureus* can still plague individual farms, and depending on chronicity and transmissibility within herd, it can be difficult to eradicate and can significantly impact milk quality. .

Pathogenesis

Intramammary infection occurs by *S. aureus* entering the teat orifice, breaching the streak canal, and entering the mammary gland. Teat skin condition and damage to streak canal keratin can impact the likelihood of IMI with chapped or injured teats being more likely to develop IMI. Once in the mammary gland, *S. aureus* adheres to mammary epithelial cells decreasing the likelihood of the bacterium being washed out of the gland by milking. The primary defense against IMI is phagocytosis of *S. aureus* by neutrophils, the primary somatic cell involved in clearance of pathogens from the mammary gland. However, neutrophil phagocytic function is impeded in the presence of milk, and *S. aureus* produces a number of antiphagocytic factors, such as protein A, capsule, and pseudocapsule that decrease its likelihood of phagocytosis by neutrophils. Furthermore, the concentrations of opsonizing antibodies and complement, factors which facilitate uptake of *S. aureus* by neutrophils, are low in milk further impeding *S. aureus* clearance (Sutra &Poutrel, 1994). .

Once established in the gland, *S. aureus* IMI can lead to ulceration and erosion of the lactiferous sinus and ducts, infiltration of the parenchymal tissue with inflammatory cells, and ultimately damage to the mammary secretory epithelial cells leading to occlusion of the ducts and alveoli which can trap *S. aureus*. These trapped bacteria can become a nidus for infection of other portions of the gland or lead to granulomas and microabscesses (Sutra & Poutrel, 1994). Tissue damage in the mammary gland is further compounded by toxins and extracellular enzymes produced by *S. aureus*, including α, β, γ, and δ toxins, toxic shock syndrome toxin (TSST-1), enterotoxins, nuclease, coagulase, catalase, hyalurodinase, phosphatase, lipase, staphylokinase, and proteases. Toxins can have direct effects on tissue or lead to an uncontrolled host inflammatory response by acting as superantigens. The involvement of enzymes in mastitis pathogenesis is not well understood, but it is hypothesized that they may facilitate pathogen adaptation to growth in milk (Sutra & Poutrel, 1994).

Sources of infection and epidemiology

The lactating cow's udder is considered the major reservoir of infection for *S. aureus* IMI. While *S. aureus* can be isolated from environmental sources on farms, the cow's environment is not considered the major reservoir for infection. The major mode of transmission for *S. aureus* mastitis is from cow-to-cow via fomites, such as milking unit liners, at the time of milking. .

The replacement heifer can be a significant source of *S. aureus* IMI. In some areas of the United States, for example, the prevalence of *S. aureus* IMI in pre-partum heifers exceeds 30%. A report from Louisiana recorded a prevalence of 37% (Trinidad et al., 1990) and work by the author in Missouri has recorded a prevalence of approximately 30% (Middleton, unpublished data), while heifers in Northern areas of the country seem to have a lower prevalence of pre-partum *S. aureus* IMI (Fox et al., 1995). The heifer's mammary gland may become infected as early as six months of age and

she may harbor these infections throughout the first lactation. However, Fox et al. (1995) reported that heifers are most susceptible to infection during the last trimester of gestation. It is presumed that heifers become infected by skin inhabitants being introduced into the mammary gland via the teat end. Roberson et al. (1998) reported that possible sources for *S. aureus* in heifer colostrum at parturition were milk of lactating cattle (70%), heifer body sites (39%), environmental sites (28%), or no identified source (16%). Similarly, Middleton et al. (2002c) reported that 71% of *S. aureus* strains isolated from heifer mammary secretions at parturition were the same as those isolated from milk of lactating cattle and 47% of *S. aureus* strains isolated from heifer teat and udder skin were the same as those isolated from heifer mammary secretions at the time of parturition. .

Owens and co-workers (1998) reported that horn flies (*Haematobia irritans*) were capable of transmitting *S. aureus* to heifers causing new IMIs, and that scabs on teats may be a source of fly colonization with *S. aureus*. Herds that practice effective fly control seem to be at lower risk of early lactation heifer mastitis caused by contagious pathogens than herds without fly control or ineffective fly control (Nickerson et al., 1995; Piepers et al., 2011). Data also suggests that herds that purchase replacement heifers may have a higher prevalence of *S. aureus* IMI than herds undergoing expansion with purchased lactating cattle (Middleton et al., 2002c). Additionally, herds that purchase replacement heifers may have more strains of *S. aureus* and more new strains of *S. aureus* enter the herd than closed herds which rear their own replacements (Middleton et al., 2002c).

With the advent of robust DNA-based strain-typing techniques, it has been recognized that many different strains of *S. aureus* exist with most herds tending to have a predominant strain-type (Middleton, et al., 2002b). It has also been recognized that differences exist between strains with regard to transmissibility, udder inflammation, persistence of infection and impact on milk production (Barkema et al., 2006; Middleton & Fox, 2002a). Individual strain characteristics impact host-adaptability and thus pathogen survival. Strain-specific factors including antimicrobial resistance and ability to survive in biofilms or intracellularly may also influence the outcome of response to antimicrobial therapy.

Studies from Switzerland used ribosomal spacer (RS)-PCR to characterize *S. aureus* isolated from bovine milk samples and found various genotypes of *S. aureus* with different virulence and pathogenicity factors (Fournier et al., 2008; Boss et al., 2011; Syring et al., 2012). Fournier et al. (2008) identified Genotype B (GTB) and Genotype C (GTC) commonly, while other genotypes (GTOG) were found rarely. Overall, Genotype B isolates were found to be highly contagious and highly pathogenic, whereas GTC and GTOG only caused infections in one to three cows per farm and were therefore not considered highly contagious. Fournier et al. (2008) were also able to show an association between RS-PCR genotype and virulence gene patterns. Specifically, GTB was characterized by the presence of *S. aureus* enterotoxin A (*sea*) and D (*sed*), and by a polymorphism within the leukotoxin E gene (*lukE*) caused by a point mutation, classified as *lukEB* (Fournier et al., 2008). Most recently, we have characterized a series of isolates to determine whether Genotype B could be found in the USA. Isolates were selected from a cryopreserved collection of previously characterized *Staph. aureus* isolates from eight dairy farms in the Pacific Northwest of the USA (Middleton et al., 2002b). Milk somatic cell count and *N*-acetyl-β-D-glucosaminidase (NAGase) activity associated with each of the mammary quarters from which the isolates were harvested was known and used to evaluate strain pathogenicity. Ribosomal spacer PCR was performed and standard PCR was used to confirm the presence or absence of the leukotoxin E gene (*lukE*) and staphylococcal enterotoxin (SET) genes, including *sea*, *sed, seg, sei, and sej*. Overall, the SET genes were uncommonly identified among these isolates, with 80% (52/65) of the isolates testing negative for all five of the tested SET genes. The most common toxin gene identified was *lukE* (87.7% of isolates), followed by *sei* (12.3%). Enterotoxin genes *sed* and *sej* were not identified in any of the tested isolates. None of the isolates were characterized as Genotype B, based on previously published RS-PCR banding pattern or toxin gene profile. Based on RS-PCR banding patterns, five different genotypes were identified. The discriminatory powers were 1.0 and 0.46 for PFGE and RS-PCR, respectively. A total of 35 different strains were identified by PFGE vs five by RS-PCR. No association between RS-PCR genotype and milk SCC or NAGase activity was found. These data suggest that PFGE is more discriminatory than RS-PCR for strain typing of *S. aureus* isolates of bovine origin, and enterotoxin genes are less prevalent in *S. aureus* isolated from this region of the USA compared with reports from Europe.

Detection and diagnosis

A definitive diagnosis of an IMI is usually based on detection of bacteria in milk. Elevations in bulk tank, cow-level, or mammary quarter-level milk SCC can be indicative of subclinical IMI, but these measures are not pathogen-specific. Hence, SCC can be used as a screening tool to detect IMI, but further testing will be needed to confirm the diagnosis. The sensitivity (Se) and specificity (Sp) of the California Mastitis Test (CMT) for detection of mammary quarters infected with *S. aureus* using a trace threshold has been previously reported as 0.62 based on a single milk culture and 0.86 when two concordant milk cultures were used to make the diagnosis (Middleton et al., 2004). Similarly, the Se and Sp of mammary quarter milk SCC for detection of mammary quarters infected with *S. aureus* using a 100,000 cells/mL threshold was 0.96 based on a single milk culture and 0.95 when two concordant milk cultures were used to make the diagnosis (Middleton et al., 2004).

Diagnostic Se and Sp will be impacted by the inoculum volume used to perform the milk culture and also the bacterial colony count threshold used for determination of a positive diagnosis. In the case of *S. aureus*, because the pathogen can be found in extramammary sites and on the udder skin, aseptic sample collection is critical for accurate interpretation of the results. .

Polymerase chain reaction (PCR) based diagnostic methods can be employed to detect *S. aureus* and Se and Sp are comparable with milk culture. At the bulk tank level, detection of *S. aureus* usually indicates presence of one or more cows in the herd with an IMI. As with cow or mammary quarter-level cultures, however, one must take into account that *S. aureus* can come from extramammary sites.

Treatment

Many approaches have been taken to the treatment of *S. aureus* mastitis including intramammary antimicrobial therapy, systemic antimicrobial therapy, and vaccination in conjunction with antimicrobial therapy. Cure rates have ranged from 3-74% depending treatment product, length of treatment and whether treatment was administered during lactation or during the dry period, or in the case of heifers, shortly before calving (Barkema et al., 2006). Barkema et al. (2006), in their review concluded that "the probability of cure depends on cow, pathogen, and treatment factors". Cure rates are impacted by increasing cow age, increasing SCC, increasing chronicity of infection, increasing bacteria counts, and increasing numbers of mammary quarters infected. They concluded that "the most important treatment factor affecting cure was treatment duration". Further, they suggest "treatment of young animals with penicillin-sensitive *S. aureus* infections is often justified based on bacteriological cure and economic outcome, whereas treatment of older animals, chronic infections, or penicillin-resistant isolates should be discouraged." Similarly, Roy & Keefe (2012) in a systematic review of the literature on the treatment of *S. aureus* mastitis during lactation concluded that extended intramammary therapy for 5-8 days was the best therapeutic option. However, this recommendation should be tempered with the knowledge that extended therapy regimens can be associated with clinical mastitis with secondary organisms such as yeast and coliforms in some cases (Roy & Keefe, 2012; Middleton and Luby, 2008). Furthermore, overall long-term cure rates in some herds even with 8 days of intramammary antibiotics can be quite poor. In one study, Middleton and co-workers, showed that while cure rates were quite high 4 days after treatment (80%), by 28 days post-treatment most infections had recrudesced with the overall cure rate dropping to 29% (Middleton et al., 2007).

Pre-partum treatment of heifers with intramammary antibiotics has been extensively studied. Generally heifers are treated with an intramammary antibiotic 2-4 weeks prior to parturition in an attempt to clear IMI caused primarily by coagulase negative staphylococci, but some efficacy has been noted against *S. aureus*. While cure rates can be quite promising, the impact of such therapy on 1st lactation performance including milk production and SCC over not treating varies from herd to herd with some herds showing no benefit (Middleton et al., 2005). As illustrated above, there is also a risk of introducing an IMI and causing clinical mastitis.

Some extended intramammary therapy regimens as well as pre-partum treatment of heifers with intramammary antibiotics constitute Extra-label Drug Use and can only be performed under a valid Veterinarian-Client-Patient-Relationship.

Prevention and control

The goal of a *S. aureus* mastitis control program is to prevent new IMI or facilitate clearance of new IMIs as soon as possible. Because the primary reservoir of infection is the cow's mammary gland and transmission occurs during milking, the major focus of *S. aureus* mastitis control has been milking time hygiene including single-use udder towels, having milkers wear gloves, and dipping teats in a post-milking germicide to eliminate *S. aureus* that may have colonized the teat skin from contaminated fomites such milking unit liners during milking. In addition, routine use of long-acting intramammary antibiotics during the dry period is advocated to eliminate existing IMI. The decision to treat infected cattle during lactation will be based on historical knowledge of the contagiousness of the disease in the herd and previous response to therapy. Additional control procedures include segregation and milking of infected cattle after non-infected cattle, culling of chronically infected cattle, maintenance of milking equipment, and prescreening of replacement heifers and purchased lactating cattle prior to inclusion in the lactating population. While milking time hygiene is the main control measure, in some herds it may not be sufficient during an outbreak (Smith et al., 1998). In such cases, more aggressive measures such as accelerated culling of infected cattle, strict segregation and milking infected cattle last, and drying-off infected mammary quarters may be needed (Middleton et al., 2001).

While *S. aureus* can be cultured from the milk of at least some cows in the majority of herds, *S. aureus* mastitis may not always have a major impact on bulk milk quality. While herds can have a predominant strain suggesting contagious spread from cow-to-cow, other herds may have a few cows with many different strain-types and no consistent strain causing IMI between cows. In the latter instance, it is hypothesized that the isolated *S. aureus* strains are not host-adapted and may be regarded as sporadic non-contagious pathogens similar to environmental mastitis pathogens. Therefore, management decisions on infected cattle, e.g., segregated milking, treatment, and/or culling may be different

in herds with non-host-adapted strains vs those herds where *S. aureus* has been clearly demonstrated to readily transmit from cow-to-cow.

Vaccination

Vaccination against *S. aureus* mastitis has been studied for many years, but none of the vaccines studied to date have consistently prevented *S. aureus* IMI. Similar to the goal of the prevention and control procedures outlined above, the goal of a *S. aureus* mastitis vaccine should be to prevent new IMI or facilitate clearance of new IMIs as soon as possible after infection. Currently there are two commercially available *S. aureus* mastitis bacterins; one available in the United States (Lysigin, Boehringer Ingelheim Vetmedica, Inc.) and the other in Europe and Canada (Startvac, HIPRA).

Lysigin is a multivalent whole cell lysed *S. aureus* bacterin that contains the most common serotypes of *S. aureus* that cause bovine mastitis in the United States. Williams et al. (1966 and 1975) studied Lysigin in both field and experimental challenge studies. Vaccinated cattle had lower clinical scores, lower SCCs, and fewer cases of chronic mastitis following intramammary challenge with *S. aureus* than unvaccinated controls. In the field trial, Williams et al. (1966) showed a reduction in new IMI rate between vaccinated and non-vaccinated cattle, but IMI still occurred in some vaccinates. More recently, the efficacy of commercially available Lysigin was compared with two experimental Lysigin formulations and unvaccinated controls in primiparous heifers in a *S. aureus* challenge trial (Middleton et al., 2006). All cattle became infected with *S. aureus* after challenge. Cattle vaccinated with commercially available Lysigin had a significantly lower mean duration of clinical mastitis and lower total mastitis score post-challenge than controls. However, there was no evidence that any of the vaccinated groups had a lower mean somatic cell count (SCC) than control, and no evidence that vaccinates had greater milk yield than controls post-challenge. Furthermore, opsonizing antibody levels in milk were no different from control. A follow-up field study in lactating dairy cattle of multiple parities showed that, in a herd with a 5% prevalence of *S. aureus* and a 40% prevalence of coagulase negative staphylococci, the vaccine did not reduce the new staphylococcal intramammary infection rate and the data suggested that there may be insufficient vaccine-induced opsonizing antibody in milk to facilitate phagocytosis and clearance of staphylococci from the mammary gland (Middleton et al., 2006).

In contrast, Nickerson and co-workers (1999) vaccinated heifers with commercially available Lysigin at 6-months of age followed by a booster 2-weeks later and subsequent booster vaccinations every 6-months until calving. Vaccinates had a 45% reduction in both new *S. aureus* IMI during pregnancy and new *S. aureus* IMI at calving relative to controls. In addition, vaccinates had a 30% reduction in new CNS IMIs which became chronic and a 31% reduction in new CNS IMI at calving relative to controls providing evidence that Lysigin may be of use in reducing staphylococcal mastitis in periparturient heifers vaccinated early in life with frequent follow-up vaccinations.

Vaccination with Lysigin has also been studied as an adjunct to antimicrobial therapy. In one study cattle vaccinated with Lysigin and treated with extended intramammary pirlimycin had a higher cure rate than non-treated controls (Smith et al., 2006), whereas two studies evaluated Lysigin in conjunction extended pirlimycin therapy vs extended pirlimycin therapy alone and showed no difference in cure rates between groups (Luby & Middleton, 2005; Middleton et al., 2007).

Startvac is a multivalent vaccine marketed by HIPRA that contains inactivated *Escherichia coli* (J5) and inactivated *S. aureus* (CP8) SP140 strain expressing slime associated antigenic complex (SAAC). The label indication is to reduce the incidence of subclinical mastitis and decrease the clinical severity of mastitis caused by *S. aureus*, coliforms, and coagulase negative staphylococci. Preliminary studies on the immunogenicity of SAAC showed that immunization did not prevent IMI, but did stimulate antibody in milk and reduce bacterial loads in milk (Prenafeta et al., 2010). Further studies showed Startvac to be immunogenic, stimulating antibody production in blood and ameliorating inflammation in the mammary gland following intramammary challenge with an inactivated strain of *S. aureus* (Piepers et al., 2012). While vaccination does not completely prevent IMI, results of a recent field trial demonstrate a decreased duration of IMI and decreased transmissibility of infection (Schukken et al., 2012). Reductions in the basic reproduction ratios in vaccinates for *S. aureus* and coagulase negative staphylococci were 45% and 35%, respectively. Efficacy was age dependent with a greater reduction in heifers than parity 3 or greater. Results varied between the study herds.

Conclusions

Have we learned anything about *S. aureus* mastitis in the last 50 years? Yes. Has what we've learned changed the basic premise that milking time hygiene is the main critical control point and *S. aureus* can be refractory to treatment? No. Ultimately, decisions about how to manage *S. aureus* mastitis in a given herd will depend on the contagiousness, persistence and inflammatory nature of the infecting strains. Use of historical data on new infection rates, SCC, and response to therapy will be valuable tools in making management decisions. It may be necessary in some herds to culture cows and have the isolates strain-typed to better understand how contagious *S. aureus* is in a given herd. If most infections are caused by a single strain that causes chronic infections which are refractory to treatment accelerated culling may be the only mechanism to maintain milk quality in the bulk tank. Alternatively, sporadic cases of *S. aureus*

IMI caused by isolates with no consistent strain-type may not have herd-wide implications and management strategies can be applied at the level of the individually infected cow. Vaccination may be a useful adjunct measure in a *S. aureus* mastitis control program; however results may vary based on the vaccine used, herd, and age of the vaccinated population.

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Occurrence of mastitis around insemination reduces the establishment of pregnancy by 20%

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Few data are available on the relationship between reproduction and udder health, in spite they are key critical parameters for the success of dairying. The relationship may be direct, perhaps through consequence of a systemic inflammatory status on conception, or indirect through the effect of common risk factors such as the coverage of energy and/or protein nutritional requirements.

In order to describe the link between somatic cell counts (SCC) and conception at first artificial insemination (FAIC) or for all AI (AAIC), data from the national French dairy milk improvement system and data on AI were examined from 2008 to 2012. The binary results of AI were explained with SCC thanks to a mixed binomial model with herd as random effect. SCC were categorized as Low or High relative to a threshold of 300,000 cells/mL. Four categories (LL, and LH, HH, HL) were defined according to their SCC before and after insemination. The models were adjusted by the lactation stage, milk yield, parity, urea and the fat: protein ratio (FPR) as a proxy of the energy balance.

The relative risks (RR) of FAIC were 0.84 (95% CI = 0.82-0.85), 0.94 (95% CI = 0.91-0.95) and 0.82 (95% CI = 0.80-0.84) for LH, HL and HH compared to LL, respectively. The RR of AAIC were 0.81 (95% CI = 0.78-0.82), 0.93 (95% CI = 0.90-0.95) and 0.79 (95% CI = 0.76-0.81) for LH, HL and HH compared to LL, respectively. Results were not sensitive to the analyzed year. Inclusion of fat, protein or FPR did not change the RR of FAIC or AAIC reported above. Similarly, excluding insemination around high negative energy balance (NEB) periods (high fat, low protein, or high FPR) did not lead to changes in these relative risks.

The present results show that the conception success is reduced by around 20% for cows with high SCC or with an increase of SCC around AI. The same is seen when indicators of NEB are included and when models were ran with or without cows with NEB, showing that the relation between udder health and conception is a direct link and is poorly modulated by ketosis.

Clinical manifestation and outcome of *S. uberis* mastitis in relationship to cow- and management factors: a field study

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Knowledge of factors influencing incidence, manifestation and outcome of mastitis is useful for good managerial decision on prevention and treatment of mastitis.

In this paper, the influence of cow factors (Parity, DIM, Milk yield and milk fat and -protein) and management factors (housing, bedding, pre and post dipping, treatment) on the clinical manifestation (severe (both local and general symptoms, n = 41), Non-Severe, first case (only local symptoms, n = 110), Non-Severe, repeated case (only local symptoms, n = 75) or subclinical mastitis (n = 25)) and mastitis outcome ("cure" = at most one monthly individual cell count > 200.000 cells/mL during the three months after mastitis) of *S. uberis* mastitis (n = 251: Monoculture on blood agar containing Colistin and Nalidixic Acid, negative in catalase reaction and positive in Esculin reaction and sensitive to penicillin in disc diffusion test) are described.

Chi-square tests were performed using Statistixs®: $p < 0.05$ was considered as significantly different. Severe mastitis happened more often in first lactation cows and cows in early lactation (< 100 DIM) and Non severe, repeated cases more often in third and more lactation cows. Farms that use post-dipping procedure have significantly less cases of severe mastitis and farms that do not use post dipping have more non-severe, repeated cases and subclinical cases. The cure rate of mastitis was not different for the different clinical manifestation forms of mastitis. Cure was overrepresented in first- and in second lactation cows and underrepresented in third and more lactation cows. The choice of treatment (IMAM antibiotics, parenteral antibiotics, anti-inflammatory drug or a combination) did not influence cure.

It can be concluded that cow and management factors do influence clinical manifestation of mastitis and cure rates: considering farms specific information concerning those factors might improve treatment and and prevention protocols.

Systemic immunization of heifers against *E.coli* poorly protects against experimental mastitis in early lactation

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Escherichia coli is a major causal agent of mastitis and despite increasing control hygienic measures, its incidence remains high in all dairying systems. Mastitis impacts in multiple ways on food security, productivity, dairy cow health and welfare. Hence improved preventive and/or therapeutic options are required. Better control of mastitis might be achievable through vaccination. Some vaccines against mastitis are available although these are not widely licensed or have limited efficacy.

Objectives

For the purpose of improving mastitis control and identifying T cell-associated determinants of protective immune responses, we evaluated the protection afforded by immunization with *E. coli* in an adjuvant favoring cell-mediated immunity.

Methods

A group of heifers (n = 6) were immunized twice by intramuscular injection of heat-killed *E. coli* P4 bacteria with Montanide adjuvant. Control cows (n = 6) received the adjuvant only. One month after parturition, the cattle were challenged with live P4 bacteria (10e3 CFU) through the teat canal of one quarter, and their response compared to that of control cows. Local and systemic clinical evaluations, in addition to laboratory tests, were performed during two weeks upon inoculation.

Results

Results indicated that, despite strong cell-associated immunity detected in the blood of immunized cattle, the reduction of clinical signs at the acute phase is low. No difference of local inflammation was noticed in immunized cows, and the mammary inflammation tended to persist longer than in control cows. However, the reduction of milk production during the acute phase was significantly lower in the immunized group ($p < 0.01$, repeated measures ANOVA).

Conclusions

Immunization through the intramuscular route with a vaccine preparation containing an adjuvant that promotes cellular immunity does not fully protect against *E. coli* mastitis. These results indicate that autogenous vaccines are probably not able to improve significantly the conditions against *E.coli* mastitis. Further studies are needed to determine mechanisms affording protection against Gram-negative mastitis.

Uterine health and reproductive performance of dairy cows fed CLA during transition period

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Negative energy balance (NEB) during transition period is a major cause of poor reproductive performance in dairy cows. Feeding conjugated linoleic acid (CLA) feeding to dairy cows during transition period reduced the NEB, therefore the aim of this experiment was to study the effects of CLA supplementation during peripartum on reproductive performance of dairy cows.

75 multiparous Holstein cows received a basal total mixed diet with 75 g/d rumen protected CLA (CLA, n = 37) or 75 g/d palm oil (C, n = 38) from 21 d before calving to 42 days postpartum (dpp). Cows metritis status were evaluated daily between 7 to 10 dpp for uterine discharges using a 4 point scoring system: clear mucus (score1), clear mucus with flecks (score 2), mucopurulent (score 3) and purulent (score 4). Clinical endometritis was evaluated at 29 ± 2 dpp (Mean±SEM) by assessing vaginal mucus and cervix diameter by ultrasonography. A presynchronization protocol were initiated on day 30 postpartum with two injection of PGF2α, 12 days later a CIDR-Ovsynch protocol was carried out and all cows were timed artificial inseminated (TAI) on day 65 postpartum. Pregnancy diagnosis was carried out by transrectal ultrasonography on day 30 post TAI. Pregnancy reconfirmed at 60 days after first and second TAI for pregnant cows and pregnancy losses determined.

Feeding CLA during peripartum period non significantly reduced proportion of metritic cows (13.2%, C Vs. 5.4%, CLA) and cows with mucopurulent (36.8%, C Vs. 24.3%, CLA; p = 0.18) discharge. Proportion of cows with clinical endometritis were reduced from 36.8% in palm to 24.3% in CLA fed cows (p = 0.4). Treatment had no significant effects on first (32.4%, C Vs. 24.3%, CLA; p=0.44), second (8%, C Vs. 21.4%, CLA; p = 0.19) and sum of first and second (37.8%, C Vs. 40.5, CLA; p = 0.81) insemination conception rates. At this study, numerically less pregnancy loss (30.8%, C Vs. 13.3%, CLA; p = 0.27) resulted in more cows pregnant before 150 DIM (32.4%, C Vs. 46%, CLA; p = 0.23), although days open of pregnant cows was the same (81d, C Vs. 88.4d, CLA; p = 0.56) and the differences were not significant. Although, our data were not significant, it seems the feeding CLA during transition period might be improved uterine health and some aspects of reproductive performance of dairy cows.

Preventive and treatment strategies for metabolic disorders of dairy cows

J. REHAGE

Economic losses due to left displaced abomasum and the financial evaluation of the surgical correction. A case study

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Left displaced abomasum (LDA) causes significant losses in large-scale dairy herds. The objective of this study was to quantify the impact of LDA on production and economic performance, and to assess the economic outcome of the surgical correction of LDA by left flank abomasopexy on a dairy farm.

A case-control study was set up to quantify the production losses caused by LDA, based on the data of a 930-cow Hungarian dairy herd between 01. January 2012 and 31. December 2014. A partial budget model was developed to evaluate the economic impact of the disease and a decision tree analysis was performed regarding the profitability of the surgical correction of LDA. The LDA-related losses were quantified based on the performance of 178 LDA-positive cows, whereas the postoperative production of 166 cows was included in the decision analysis.

The average annual prevalence of LDA was 6.6% in the herd and it was the highest in the first lactation. LDA-positive cows produced 303 kg less milk throughout their lactation and 1339 kg less milk annually than the control group. The LDA significantly deteriorated the cows' reproductive parameters ($p < 0.0001$); the calving interval was 58, the lactation 50 days longer, respectively, and the cows required 1.29 more inseminations and additional 57 days to conceive compared with the control cows. Average losses related to LDA summed up to € 612.2 per case on average. Increased culling rate was the major source of loss, which caused 42.6% of the losses, followed by the increased mortality (14.2%) and reduced milk yield (14.1%). Nine percent of the cows died after surgery in the affected lactation, 42.2% was culled due to LDA or other reasons and 48.8% remained in the herd at least until drying-off.

The decision analysis revealed that the average loss was € 119.9 after the LDA operation, but immediate culling of the cows with LDA would have led to € 1255.4 loss per case. It was concluded that the surgical correction was more beneficial from an economic point of view, since € 1135.5 loss could be avoided by the operation.

Prevalence of *postpartum* hyperketonemia, endometritis and prolonged anovulation in dairy herds and their association with poor reproductive performance at first service

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Postpartum hyperketonemia, endometritis and prolonged anovulation are known risk factors for poor reproductive performance at first service at the cow level. However, it remains unclear what the acceptable prevalence of these conditions is at the herd level. The objective of this study was to quantify the prevalence of these conditions in dairy herds and to determine the optimal prevalence thresholds associated with poor reproductive performance at first service at the herd level. A total of 100 Holstein dairy herds from the province of Québec (Canada) were conveniently enrolled in this observational study. Herds were visited every other week and a total of 15 cows per herd were randomly selected and followed from parturition until 200 days in milk (DIM). Hyperketonemia was defined as having blood beta-hydroxybutyrate acid ≥ 1.4 mmol/L using the Precision Xtra meter at 1-14 DIM; endometritis was defined as having mucopurulent or purulent vaginal discharge using the Metricheck device at 30-43 DIM; prolonged anovulation was defined as having serum progesteronemia < 1 ng/mL using the Immulite laboratory technique at both 37 (± 7) and 51 (± 7) DIM; success at first service was defined as pregnancy diagnosed by transrectal palpation at 33 to 46 days after breeding. Statistical analyses were performed using logistic regression models in SAS. Herd-level median prevalence of hyperketonemia, endometritis, prolonged anovulation, and success to first service were 22%, 25%, 35%, and 30%, respectively. Poor success at first service was defined as < 30%. Optimal disease prevalence thresholds for predicting herd-level poor success at first service were ≥ 20% for hyperketonemia ($p < 0.01$), ≥ 20% for endometritis ($p < 0.01$), and ≥ 30% for prolonged anovulation ($p < 0.01$). These results demonstrate the high prevalence of *postpartum* hyperketonemia, endometritis and prolonged anovulation in this population of dairy herds. They also show that herd-level thresholds can be used to identify herds with poor success at first service. Overall, these findings highlight the potential usefulness of herd-level disease prevalence for helping farmers and veterinarians in improving dairy herd management.

Reducing the curative antibiotic use up to 25% by controlling subclinical ketosis

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Good herd management is known to mitigate antimicrobial use, but few research quantify it. Subclinical ketosis (SCK) is known to promote the occurrence of infectious diseases which require antibiotics for curative treatment. The control of SCK is based on peripartum management and/or use of preventive monensin bolus for cows at risk for SCK.

A stochastic static model was built so as to assess the changes in preventive and curative antibiotic use when farmers (i) reduce the prevalence of cows at risk for SCK or (ii) use monensin bolus to prevent SCK (with fear or good targeting of cows). Most popular scenarios included: prevalence of cows at risk for SCK between 0.1 and 0.8; efficacy of monensin to prevent SCK fixed at 0.45, 0.66 and 0.85; or for cows at risk for SCK to really have SCK fixed at 2.2 or 4.5. Calibrations were provided by literature review, meta-analysis and expert opinion.

The decrease in prevalence of cows at risk for SCK from 80% to 10%, corresponding to a decrease of SCK prevalence from 68% to 17%, led to a decrease in the curative antibiotic use by 25%. The relationship is linear. The low decrease in the curative antibiotic use compared to the decrease in SCK prevalence is in agreement with the base level of curative antibiotics use in cows without SCK.

Using monensin with very good to good accuracy (perfect targeting or 80% of cows well targeted, respectively) allowed a decrease up to 20-25% in the curative antibiotic use when the prevalence of cows at risk for SCK was high. The curative use of antibiotics is stabilized at the level observed with low prevalence of cows at risk for SCK, when this prevalence is high and monensin bolus is used.

As conclusion, reducing the prevalence of SCK is a powerful way to reduce curative antibiotic use in dairy, when compared to the 25% decrease objective provided by French policy-makers (EcoAntibio). The hazard regarding antimicrobial resistance that is linked to either use of monensin or curative antibiotics such as cephalosporin has now to be assessed.

Optimising reproductive performance of beef cows and heifers

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Introduction

Worldwide, beef cows serve a unique role in converting low quality forage to high quality protein for human consumption and are often maintained on unsuitable for more intensive agricultural enterprises. Notwithstanding this beef cow systems vary enormously across countries in terms of herd size, stocking density and level of output. Despite this, however, in general the system is characterised by profitability as a consequence of low biological and economic efficiency and is frequently cited as having a relatively high carbon footprint. In the beef cow herds reproductive performance is a key driver of efficiency and profitability. Regardless of geographic location, because of the dependence on grazed forage, the vast majority of beef cowherds tend to be based on seasonal calving with calving occurring at, or around, the time of onset of pasture growth. As the calf is largely the sole output in beef cow enterprises, reproductive efficiency is a key determinant of profitability, irrespective of the system of production employed. The objective of this paper is to review the predominant factors affecting reproductive efficiency of beef cow herds, including management of replacement heifers.

Reproductive targets for a beef cow herd

The following are the generally agreed reproductive targets for a beef cow herd:

- 365 d –calving-to-calving interval,

- < 5 % cows culled annually as barren,

- > 95% of cows calving to wean a calf,

- heifers calving at 24 months of age,

- compact calving with 80% of cows calved in 42 days,

- replacement rate 16-18%,

- sustained genetic improvement of the cow herd for economically important traits relating to reproduction, calving ability and calf weaning weight,

- close alignment of calving date with onset of pasture availability in the spring (Diskin & Kenny, 2013).

There are four key benchmarks that must be achieved in a timely fashion in order to meet the above targets. These are:

- timing of puberty in heifers,
- resumption of oestrous cycles post calving,
- expression and detection of oestrus if AI is used; and
- breeding and the establishment of pregnancy.

Timing of puberty in replacement heifers

Puberty in the heifer can be defined as the developmental stage that supports normal ovarian cyclicity combined with the ability to become pregnant. A prerequisite for puberty and the initiation of oestrous cycles is the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus, at an adequate pulse frequency and amplitude, to stimulate sufficient luteinizing hormone (LH) pulsatility from the anterior pituitary (Allen, 2012). The onset and regulation of puberty in heifers is governed by a complex neuroendocrine control system and is responsive to a myriad of factors including nutritional status (particularly during early life), breed (Table I), genotype, season of birth as well as bull exposure. However, genetics and nutritional status seem to be the predominant influencing factors.

Table I: Summary of the relative age at puberty of cattle breeds (adapted from Hall, 2014)

Age at puberty	Breeds
Very early (< 9 months)	Jersey
Early (9 - 12 months)	Holstein, Brown Swiss, Gelbvieh, Red Poll, South Devon, Tarentaise, Pinzgauer
Moderate (12 - 14 months)	Simmental, Hereford, Angus
Late (14 - 16 months)	Limousin, Charolais, Blonde d'Aquitaine, Chianina, Brangus, Santa Gertrudis
Very late (> 16 months)	Brahman and Sahiwal

The secretory control of GnRH and LH involves the interplay between complex neural pathways involving neurohormones, peptides (e.g. neuropeptide Y; NPY) agouti-related peptides dopamine, opioid peptides, RF amides (kisspeptin and its receptors; see recent reviews by Williams & Amstalden, 2010; Ahmadzadeh et al., 2011; Pinilla et al., 2012). These biochemicals interact with various metabolites and metabolic signalling hormones including glucose, fatty acids, leptin, adiponectin, ghrelin, insulin like growth factor-I and insulin ultimately leading to increased GnRH and LH pulsatility leading to ovulation and the commencement of oestrous cycles (Pinilla et al., 2012). Timing of puberty is highly variable even within breed and is dependent on a number of significant genetic and environmental factors and these are discussed later.

Replacement heifers are an important resource in the beef herd and in theory should, on average, be genetically superior for commercially important traits compared to their dams. Given the not insignificant costs involved in rearing these animals it is imperative that they become pregnant early in their first breeding season, encounter minimal dystocia, are successfully rebred to attain a 365 inter-calving interval and ultimately have a long (> 6 lactations) and productive life within the herd. Because of the seasonal calving pattern within beef cow herds, heifers invariably are either 2 or 3 years of age at time of first calving, with a preponderance of animals, in most countries, calving closer to 3 rather than 2 years of age.

It has been shown that beef heifers that conceived early during their initial breeding season and calved as 2-year-old females had a greater probability of becoming pregnant as primiparous cows, have greater lifetime production reflected in greater weaning weights, and tend to calve earlier in subsequent years compared with females that conceived later in their first breeding season (Day & Nogueira, 2013). Thus, the age at which puberty occurs and its timing relative to the onset of the breeding season will impact the reproductive efficiency of the heifer cohort as well as, ultimately, the entire herd. Byerley et al. (1987) recorded a 21 percentage point lower conception rate when beef heifers were bred at their first rather than third post pubertal oestrus. Indeed, the timing of puberty and the proportion of heifers that are pubertal before the planned onset of their first breeding season is critically important to overall herd reproductive efficiency (Perry, 2012).

Genetic effects on timing of puberty

Heritability estimates (h^2) for age at puberty range from 0.07 to 0.67 and average 0.4 (see review by Martin et al., 1992). Heterosis, by definition is the difference between the mean for reciprocal F_1 crosses and the mean of the respective parental purebreds and is caused by non additive genetic effects. Crossbred heifers reach puberty at a younger age than the average of their parental breeds (Wiltbank et al., 1966; Gregory et al., 1991). Estimates of the effect of heterosis on age of onset of puberty vary from about 9 days (Gregory et al., 1991) to up to 41 days (Wiltbank et al., 1966) with evidence that heterotic effects begin to decrease with increasing age (Martin et al., 1992). There are a number studies that have reported both between and within breed effects on age at puberty (Martin et al., 1992). Larger European continental breeds of cattle are typically older at puberty than traditional British beef breeds or dairy breeds.

Nutritional status, body weight and puberty

There is some variation in the published literature on the proportion of mature bodyweight which heifers must attain before undergoing puberty. Despite this it is clear that differences exist between breeds and breed types with values of 60% typical for European derived beef breeds, 55% for dual purpose beef/dairy breeds and 65% for *Bos indicus* cattle (Larson, 2007). A number of recent reports, however, have suggested that threshold weight may be lowered to 0.50 to 0.57 of mature body weight for beef heifers (Funston et al., 2012; Endecott et al., 2013). However, in these studies the proportion of heifers pubertal prior to the start of the breeding season and the percentage of heifers pregnant early in the breeding season was generally worse for lower compared to higher threshold weights (Perry et al., 2012; Gasser, 2013). Accelerated growth during early calfhood influences age at puberty. Early weaned heifers offered diets that promote rapid rates of bodyweight gain between 3 and 7 months of age reached puberty earlier and at lighter bodyweight than diet restricted heifers with lower weight gains (Gasser et al., 2013). Increased bodyweight gain later during calfhood apparently does not have the same impact. Therefore, a critical window for nutritional imprinting of neuroendocrine functions that regulate age at onset of puberty appears to exist early in juvenile development (Allen et al., 2012; Pinilla et al. 2012).

Resumption of oestrous cycles post calving

Following an uncomplicated calving, about 30 days are required for completion of uterine involution in beef cows. Resumption of normal ovarian cycles and oestrus is dependent on the recovery of the hypothalamic-anterior pituitary–ovarian axis and in particular attainment of a GnRH/LH pulse frequency of 4-5 pulses per 10 hour period (see review by Crowe et al., 2014). Our own data (Mackey et al., 2000) indicate that the hypothalamic-anterior pituitary-ovarian axis has potentially recovered in terms of the ability of the anterior pituitary to synthesise LH and the ovaries to respond to the increased LH pulse frequency by day 30 post calving in the majority of suckled beef cows. However, the synthesised LH is largely sequestered in the anterior pituitary and normal resumption of ovulation is thus prevented by inadequate LH pulse frequency as a result of the suckling–maternal bond that exists between the cow and her calf and/or by her nutritional status also affecting GnRH/LH pulse frequency. Therefore, the major factors affecting the duration of the *postpartum* interval in suckled beef cows are maternal bond, nutrition, parity and season. (Crowe et al., 2014).

Suckling-maternal bond

It is now well established that neither chronic sensory stimulation of the teat nor indeed the sensory pathways within the teat and udder are necessary for the suppression of LH pulse frequency in the suckled beef cow. Indeed, neither surgical denervation of the udder (Williams et al., 1993) nor mastectomy (Viker et al., 1993) shortened the *postpartum* anoestrous interval where calves remained with their dams. This confirms that factors other than direct suckling are responsible for the longer duration of the *postpartum* anoestrous interval in beef cows nursing calves compared with milked dairy cows.

Use of calf separation / isolation to stimulate recommencement of oestrous cycles

The studies of Stagg et al. (1998) and Sinclair et al. (2002) clearly show that the use of calf separation /isolation, commencing at around day 30 *postpartum*, results in an immediate (within 2-5 days) increase in LH pulse frequency with about 85% of cows responding by ovulating the first dominant follicle that develops and is exposed to this increased LH pulse frequency. For optimal effect, the data of Stagg et al. (1998) would clearly imply that calves must be isolated from their dams with no tactile contact permitted between them. In the study of Stagg et al., (1998) a total of 43%, 65% and 90% of cows had ovulated by 80 days *postpartum* for cows with ad libitum calf access, suckled once daily suckling with calves penned adjacent to their dams and suckled once daily suckling with calves isolated from their dams, respectively. Interestingly, in both of the studies of Stagg et al. (1998) and Sinclair et al. (2002) a small proportion (circa 15%) of cows failed to respond to the removal of the suckling/maternal calf bond and showed no increase in LH pulse frequency and no evidence of an increase in the circulating concentrations of oestradiol. These "non-responders" typically had prolonged *postpartum* anoestrous intervals and could be described as being in "deep anovulatory anoestrus" (Sinclair et al., 2002). In the latter study there was evidence of an association between low circulating concentrations of insulin, during the early *postpartum* period, and the failure to record an ovulatory response to calf separation/isolation thus emphasising the importance of nutrition as a regulator of *postpartum* interval. The importance of nutrition is also clear from the study of Bishop et al., (1994), where the authors reported that all (100%) cows in a BCS of greater or equal to 5 (scale 1-9) at calving had ovulated by day 60 days following weaning at day 35 day post-calving. In contrast, only 40% of cows in a BCS of less than 5 had ovulated at the same time. Therefore, the available evidence suggests that calf isolation and restricted suckling might be a management option to shorten the *postpartum* anoestrous interval in beef cows but with lower and more variable response expected in cows that are in a low BCS at calving.

Nutrition and postpartum interval in beef cows

Nutrient intake, before and after calving, is a major factor affecting the duration of the *postpartum* anoestrous interval and subsequent calving-to-conception interval and overall pregnancy rate. If nutrient intake is inadequate, cows body reserves become depleted and body condition declines. Cow body reserves and/or body condition score (BCS) at any one time is a reflection of previous nutrient intake and is a more reliable indicator of cow nutritional status than cow body weight which is affected by cow frame size and products of conception, which vary with gestational age. Body condition scoring of beef cows is considered an objective, repeatable and easily applied measure of a cow's fat reserves and is frequently advocated as a practical tool for the nutritional management of beef cows and to level out peaks and troughs in seasonal feed supply, as well as the overall nutritional status of a herd at a given time point. In Britain and Ireland a scale of 0-5 (Lowman et al., 1976) is used while in most other countries a scale of 1-9 (Richards et al., 1986) is used.

In a recent review including a statistical re-evaluation of published literature, Hess et al. (2005) found that the length of the *postpartum* interval (PPI) was negatively correlated with BCS at calving ($r = 0.75$) and prepartum energy balance estimated from changes in prepartum BCS ($r = 0.52$) but not with prepartum change in BCS ($r = 0.35$). Results of a multivariate stepwise regression demonstrated that BCS at calving was the only variable to consider for estimating

effects of prepartum plane of nutrition on PPI (R^2 = 0.57). Interestingly, in the transnational study of Sinclair et al. (2002) which employed very divergent beef genotypes, it was clearly shown that both BCS at calving (2 v 3; scale 0-5) significantly affected LH pulse frequency (1.64 v 3.2 pulses/10 hours) and mean concentrations of insulin (6.3 v 8.5 mIU/L) at about 27 days *postpartum*, both of which are key drivers of onset of ovulation post calving.

From the analysis of published data Hess et al. (2005) concluded that:

- prepartum nutrition is more important than *postpartum* nutrition in determining the duration of *postpartum* anestrus,

- energy is the primary nutrient regulating reproduction in female beef cattle and inadequate dietary energy during late pregnancy lowers reproduction even when dietary energy is sufficient during lactation,

- a BCS ≥ 5 (scale 1-9) will ensure that body reserves are adequate for *postpartum* reproduction,

- severity and duration of negative energy balance during the early *postpartum* extends the *postpartum* anestrous period and negatively affects reproductive performance.

Postpartum nutrition and duration of the anoestrous interval

The reported effects of increased nutrient intake after calving on PPI are inconsistent with positive effects (Stagg et al., 1995; Vizcarra et al., 1998) or no effect (Whittier et al., 1988; Stagg et al., 1998) on duration of the *postpartum* anovulatory interval. This lack of consistency among studies may reflect variation around dietary energy intake, duration of the feeding period, BCS at calving, age of cows etc. Similar to their review and statistical re-evaluations of published literature, Hess et al., (2005) found that BCS at breeding ($r = 0.41$; $p < 0.001$), but not BCS at calving ($r = 0.10$; $p = 0.41$), was correlated with PPI. Both BCS change ($r = 0.47$; $p < 0.001$) and energy balance estimated from BCS change ($r = 0.43$; $p < 0.001$) were also correlated with PPI. Results of a stepwise regression analysis, however, indicated that BCS at breeding was the only predictor of PPI. A large portion (47%) of the variation in BCS at breeding was, however, explained by BCS at calving.

There is evidence that thin cows at calving and particularly primiparous cows respond to increased *postpartum* nutrient intake with enhanced reproductive performance (Richards et al., 1986; Spitzer et al., 1995; Ciccioli et al., 2003), although reproductive performance may still be less than adequate. In the study of Ciccioli et al., (2003), primiparous cows that calved in a BCS of 4 or 5 (scale 1-9) had similar endocrine function and reproductive performance at the first *postpartum* oestrus. However, increased nutrient intake for a period of 71 days post-calving shortened the interval from calving to 1st ovulation followed by a normal luteal phase from 120 to 100 days indicating earlier resumption of cyclicity *postpartum*. Increasing feed intake promotes fat deposition which may be a prerequisite to re-establish ovarian function in *postpartum* cows. Increased BCS is required for the resumption of oestrous cycles in nutritionally induced anoestrous cows (Richards et al., 1989) and heifers (Bossis et al., 2000), and body energy reserves influence the interval to ovulation after early weaning of beef cows (Bishop et al., 1994). This suggests that adiposity, or at least the achievement of a certain threshold level of adiposity may be a prerequisite for occurrence of puberty and resumption of *postpartum* ovarian cyclicity. Although the precise chemical or hormonal signals that link bodyweight and adiposity to pubertal onset have not been clearly defined, an increase in circulating concentrations of leptin, an adipose-derived hormone that regulates a wide variety of physiological processes, has been shown to precede the onset of puberty in several species, including cattle (Perry, 2012) leading to postulation that leptin acts as a permissive signal to the occurrence of puberty. Furthermore, administration of recombinant ovine leptin stimulated secretion of GnRH and LH in undernourished heifers, but was not capable of accelerating puberty onset in well nourished heifers (Maciel et al., 2004; Zieba et al., 2004). However recent findings show that although exogenous administration of leptin temporarily enhanced the rate of follicular growth, it does not accelerate puberty (Carvalho et al., 2013). Collectively these data indicate that while leptin plays an important role as a signal linking nutritional status to the central reproductive axis in cattle, other factors are also involved.

Use of body condition scoring

Body condition scoring has been frequently advocated as a practical tool for the nutritional management of beef cows. From the foregoing and from published literature it is clear that the critical time to achieve a minimum target BCS is at calving (Hess et al., 2005). The recommended BCS at calving for mature cows and 1st and 2nd caving cows heifers are 5 and 6 (scale 1-9), respectively or score 2.5 and 3.0 on a scale of 0-5 (Lowman et al., 1976). A somewhat higher BCS (+ 1 unit; Scale 1-9) is warranted for younger cows and heifers because, after calving, they have an additional feed requirement for growth together with their requirement for maintenance and lactation. Excessive adiposity at time of calving, however, is not recommended and is associated with greater dystocia and may contribute to lower feed intake *postpartum*. It is difficult, unless there is an abundance of cheap high quality feed, to economically improve the BCS of cows during early lactation. Similarly, waiting until calving to commence improvement in BCS is not recommended and will generally be difficult to achieve, particularly in a cost effective manner and without resorting to

concentrate/energy supplementation. Equally and from a practical perspective, BCS at breeding is less useful as a management tool than BCS at calving because it is very difficult, if not impossible, to sufficiently improve BCS after the onset of the breeding period. Furthermore, as the reproductive response to improving nutrition is not immediate, the commencement of enhanced feeding only during the breeding period will not immediately induce oestrous cyclicity in anoestrous cows though may improve conception and final pregnancy rate. It is also clear that improvements of one unit BCS (scale 1-9) requires an improvement in live weight (independent of changes in the products of conception or gut fill) which, will depend on current cow/heifer weight and BCS (Table II.). Where the 0-5 scale is used to measure BCS, a one unit increase in BCS equates to about 70 kg increase in body weight (Wright and Russell, 1984). Therefore, for cows in a low BCS, adequate time must be allowed for BCS to improve. It is frequently recommended to body condition score cows during the late summer/early autumn period, particularly young cows and if feed supply is becoming scare or deteriorating in nutritive value. This would allow the identification and selection of cows in low BCS for either early weaning or supplementation to improve BCS. Typically, cows should be approaching mid gestation at this time and their feed requirements are low compared with early lactation and it is therefore, more economical to improve BCS through grazing. Once the target BCS is achieved the objective should be to maintain it to calving. Attempting to improve BCS in late gestation can frequently be difficult because the nutrient demands of the rapidly growing foetus are increasing at this time as well as the increased nutrient demands for mammary regeneration and colostrogenesis in preparation for lactation. Frequently, in harsher climates, weather conditions and feed supply can be additional challenges. For cows in very good BCS (score ≥ 7; Scale 1-9) there is evidence that tissue reserves can be utilized during late gestation without compromising subsequent reproductive function.

Table II: Weight changes and time needed to increase one unit of BCS (scale 1-9) in cows of different bodyweights and BCS

(Adapted from Nutrient requirements of beef cattle 7th Revised Ed, 1996, National Acad Press, Washington DC, USA)

BCS	Cow/heifer bodyweight (kg)	Bodyweight gain (kg)	Time required (d) at gain of 0.5 kg/day
3	395	27	55
	475	33	65
	554	38	76
4	423	32	64
	507	38	76
	592	45	89
5	455	63	73
	545	44	87
	636	51	102
6	491	45	91
	589	55	109
	687	64	127

Bull Exposure and induction of postpartum cyclicity in cows

Similar to pre-pubertal heifers, there is evidence that the exposure of anoestrous *postpartum* cows to bulls hastens the onset of recommencement of oestrous cycles though the effects have not been consistent across all studies (Fiol & Ungerfeld, 2012). It would also appear that a continuous stimulus from the male is necessary to obtain a positive response on induction of cyclic activity. Based on the published evidence it may be advisable to accommodate *postpartum* cows' adjacent to bulls or run teaser bulls with them (prior to the onset of the breeding season or where AI is being used). If this is to be practiced, it should commence shortly after calving. However, it is highly unlikely that the stimulatory effects of bull exposure will overcome low BCS at calving and/or nutritional anoestrous.

Nutritional effects on conception and pregnancy rate

Dietary energy

While pre-partum nutrition plays a key role in regulating the interval to resumption of *postpartum* ovulation, mainly through its modulating effects on BCS, both concurrent plane of nutrition as well as dietary chemical composition during the breeding season, has been shown to affect conception and pregnancy rates. Some studies have also reported latent effects of early *postpartum* plane of nutrition on subsequent fertility during the breeding season. For example, Ciccioli et al. (2003) reported a higher pregnancy rate for beef cows maintained on a moderate, compared with a low plane of nutrition for 10 weeks *postpartum* and associated this with the higher systemic concentrations of IGF-1 and leptin observed. In our own studies (Dunne et al., 2000) we observed ~ 50% reduction in conception rate of heifers offered a low compared with high post AI diet, where both groups were accustomed to a high plane of nutrition pre-AI. Additionally, there was no evidence in that study that systemic concentrations of progesterone were implicated in the conception rates recorded.

There has been much interest over the past decade in the potential of dietary fat supplementation to improved reproductive performance of cattle. Strategies have included the use of fat supplements to augment energy intake but mainly to examine effects of their constituent fatty acids, on various aspects of the reproductive performance (Santos et al., 2008). Inconsistencies in fertility outcomes have often been suggested as being the result of differences in the fatty acid composition of the supplement (i.e. n-3 v n-6 fatty acids) or status of the animals employed (heifers v cows). While many studies have examined the effects of various fatty acid based supplements on aspects of the reproductive process in dairy cows, there have been few reports for beef cows. Scholljegerdes et al. (2009) recorded lower tissue concentrations of LH and IGF-1, follicle numbers, systemic oestradiol and overall conception rate in beef cows supplemented with high-linoleate safflower seeds compared with unsupplemented controls. SimiIarly, Martin et al. (2002) observed no advantage of supplementing heifers with soyabeans in either productive or reproductive efficiency. In a series of experiments conducted with beef heifers, Childs et al. (2008 a,b,c) failed to establish any positive effects of enriching diets with rumen bypass n-3 fatty acids on a range of reproductive variables including steroid concentrations, follicle size and on the quantity or quality of embryos, following superovulation. The lack of a positive effect of fat supplementation on beef females may be due, in part, to their overall general positive metabolic energy status in comparison to their dairy counterparts.

Dietary protein

Some authors have raised concern in the past over possible deleterious effects of high protein diets on reproductive efficiency of dairy cows and heifers in particular (Butler, 2000). While beef cows or heifers are generally not exposed to excessively high dietary levels of protein, or indeed its systemic metabolites, ammonia and urea, cows managed under temperate pasture based systems, may be grazing herbage with a high rumen degradable protein (RDP) content leading to elevated concentrations of rumen ammonia and systemic ammonia and urea. Despite this, over a series of experiments conducted with beef heifers both indoors and on pasture, Kenny et al. (2001, 2002) failed to establish any effect of high protein diets or elevated systemic urea or ammonia on any reproductive variable measured.

Breeding and the establishment of pregnancy

Once oestrous cycles have commenced it is the combined effect of heat detection efficiency (submission rate) and conception rate that determines and compactness of calving and ultimately the pregnancy rates after a short defined breeding period (Diskin & Sreenan, 2000). Where an active, fertile bull(s) is used it is expected that all cows and heifers in heat should be mated and, therefore, under such circumstances, compactness of calving and pregnancy rate will be solely the function of bull fertility. While it is acknowledged that the vast majority of beef cows throughout the world are bred by natural mating, nevertheless, AI is used in many small herds and in herds where planned mating is the objective. For such herds, accurate detection of oestrus combined with the detection of a high proportion of cows that express oestrus is of paramount importance. The factors affecting both bull fertility and optimal use of AI in beef cows have recently been reviewed in detail by Diskin & Kenny (2013).

Conclusions

Compact calving is an essential component of pasture-based suckled beef production systems to ensure maximum herbage utilization and, hence, profitability. Achieving a concentrated calving pattern requires early onset of puberty in replacement heifers, together with both high submission and high pregnancy rates in both heifers and cows early in the breeding season. In beef cows high submission rate in the first 6 weeks of the breeding period is highly dependent on cows having resumed oestrous cycles by 50 days post-calving. However, beef cows typically experience considerable variability in the duration of the *postpartum* anoestrous period with mean duration often extending to beyond 80 days even in cows of moderate to good BCS. While ovarian follicular development resumes early *postpartum*, the prolonged *postpartum* interval of suckled beef cows is due to the failure of successive dominant follicles to ovulate. This is a consequence of inadequate frequency of LH pulses. While management practices such as restricted suckling and/or exposure to an intact male can cause an increase in the frequency of LH pulses and hasten the resumption of ovarian cyclicity in some cows, these approaches are often viewed as impractical and or labour intensive at farm level. Maximizing the proportion of cows that establish pregnancy within the first 42 d of the breeding season decreases the incidence of extended calving patterns (McDougall, 2006). Later-calving cows with an extended *postpartum* anoestrous interval can disrupt the seasonal calving pattern and result in extended duration of calving. Furthermore, in beef cows nursing their calves, the expression of overt signs of oestrus are reduced, thus further increasing the difficulty of oestrous detection which is a prerequisite for using AI. Subfertility of bulls, although often a transient condition, can have devastating effects on achieving high herd fertility and requires on-going vigilance. While, increased efforts are being made internationally to genetically identify and select for more reproductively efficient beef cows, this is a more long-term strategy and will not replace the need for a high level of technical efficiency and management practice at farm level.

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Use of ultrasonography in practical reproductive management

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Poor oestrous behaviour and increased susceptibility to metabolic and infectious disease are typical of high yielding dairy cows. Poor reproduction is now a usual issue in most dairy herds. Our reproductive management is based on regular visits in farms with systematic examination of cows after calving, at the time of breeding and at the beginning of pregnancy.

Ultrasound has allowed us to be much more precise in our reproductive diagnosis. It has become an essential tool in reproductive management. New services allowed by ultrasonographic examination are the following: subclinical endometritis or pyometra diagnosis, cyst diagnosis, assessment of cyclicity, prediction of heat date, pregnancy diagnosis, diagnosis of age of the foetus, sex diagnosis, and obviously follow-up of good embryonic/foetal development. Different ultrasonographic views are presented to illustrate these different items.

This tool is now essential for accurate reproductive diagnosis and has greatly improved our herd reproductive management. By this mean, no systematic treatment are performed and every cow with reproductive problem gets the right treatment. Reproductive management is just a part of global herd management. It may be a door for veterinarians to go inside the farms and offer their ability to manage other topics, like mastitis management or dairy cow's welfare.

Early pregnancy diagnosis by per rectum amniotic sac palpation on pregnancy loss, calving rates and abnormalities in newborn dairy calves

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The present study evaluated the per rectal amniotic sac palpation (ASP) for pregnancy diagnosis during late embryonic period on pregnancy loss, calving rates, and abnormalities in newborn calves.

A controlled randomized blocked double blind experiment containing 680 lactating pregnant dairy cows with a viable embryo diagnosed by transrectal ultrasonography (TRUS) were used. Cows from two farms were randomly divided into control (CON) and ASP groups. CON group was not subjected to per rectum palpation (PRP).

All ASP examinations were performed by one experienced veterinarian between days 34 and 45 after estrus. All cows were reevaluated only by TRUS between 2 and 4 weeks later. Calving rates one and two were calculated.

In Farm A, the early pregnancy loss percentage for the CON group was 11.5% and for ASP group, it was 13.2% ($p = 0.64$). In Farm B, the early pregnancy loss percentage for the CON group 11.2%, and for the ASP group, it was 8.8% ($p = 0.48$).

In Farm A, the late pregnancy loss percentage for the CON group was 7.6% and for the ASP group, it was 5.2% ($p = 0.39$). In Farm B, the late pregnancy loss percentage for the CON group was 3.7%, and for the ASP group, it was 6.3% ($p = 0.32$).

In Farm A, the early pregnancy loss percentage was higher than the late pregnancy loss percentage (12.4% vs 6.3%; $p = 0.01$) and in Farm B, the same results were detected, respectively (10.0% vs 4.9%, $p = 0.02$).

In Farm A, calving rate one for the CON group was 81.2%, and for the ASP group, it was 80.8% ($p = 0.92$). Calving rate two for the same groups was 92.4% and 94.8%, respectively ($p = 0.68$). In Farm B, calving rate one for the CON group was 77.7%, and for the ASP group, it was 74.8% ($p = 0.55$). Calving rate two for the same groups was 87.4% and 82.1%, respectively ($p = 0.20$).

Two female calves with atresia coli were diagnosed only in the CON group. It was concluded that ASP for pregnancy diagnosis during the late embryonic period did not increase the pregnancy loss, affect calving rates, or produce abnormalities in calves.

Are Chlamydiales responsible for abortions of cattle in France and Belgium?

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While cattle abortions have still considerable economic impact on the productivity of herds in France and Belgium, the implementation of exhaustive analysis to identify putative pathogens leading to these abortions remains limited.

In France, a recent National Plan for the diagnosis of abortifacient agents in cattle has established a first list of main pathogens which are searched systematically (*Brucella spp*, BVDV, *Neospora caninum* and *Coxiella burnetii*) and a list of secondary pathogens to look for in a specific epidemiological context.

Chlamydophila abortus is part of this second list. Thus, the favorable epidemiological context is a close contact with sheep/goats herds or associated clinical signs in calves (pneumonia, otitis, kerato-conjunctivitis). But while the prevalence of seropositive animals for *Chlamydophila spp.* is quite common, the prevalence of PCR positive samples on cervical-vaginal swabs remains low (below 0.5%).

In parallel, recent publications have described the probable involvement of other Chlamydiales such as *Parachlamydia acanthamoebea* and/or *Waddlia chondrophila*, in abortions of cattle in Switzerland, the UK and Tunisia, with a prevalence of positive PCR up to 18%. These two bacteria, by having a high antigenic community with members of Chlamydiacea family, might be the cause of cross-reactions with ELISA *C. spp* tests used.

To test this hypothesis and to establish the first prevalence data of Chlamydiales other than Chlamydiacea in abortions of cattle in France and Belgium, BioSellal have developed and validated according to the French Standard NF U47-600-2, five real-time PCR: qPCR panChlamydiales, qPCR panChlamydiacea, qPCR *Parachlamydia acanthamoebea*, qPCR *Waddlia chondrophila* and qPCR *C. abortus*.

These different tools were used to analyze 103 cervical or placental swabs from aborted cows of which 66 with known status in serology *C. spp* by ELISA and 6 fetal organs. All samples were found negative with the qPCR *Parachlamydia acanthamoebea* and *Wadlia chondrophila* and only one sample was found posititive with a late Ct over 35 with qPCR Chlamydiacea. Interestingly, the prevalence of positive results with qPCR panChamydiales on the 66 samples with associated serological status was 33.3%. Further studies are being conducted to determine the Chlamydiales specie(s) detected in French and Belgian aborted cattle.

Anthelmintic resistance in gastrointestinal nematodes of cattle: towards a needed paradigm shift

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Introduction

Anthelmintics play a central and at times exclusive role in controlling cattle helminthosis. Their use has been increasing over the past decades as much in terms of the nature of the molecules used (longer acting) as the frequency of use and the categories of animals treated (young and adult). This has resulted in an overuse which can have consequences in terms of anthelmintic resistance, insufficient development of immunity, environmental impacts and economic efficiency (Stafford & Coles, 1999; Vercruysses & Claerebout, 2001).

In ruminants, anthelmintic (AH) resistance has mainly been described in gastrointestinal (GI) nematodes and secondarily in *Fasciola hepatica.* AH resistance in *F. hepatica* was described with regard to closantel, rafoxanide and nitroxynil (Wolstenholme et al., 2004). Since 1995, cases of resistance to triclabendazole were confirmed in Australia and Europe (Ireland, United Kingdom and the Netherlands) (Fairweather, 2011). This article deals with the resistance of GI nematodes.

There are three main broad spectrum anthelmintic families for GI nematodes affecting ruminants: benzimidazoles and probenzimidazoles, imidothiazoles (levamisole), and macrocyclic lactones (ML) (avermectins and milbemycins). The cattle anthelmintic market has been largely dominated by the last family (which is also efective against external parasites) since its introduction in the early 1980s. Alongside these broad spectrum products, there are two narrow spectrum anthelmintics against haematophagous nematodes (principally *Haemonchus*, and therefore of limited interest in temperate areas as France due to the quasi absence of this parasite in cattle), namely salicylanilides (closantel) and nitrophenols (nitroxinil). The "anthelminitic resources" may be considered to be limited or even stagnating if one refers to the antiparasite families (defined by the same mode of action). Nearly 30 years passed between the release of MLs (ivermectin in 1981) and the launch of monepantel. Monepantel has been authorized to be marketed for sheep in France (Zolvix©) since November 2009; it is a member of the Amino Acetonitril Derivatives (AAD) class and it has a unique mode of action. Another anthelmintic with a unique mode of action, derquantel, is a member of the Spiroindol class and was registered for sheep in 2012 in Great Britain in the form of a combination with abamectin (Startect©). This product has not been authorized to be marketed in France (Epe & Kaminsky, 2013). These two products are unavailable for cattle and it thus would be foolish to think that we can easily respond to the emergence of anthelmintic resistance with the discovery of original molecules with a different mode of action given the time needed to develop new anthelmintics in animal production.

The nematocide anthelmintics are listed in Table I. It should be noted the conditions of use and notably the withdrawal period that have recently evolved for some of them.

What is anthelmintic resistance?

For a specific population, resistance is the existence of a larger proportion of parasites able to survive a given exposure to an anthelmintic compared to a normal reference population. This characteristic is heritable. For an anthelmintic, one conventionally speaks of resistance when the reduction related to a treatment (faecal egg count or necropsy exam) is under 95% (Sutherland & Leathwick, 2011). Within a parasite population that has not been previously selected by an anthelmintic, a tiny but not nil proportion of worms have the genetic ability to resist this anthelmintic (pre-adaptive phenomenon). They must be distinguished from worms which, without selection, naturally tolerate a given anthelmintic (for example, the ineffectiveness of levamisole against whipworms) and which therefore fall outside the spectrum of the anthelmintic.

The genetic diversity of nematode populations explains this pre-existence of resistant populations with probably a very low allele frequency. Resistance develops within a parasite population when the allele frequency of one or several resistance genes increases and leads to a reduced efficacy of the treatment compared to what normally is observed. Genotypic resistance (increase in the frequency of the resistance allele) evolves slowly and silently until, after reaching

a certain allele frequency threshold, phenotypic resistance (reduced efficacy of the anthelmintic) brutally manifests itself (Kaplan & Vidyashankar, 2012).

Table I: Principal anthelmintics that can be used against GI nematodes in cattle

Family	Molecule	Pharmaceutical form	Long-acting	Activity against L4 larvae	Activity against other parasites	Can be used on dairy cows?
BENZIMIDAZOLES	Fenbendazole	drinkable suspension oral powder	none	partial	lungworms	yes, but with WPM = 6 days
	Oxfendazole	drinkable suspension	none	partial	lungworms	yes, but with WPM = 7 days
		sequential release intra-ruminal device (bolus)	release of 5-6 successive doses spaced 3 weeks apart	partial	lungworms	no (nor in future drinking milk producing pregnant heifers)
	Albendazole	medicated premix	none	partial	lungworms liver fluke	yes but only in divided doses: WPM = 1.5 days after the third administration
		drinkable suspension	none	partial	lungworms liver fluke	no or WPM = 3.5 days depending on the brand
	Netobimin	drinkable suspension	none	partial	lungworms	yes, but with a WPM = 3 days
IMIDAZOTHIAZOLE	Levamisole	drinkable solution or suspension	none	no	lungworms	no
		continuous release intra-ruminal device (bolus)	90 days	no	lungworms	no
		injectable IM or SC solution	none	no	lungworms	no
		pour on skin route solution	none	no	lungworms	no
AVERMECTINS	Ivermectin	injectable SC solution	14 to 21 days depending on the GI nematode species	yes	Depending on the molecules and the administration route: lungworms, warble flies, lice, mites, and hornflies	no
		pour on skin route solution				
	Doramectin	injectable SC solution	21 to 35 days depending on the GI nematode species			
		pour on skin route solution				
	Eprinomectin	pour on skin route solution	14 to 28 days depending on the GI nematode species			yes, but with a WPM = 0
MILBEMYCINS	Moxidectin	injectable SC solution	35 to 120 days according to the specialities			no
		pour on skin route solution				yes, but with a WPM = 6 days

WPM = withdrawal period for milk

The mechanisms involved in resistance to the three main nematocides are presented in Table II. One may distinguish specific (involving the target of the molecule) and non-specific mechanisms (detoxification). These data are complex (according to the parasite, for example) and constantly evolving, in particular for the MLs, which is notably the reason for the deficit of routine molecular diagnostic tools.

Table II: Mechanisms of action of the 3 main nematocide anthelmintic families
(Lespine et al., 2008; Martin et al., 1989; Urdaneta-Marquez et al., 2014; Wolstenholme et al., 2004)

Anthelmintic family	Resistance mechanism	Specificity
Benzimidazoles	Mutations on the β-tubulin gene Metabolic changes	Target (specific) Specific and/or non-specific
Levamisole	Changes on the nicotinic acetylcholine receptors	Target
Avermectins and milbemycins	Mutations on the glutamate/chlorine or GABA receptor genes Mutations on the dyf-7 gene (involved in the cephalic sensory function) ABC transporters (transmembrane proteins): overexpression	Target Target Non-specific

In addition to the many risk factors related to treatment practices which we will discuss later in this article, the development of resistant populations depends on numerous factors associated with the parasite's biology (Coles, 2002):

- natural frequency of resistance genes in a unselected population,

- resistance genetics (mono, multi-genes; recessive or dominant character, etc...),

- fitness (biotic potential) of resistant worms: there is little reason to believe that resistant worms are less fit than susceptible worms. Even in the absence of the anthelmintic, a resistant parasite population does not seem to return to susceptibility (Kaplan, 2004),

- number of parasites in the population *in refugia*: the population *in refugia* is defined as the parasite population (worms or infective larvae) unexposed to the anthelmintic and thus unselected during treatment; they thereby contribute to maintaining susceptibility alleles in the population. The use of "refuge" is a key component of sustainable treatment programs by limiting the selection pressure and thus slowing down the emergence of resistant populations. There are three sources of refuge: infective larvae on pastures, untreated animals, inhibited stages (for the anthelmintics which do not affect these stages). With regard to infective larvae, refuge will be weak in all situations where infestivity is low (end of winter, drought, resting plots, new pastures...).

Detection of anthelmintic resistance

The methods to detect anthelmintic resistance were the subject of a reference publication in 1992 (Coles et al., 1992) and were amended in 2006 (Coles et al., 2006). The main detection technique remains today the post-treatment Faecal Egg Count Reduction Test (FECRT); it can be used with all of the anthelmintics and is based on counting nematode eggs in faeces before and after treatment, with interval following treatment varying depending on the anthelmintic. This test also is the only one that can be conducted in the field. The necropsy examination test following an experimental infection and AH treatment could be considered as the reference method but is quite expensive and incompatible with field surveys, which limits its use to confirmation and research studies. There also are *in vitro* tests, including the egg hatch test, indicated only for benzimidazoles (ovicidal activity), and larval tests (development or motility) for the two other anthelmintic families. Lastly, PCR techniques have been published for benzimidazoles. Molecular techniques remain the domain of research and cannot yet be used for the routine diagnosis of resistance (Kaplan & Vidyashankar, 2012).

FECRT after treatment

Performing the test

The principle of the test in presented in Box 1. This test can be considered as not very sensitive. With benzimidazoles, for example, detection only is possible if over 25% of the population is resistant (Martin et al., 1989).

In cattle, the realization and interpretation of this test combine two challenges. According to the recommendations (Coles, 2006), the individual excretion level of animals before treatment must not be under 150 epg (even 100 epg) which is, even among young cattle, difficult to satisfy. Actually, significant excretions are relatively transient (few months after turnout) and obviously heterogeneous. The ideal size of groups (1 group per molecule tested with possibly a control group) is 15 animals, which, again, is not always easy to respect, particularly if all animals excreting < 100 or 150 epg must be set aside. Other difficulties specific to cattle also may be noted: compared to small ruminants, faecal egg count/parasite burden correspondence is less good and egg production is highly density dependent (negative interactions between the number of worms and female egg-laying) for some nematode species (Sutherland & Leathwick, 2011).

With regard to MLs, numerous molecules, formulations and administration routes exist and can impact the choice of the post-treatment interval and the results of the FECRT. For example, the greater efficacy observed (most often) with moxidectin compared to ivermectin on the same parasite population is related to its greater lipophilicity, persistence and potency (Bartley et al., 2012).

The molecules tested must be in an oral or injectable formulation, which ensure less dispersion in pharmacokinetic parameters (Kaplan & Vidyashankar, 2012). Moreover, numerous biases and errors can mar the conduct of the test (identification of animals, application of the anthelmintic treatment, not respecting post-treatment timing, etc...).

Another difficulty lies in the precise definition of the interval between the AH treatment and the faecal egg count. When animals have not been confined indoors when the FECRT is conducted, they continue to be re-infected by L3s. The maximum period between treatment and faecal egg count must take into account this risk of re-infection following the excretion of eggs (prepatent period). In contrast, a minimum period must be respected, particularly with MLs, due to a possible temporary suspension of egg laying. A period of 17-21 days thus was proposed for moxidectin given the risk of resistant female parasites temporarily suspending egg-laying (Kaplan & Vidyashankar, 2012). De Graef et al. (2012) confirmed the limits of FECRT to detect the resistance of *C. oncophora* to moxidectin (97% reduction on Day 7, 86% on Day 14) compared to a necropsy exam (31% reduction Day 14 after slaughter) due to a reduction in the fertility of surviving parasites on Day 14-15 after treatment. For the same reasons, Bartley et al. (2012) also confirmed the non-validity of FECRT on Day 7 in detecting resistance to ivermectin and moxidectin.

Box 1: Assessment of anthelmintic resistance in a cattle herd
(Coles et al., 2006; Levecke et al., 2012)

Setting up

Several weeks after turn out to pasture, on first season grazing animals that have not been previously dewormed to ensure maximum excretion (ideally > 100 epg individually). Two techniques are possible: carry out faecal egg counts before and after treatment (recommended with cattle) or faecal egg counts only after treatment (frequent with small ruminants). At least 15 animals per group, that is to say 30 animals to enable 1 control group (not dewormed) and one treated group (anthelmintic to be tested). The anthelmintics must be in an injectable or oral formulation (the oral route is the most effective against *Cooperia*).

Inclusion criteria

- sufficiently infected animals (excreting): ≥ 100 epg,
- strict identification of animals,
- animals which have not been dewormed for 2 months.

Implementation

- pre-visit on Day-7: Identification and faecal egg count on the maximum number of animals, select for the next step animals ≥ 100 epg,

- treatment visit (Day 0): anthelmintic treatment (groups of 15 animals, dose calculated based on the weight of the heaviest animal in the group without stop-dose, weighing or tape),

- post-treatment faecal egg count visit on a precise date (before Day 11 for levamisole, on Day 10-14 for benzimidazoles, Day 14-17 for macrocyclic lactones[1]): take individual faecal egg count samples (3 to 5 g) from control group animals and treated group animals with careful identification,

- storage of samples one night at 4°C then send to laboratory.

Faecal egg count (laboratory)

- favour the most sensitive technique (or reading),

- the faecal egg count results are expressed by the following formula (other calculations are possible including pre-treatment counts): % faecal reduction = [1 - (mean epg treated animals/mean epg control animals)] x 100

Interpretation

When the percentage of reduction is ≥ 95%, nematode populations are considered as susceptible; when it is less, there is a suspicion of resistance.[2] Coproculture on the control and treated groups inform on the type(s) of nematode involved in the resistance.

Errors to avoid

- animals which are poorly infected render interpretation impossible,
- errors of identification,
- errors of treatment resulting from incorrect dosage or incorrect assessment of weight.

[1]for moxidectin, this period may prove to be too short due to a possible temporary suppression of egg laying. In contrast, an overly long period may lead to reinfection with new egg ouput.

[2]it is preferable to talk about suspicion of resistance with FECRT and about confirmation with the controlled test (necropsy) especially for new records

Critical analysis

The critical analysis of this protocol, applied to a system without a control group (animals sampled before and after treatment) has shown that the interpretation of FECRT depended on the initial excretion level and the distribution of this excretion (aggregation), and that FECRT had a weak diagnostic value for the detection of small excretion reductions in the absence of a control group (Levecke et al., 2012). The group sizes, epg aggregation, post-treatment excretion level and sensitivity of the faecal egg count analysis technique thus determine the accuracy and therefore the detection capability of FECRT at a 90-95% confidence level. Others confirm the importance of adopting a more sensitive faecal egg count technique for cattle, such as the Wisconsin technique, or to carry out three faecal examinations per animal (Gasbarre, 2014; Kaplan & Vidyashankar, 2012).

The importance of these points is illustrated in the study made by El-Abdellati et al. (2010) on 6-18 month old heifers on 84 farms in Belgium and Germany where a first survey with a simplified FECRT (10 animals randomly selected before and after treatment, McMaster technique with a 50 epg sensitivity) had found resistance prevalence of 25 to 39% (cut-off at 95%). In a second step, four farms with resistance were re-tested the following year with a standardized FECRT using the identification of animals, the inclusion of only excreting heifers and another method to calculate the reduction; this resulted in the confirmation of resistance on only one farm out of the four.

The calculation method

The method used to calculate the post-treatment reduction in faecal excretion also is important. Numerous studies have examined the importance of including a control group, the faecal egg counting before and after treatment, the use of arithmetic or geometric means, the faecal egg count reduction at the individual level (compared to group means) and finally the use of different formulae with, in certain cases, a re-sampling system (bootstrapping) (Cabaret & Berrag, 2004; Falzon et al., 2014). In cattle, Dobson et al. (2012) emphasize the importance of density-dependence control on the fertility of *O. ostertagi* females in young cattle which can generate excretion variations over short time periods on treated and untreated animals (increase or reduction of epg respectively). These authors propose a completely different calculation method for FECRT based on the distribution type of faecal egg count data. It should be mentioned that new guidelines on faecal egg count reduction test from the World Association for the Advancement of Veterinary Parasitology are in progress (Levecke et al., 2015, WAAVP Conference, Liverpool, 16-20 August 2015).

The key elements to conducting the most reliable FECRT on cattle are thus:

- use a sensitive faecal egg count technique,
- only retain positive faecal egg count animals,
- try for groups of 15 animals,
- include a control group,
- use anthelmintics with oral or subcutaneous routes of administration,
- adapt the timing of the post-treatment faecal egg count to the molecules used.

***In vitro* tests**

These tests are conducted in the laboratory. The egg hatch test used to determine the sensitivity of parasite populations to benzimidazoles was updated in 2006 (Coles et al., 2006) and its protocol was standardized (Von Samson-Himmelstjerna et al., 2009), adapted and assessed on cattle by Demeler et al. (2012). This test relies on isolating nematode eggs from faecal material before treatment and their exposure to increasing concentrations of thiabendazole in order to assess the larval hatching rate. The different results published show a good match with the FECRT and even suggest greater sensitivity to detect BZD resistance. For the other molecules, different tests on larvae exist (development test, motility test, migration test). The larval migration inhibition test was recently the subject of a vast inter-laboratory trial in Europe on known resistant parasites. The trial assessed resistance to ivermectin, and a standardized procedure was proposed (Demeler et al., 2010). However, these results were obtained on monospecific strains (mainly *Cooperia*) and an assessment of this test on mixed populations (*Ostertagia, Cooperia* even *Trichostrongylus*) has yet to be carried out.

Test by experimental infection, treatment, slaughtering and worm counts (controlled test)

This approach is the gold standard for resistance. Coupled with pharmacological monitoring on treated animals (allowing all anomalies to be set aside at this level), it allows the species involved in the resistance to be confirmed. This test can be considered as key point for the first descriptions of resistance in a given situation.

Epidemiology of anthelmintic resistance

Reports of resistance in cattle nematodes are less frequent and more recent than for small ruminants (Sutherland & Leathwick, 2011). Since 2000-2005, the reports seem to have multiplied and the countries most affected are New Zealand (up to 90% of farms), Argentina and Brazil. Overall, all nematode species and all three anthelmintic families are involved, but the reports are much more frequent for the genus *Cooperia* and the macrocyclic lactones. Reports of resistance to more than one anthelmintic and for more than one nematode species also have been published, which suggests, if one looks at the history of AR in small ruminants, that the development of AR is probably older in cattle than the reports would lead to believe (Sutherland & Leathwick, 2011). There are, however, more reports than true prevalence surveys, which impedes a more precise vision of the importance of the phenomenon (Kaplan & Vidyashankar, 2012).

The situation in Europe and France

The first case of resistance in cattle in Europe was described in 1999 by Stafford & Coles (1999) on a dairy farm in the United Kingdom. This case was confirmed by experimental infection and necropsy exam and concerned *Cooperia oncophora* and ivermectin. In 2010, a FECRT conducted in Scotland demonstrated resistance to ivermectin involving *Cooperia* on 3 of the 4 dairy cattle farms surveyed (McArthur et al., 2011). Later, the study of 2 nematode populations from beef cattle farms in Scotland demonstrated resistance to ivermectin and moxidectin through necropsy exams (Bartley et al., 2012). The first large survey, using FECRT, was then conducted in 2006-2007 on 20 dairy herds in Germany, Belgium and Sweden (Demeler et al., 2012). For 6 out of 20 herds, the faecal egg count reductions following treatment with ivermectin were under 95%, the larvae discovered through coproculture were the genera *Cooperia* (mainly) and *Ostertagia*. In the same study, the efficacy of albendazole was tested on 12 herds with faecal egg count reductions of 100% in all of the groups. One of the farms surveyed was monitored over 4 years, which enabled the demonstration of an increase of resistance to ivermectin (FECRT dropped from 73 to 0%) and the inefficacy of moxidectin (El-Abdellati et al., 2010). In 2011-2012, a second large survey including Germany, Italy, the United Kingdom and France (Geurden et al., 2013) demonstrated low post-treatment faecal egg count reductions (< 95%) in 10 to 60% of farms depending on the country (less resistance in Italy) and the molecule tested (ivermectin or moxidectin). In France, the study was performed on 8 dairy herds: in 3 herds out of the 8 for ivermectin, and 5 herds out of the 8 for moxidectin, the post-treatment faecal egg count reductions were under 95%, with the post-treatment larvae being mainly *Cooperia* (Geurden et al., 2013). On 3 farms, reductions of < 95% for both molecules were found. These are the only data available today for France.

Several conclusions can be drawn from these studies of the resistance of GI cattle nematodes in Europe:

- the resistance phenomenon is clearly confirmed by necropsy exams, notably for MLs and *Cooperia*,

- the phenomenon exists in dairy and beef cattle,

- large-scale studies are still very scarce and rely on post-treatment faecal egg count reduction tests; the conclusions drawn must be cautious due to the numerous variability factors (see above). However, it appears that a reduction in efficacy of MLs (ivermectin and moxidectin) is occurring in numerous herds, notably for *Cooperia*, and a suspicion of resistance can be legitimately evoked,

- these same studies give little information on the selection criteria for sampling the farms as well as the possible risk factors related to ML resistance,

- the limited data available for BZDs suggest the efficacy of these molecules has been maintained.

Why so few cases reported in cattle?

For many years, it was thought that cattle were little affected (compared to small ruminants) by the problem of anthelmintic resistance in nematodes. The main reasons evoked were (Coles, 2002):

- the low frequency of AH treatments on cattle due to the strong development of immunity,

- pasture practices, notably for beef cattle, which favours a permanent dilution of parasites between treated young cattle and untreated immune adult cattle. In contrast, raising young dairy heifers on the same pastures year after year in a context of repeated treatments seems to be an at-risk practice,

- the biology of the parasites, and notably their relatively short life span (25 to 50 days) for *O. ostertagi*, the survival of larval stages in cow pat with a reservoir effect compared to the faeces of small ruminants quickly prone to dry.

Another possible reason for this low number of cases is related to the circumstances of reporting. As we have noted, it is most often due to insufficient faecal egg count reductions following treatment with MLs and involves *Cooperia*. This genera (in particular *C. oncophora*) is considered to be less pathogenic than *O. ostertagi* and furthermore generates an immunity which develops faster (couple of months). The production consequences, and even more the clinical consequences, are scarcely noticeable (Sutherland & Leathwick, 2011), suggesting a likely silent development of resistance in herds. One must remember here that the first reports of resistance in small ruminants mainly involved *Haemonchus contortus* in a context of pronounced clinical signs (Kaplan, 2004).

The low pathogenicity of *Cooperia* must, however, be nuanced by the intensity of the infection and the strain (or species) involved, resistant strains (or *C. punctata*) seem to be more pathogenic (Kaplan, 2004; Stromberg et al., 2012). Furthermore, it must be emphasized that reporting linked to clinical cases (failures of treatment) provides late information as the resistant population has already reached a certain level (Kaplan, 2004).

Why mainly Cooperia?

Cooperia spp is considered to be the "dose-limiting" nematode (the least susceptible species) for MLs in cattle (Coles, 2002). The lower susceptibility of this parasite to MLs, possibly coupled with lower concentrations of anthelmintics in the small intestines (compared to abomasums), associated to its greater fecundity (compared to *Ostertagia*), are the elements put forward to explain the more frequent resistance of this genera (Demeler et al., 2009).

Economic consequences of resistance

The cost of resistance is difficult to measure. The only estimates were made in Brazil and New Zealand with parasites that were sometimes different (*Cooperia* but also *Haemonchus*) and therefore difficult to extrapolate to European conditions (Leathwick & Besier, 2014).

Factors influencing selection pressure for resistance

Relying primarily on small ruminant data but also on cattle data for countries where AR is very developed, several risk factors can be put forward (Sutherland & Leathwick, 2011):

Indiscriminate and excessive use of anthelmintics

This factor, clearly highlighted in sheep, also probably plays a role in cattle. Numerous publications suggest excessive use of anthelmintics in Northern Europe on first season grazing heifers with treatments applied later in the life of the animals due to an immunity that is not completely developed (Coles, 2002; Sutherland & Leathwick, 2011; Vercruysse & Claerebout, 2001). According to Stafford & Coles (1999), 57% of dairy cattle farmers treat their second season grazing heifers. This non-rational and heavy use may be due to the low price of MLs (generic drugs) and a difficulty in assessing parasite risk.

Use of long-acting anthelmintics

The majority of MLs used are pour-ons with variable but significant activity persistence. The selection pressure on the ingested larvae is thus prolonged over time (see Box 2). For some authors, a reduction in the post-treatment egg reappearance period (protection period + prepatent period) represents a strong suspicion of resistance.

Box 2: The potential effect of long-acting anthelmintics in relation to anthelmintic resistance
(Leathwick & Besier, 2014)

Following an anthelmintic treatment, long-acting or other, the population of resistant worms which have survived the treatment will contribute exclusively to the emission of eggs in the faeces. They will continue to do so during at least the prepatent period, namely the period during which new infections from infestive larvae ingested after treatment turn into adults. When a long-acting anthelmintic is administered, this period is lengthened. This is called "head selection". When the concentration of the molecule diminishes, efficacy in relation to larvae is probably variable depending on their resistance alleles (low for resistant larvae, higher for susceptible larvae). In this case, the parasite population continues to be selected through both the surviving parasites as well as the incoming ones. This is called "tail selection".

Variability in pharmacokinetics and efficacy of pour-on MLs

Research studies on ivermectin, eprinomectin and moxidectin pour-on formulations have shown variability in bioavailability and efficacy in connection with variability in absorption or with a licking phenomenon (Bousquet-Mélou et al., 2011; Sutherland & Leathwick, 2011). This variability in parasite exposure to the drug, particularly when underexposed, could result in a heightened resistance selection process.

Under-dosing

This was experimentally demonstrated as a factor of selection of resistant populations for MLs (Demeler et al., 2009).

Lack of nematode populations in refugia

The selection pressure resulting from repeated and/or long-acting treatments on young cattle can lead to impairment of immunity development and then to treatments of older animals or even adult dairy cattle (Vercruysse & Claerebout, 2001) and generate, notably for MLs, an overall selection pressure by diminishing the number of untreated animals and thus the parasite populations *in refugia*.

Lack of effective quarantine AH treatments

As quarantine procedures and associated AH treatments are poorly practiced and cattle trade between herds is frequent, the probability of buying resistant worms with the introduced animals is high.

How to delay the development of resistance in cattle farming?

Different aspects can be put forward to limit the risk of the development of anthelmintic resistance. Despite the scarcity of specific information on cattle, it is likely that the recommendations below are relevant for all ruminants. In contrast, a major challenge lies in how this risk is taken into account and in the acceptability of novel control measures to farmers. These measures mainly involve rationalising anthelmintic use, conserving a parasite population *in refugia*, and controlling the introduction of resistance alleles on the farm. These are summarized in Table III.

Table III: Measures to prevent or manage anthelmintic resistance
(Coles, 2002; Gasbarre, 2014; Leathwick & Besier, 2014)

Reduce at risk practices	Reduce the frequency and impact (duration) of treatments	First Grazing Season (FGS) cattle: priority target; treatment not systematic but relying on group diagnostic tools Promote the acquisition of immunity Second GS and adult cattle: no treatment unless precise justification/indication
	Limit under dosing	Weighing, barymetric tape, groups by weight (dosage based on heaviest animal)
	Avoid treatment when pastures are relatively uncontaminated	No dose-and-move, no treatment before drought
Keep a population *in refugia*	Selective treatment (at on individual or group levels)	Identify the individuals and/or groups at risk
	Dilution of resistance alleles	No pastures permanently dedicated for FGS cattle; alternate young/adults grazing Regularly change pastures for young cattle
Choice of anthelmintics	Long-acting and/or pour on anthelmintics	Privilege oral or subcutaneous routes of administration
	Combination of anthelmintics and new molecules	No information in Europe Risk-benefits unknown
	Anthelmintic rotation	Each year (empirical)
Prevent the introduction of resistant worms	Treatment during quarantine	Treatment with two anthelmintics, wait 48h and turn out on contaminated pastures (dilution)

One particular point concerns the use of pour-on and more generally of long-acting products. This mode of presentation and this type of molecule constitute much of the anthelmintic arsenal used on cattle. Yet as noted above, the potential effect of long-acting molecules on the emergence of resistant nematode populations has been mentioned for several years. Furthermore, the variability of pharmacokinetic parameters obtained with pour-on MLs raises the question of their efficacy and eventual impact on the emergence of resistant worms, notably for *Cooperia* (Leathwick & Besier, 2014). Under these conditions, the use of these molecules should be subject to rationalisation and limitations. The efficacy of the other anthelmintics, benzimidazoles and levamisole, is probably satisfactory but specific surveys should be carried out to precisely address this question (Stafford et al., 2010).

The combination of two nematocides is practiced on sheep in New Zealand and Australia and appears in simulation models to be more effective in slowing the development of resistance than alternating between two families. This strategy is developed notably with the new molecules available for sheep. Applying this principle to cattle, one could combine a ML with a BZD (or levamisole), which would destroy ML resistant worms while protecting against BZD or

levamisole resistance (Gasbarre, 2014). This approach remains nonetheless very theoretical and runs up against many practical difficulties.

The measures summarized in Table III represent an important, indeed radical, change in helminth control practices in several respects, for example, the selective treatments and taking into account parasite populations *in refugia*. These last two points are not easy to implement and complementary research is underway to define treatment rules which are at once easy to follow and do not penalize production. It must, however, be emphasized that mathematical models have shown that a low to very low percentage of untreated animals (sometimes less than 5%) may be enough to significantly reduce selection pressure (Leathwick & Besier, 2014).

These measures also illustrate the necessity of reconsidering the evolution of anthelmintic practices over the last few decades and turning towards a rationalised use by targeting interventions on the groups of animals at risk during the periods at risk. The promotion of the development of good immunity against gastrointestinal strongylosis is one of the main levers to optimise the use of anthelmintics. The need to have sufficient exposure at the end of the first grazing season to diminish the production impact of strongylosis in the second year of grazing or for adulthood was highlighted over 20 years ago for dairy cattle (Dorny et al., 1999; Ploeger et al., 1990) and must be again taken into consideration. This requires in return the availability of simple and reliable risk prediction and diagnostic tools.

Conclusions

For the past 30 years, anthelmintics have represented an increasingly powerful therapeutic arsenal which is more and more adapted to the requirements of veterinarians and farmers. The objective has shifted from disease control to production increase. We have now reached a point where this system must be rethought in terms of a dual objective, a quest for production performance but, at the same time, reduced selection pressure and less development of resistant parasites. The development of approaches integrating the use of alternative chemical products, taking into account grazing and herd management practices and the development of strategies to maintain parasites *in refugia* are required (Figure 1).

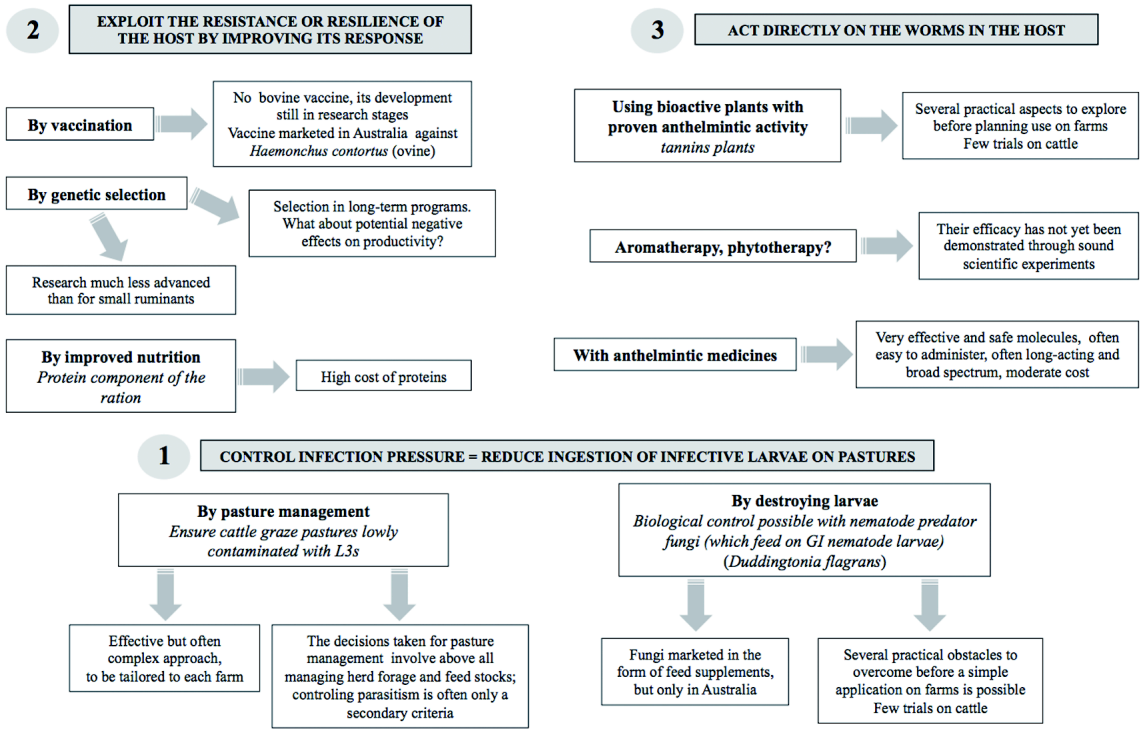

Figure 1: The integrated control of GI nematodes in cattle

AH resistance is a real threat for the sustainability of controlling strongylosis in cattle farming. Information and experience from small ruminants should be taken into account but specific data on cattle in relation to the management of parasitism, GI nematode epidemiology and the prevalence of AR are absolutely necessary to better assess and manage this risk. The technology available to demonstrate resistance, notably in relation to MLs, is either not very operational or difficult to implement with the exception of FECRT. This test requires detailed implementation rules for cattle species which must be known to veterinary practitioners.

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Methodology for optimised management of wet areas trematodosis following the new constraints regarding flukicides

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The present study evaluated the per rectal amniotic sac palpation (ASP) for pregnancy diagnosis during late embryonic In 2011, after the discovery of flukicides residues in Irish bulk tank milk, the European Medicines Agency was approached in order to establish guidelines for health protection of milk products consumers. Two simultaneous courses of action were set : i) determining safety withdraw periods regarding the use of flukicides in dairy herds when MRL were not available (then the establishing of that MRL); ii) re-examination of withdraw periods for oxyclozanid and albendazol (null-up to that time).

Those constraints lead inevitably to a more reasoned management of wet areas trematodosis, especially because the fasciolosis clinical impact is not diminishing and the paramphistomosis prevalence is growing. Besides the usually described clinical consequences of fasciolosis, the reduction of the sensitivity of intradermotuberculination tests and the modulation of Th1 immunity reactions, inducing a greater consumption of antibiotics, exacerbate its impact regarding human health, especially within the scope of the French Ecoantibio 2017 plan.

Regarding wet areas trematodosis, a precise procedure has to be set up. Every herd status has to be known. It is largely under-estimated due to the test results commonly available to farmers. In fact, the bulk milk tank kits used for fasciolosis diagnostic present a insufficient sensitivity in case of moderate prevalence. Regarding paramphistomosis, only coproscopic diagnosis is so far available and too scarcely implemented.

Then, the assessment of the prevalence and infestation areas for liver and rumen flukes, jointly with a complete knowledge of the features and constraints regarding each usable flukicide have to lead to customised plans of agronomic and medical management. Namely, from now on, the mandatory withdraw time for oxyclozanid in dairy herd forces, except for emergency treatment, to restrain that molecule to the dry period until calving or in conjunction with a medical treatment generating itself a milk withdraw delay, while respecting those parasitosis epidemiology.

Eventually, the approach and management methodology of fasciolosis and paramphistomosis in dairy herd is described in a practical way and illustrated with two cases of customised prescription in dairy herds of our practice. For the farmer, that one is detailed month by month and synthetized in a table easy to use.

To control cryptosporidiosis: a challenge for both human and veterinary medicine

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More than 100 years after the first description of *cryptosporidium* parasites by Edward Tyzzer, the treatment and prevention of cryptosporidiosis remain a challenge for scientists.

The research has generated information on many aspects of the *cryptosporidium* biology (crypto- from Greek etymology "kryptos" meaning "hidden") but recent advances in sequencing techniques will probably improve our knowledge.

From nearly 25 *cryptosporidium* species confirmed, *Cryptosporidium hominis* and *Cryptosporidium parvum* are responsible for the majority of human infections. Livestock, particularly cattle, are one of the most important reservoirs of *Cryptosporidium parvum*.

Cryptosporidium is an apicomplexan protozoan but demonstrates several peculiarities and can infect several hosts.

The first report on bovine cryptosporidiosis was published in 1971 and, in 1976, two clinical cases of human cryptosporidiosis were identified in two immunodeficient patients.

The 1980s have seen the emergence of cryptosporidiosis as a potentially severe opportunistic infection in people living with AIDS. During the same period, this protozoan parasite gained also attention from veterinarians as a pathogen of some neonatal diarrhea. An example of this increasing interest was a meeting "Cryptosporidiosis of young ruminant" organized by the French Buiatric Society in Lyon in 1984. And thirty years later, the importance of this infection of farmed animals continues to be a major concern among farmers and vet practitioners.

The greater emphasis for human health concern has followed the waterborne cryptosporidiosis outbreak which occurred in 1993 in Milwaukee and human cryptosporidiosis remains a serious infection, not only for immunocompromised individuals but also for children, particularly living in underdeveloped countries.

The vet practitioners should inform the livestock owner of the hazards that result from contact with visibly and invisibly infected animal and provide him the basic knowledge of sanitary measures required to protect society against the zoonoses.

If they know very well *Cryptosporidium parvum* as one of the most frequent causal agent of neonatal calf scours, a wider knowledge and understanding of the zoonotic potential of *cryptosporidium* should be sought for a successful One Health response.

This review is designed to summarize some publications that document zoonotic transmission of *cryptosporidium*.

Results of a multicentric coproscopic survey on coccidiosis in French dairy and lactating calves

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Purpose

The purpose of this survey (named Elanco-Pro®) is first, to clarify in France, the global prevalence of coccidiosis in french cattle herds and secondly, to evaluate the prevalence of the three pathogen species, *E. bovis, E. zuernii* and *E. alabamensis*.

Protocol

Individual faecal samples are collected, since 2013 in dairy and lactating herds, on groups of 5 calves, similarly aged (mostly between 2 and 12 weeks of age) and pooled (5 calves together).

Identification of coccidian species during the coprological examination (flotation with satured Sodium Chloride Solution 40%, sg = 1.2) allows in particular, the determination of the specific prevalence of the 3 pathogen species, *Eimeria bovis, E. zuernii* and *E. alabamensis.*

Results

176 and 356 pooled samples, collected in 2013 and 2014 reveal the same herd prevalence with 80.1%.

86.5% and 86% of the positive samples (respectively in 2013 and 2014) were infected by the 3 pathogen species : coproscopic prevalences of *E. bovis* were 70.2% and 64.1%, of *E. zuernii* 70.2% and 65.2% and of *E. alabamensis* 24.8% and 14.8%. Mixed infections were frequent (49.1% and 38.3%) then 34.7% of positive samples revealed the simultaneous presence of *E. bovis* and *E. zuernii*: it is the most usual association in France, just as well in dairy and lactating herds.

Discussion

This survey confirms the wide prevalence of coccidiosis in french cattle herds, with the predominance of the association *E. bovis* - *E. zuernii*, both highly pathogen species.

It suggests clearly that 80% of the herds are threatened with the danger of coccidiosis, either with clinical cases or more frequently, with subclinical coccidiosis and all economic losses.

So, coccidiosis should be more controlled in field conditions, with metaphylactic strategy at carefully choosen periods, according to risk analysis, specific for each herd.

The weak coproscopic prevalence of *E. alabamensis* (24.8% and 14.8%) differs seriously from a previous multicentric evaluation (75% in 2010-2011): the largest range of samples in 2013-2014 survey, seems to reflect accurately the current situation in France.

Moreover, at the opposite of northern parts of Europe, extremely few observations of clinical coccidiosis due to *E. alabamensis* (even for turning out) have been yet described in France.

Results of a French multicentric coproscopic survey on strongyloidosis in dairy and lactating calves

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Purpose

Underestimated until 2011 (owing to extreme susceptibility of *Strongyloides sp*. egg to high specific gravity of flotation solutions, if sg > 1.2), strongyloidosis proved to be widely more prevalent in cattle herds than described before.

Despite the lack of experimental proofs in cattle, *Strongyloides sp* appearing quite always before the first coccidia oocysts excretions, very probably impact the immunity building of coccidiosis.

This survey between 2013 and 2014 (named Elanco-Pro®) contribute to evaluate the herd prevalence of strongyloidosis in France.

Protocol

Individual faecal samples were collected, all over France, from 2013 to 2014, on groups of 5 dairy and lactating calves reared indoors, similarly aged (mainly between 2 and 12 weeks of age) and pooled (5 calves together).

Coproscopic examination used flotation with satured Sodium Chloride solution 40% (specific gravity = 1.2), recognized to be the best for laboratory diagnosis.

Results

176 and 356 pooled faecal samples, collected in 2013 and 2014, showed a herd prevalence respectively of 9.1% and 16%. The positive samples were found preferentially on calves between 3 and 8 weeks of age.

Discussion

This survey confirms the reality of strongyloidosis in young calves. The herd prevalence in France appears to be here lower than the one found in a previous multicentric French evaluation (25% of the sampled calves in 2010-2011).

Two main reasons could explain this difference : first, the 2010-2011 survey concerned only 14 farms with 53 groups of calves, aged from 2 to 7 weeks whereas the 2013-2014 survey concerned 150 and then 300 farms with respectively 176 and 356 groups ; secondly, the age bracket in 2013-2014 survey varied greatly from 2 to 12 weeks of age.

In 2013-2014 survey, two groups of positive pooled samples seems to be distinguished : the first one precociously, between 2 and 4 weeks of age (probably due to colostral infection) and the second, more important, between 4 and 8 weeks of age, relative to litter contamination.

Both types of breeding are concerned but it seems that strongyloidosis is more prevalent in lactating calves, according their life in barns with permanent litter.

Currently, these survey data suggest that the control of *Strongyloides sp* in infected herds, would be essential to prevent the clinical signs of strongyloidosis but also to improve the coccidiosis control.

Application, value and future of mapping *Fasciola hepatica* risk in cattle for animal health decision makers

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Several spatial modelling approaches have been used in order to predict high and low risk zones for fasciolosis in cattle. This is done by either modelling directly the presence/absence of the infection on farms or indirectly through species distribution modelling of the intermediate host *Galba truncatula*. Existing models usually focus on a region within a single country and often have a low spatial and temporal resolution. Here, we developed spatial models for *Fasciola hepatica* at different scales to inform animal health decision makers at the EU, country-specific or farm-specific level. As part of the EU GLOWORM project, using samples from 3,359 farms in 849 municipalities in Belgium, Germany, Ireland, Poland and Sweden a pan-European spatial distribution model was developed. Country-specific models showed important additional variation in infection-risk. Finally, using data from (very high resolution) satellite imagery or remotely piloted aircraft systems ("drones"), small water bodies on grazed pastures were detected and farm-specific risk maps could be obtained. When meteorological information is updated annually the risk maps can become more dynamic. Further research is required to assess how farm-specific risk maps can contribute to the economic control of fasciolosis in cattle.

Knowledge is Power
How understanding perioperative pain helps us with effective treatment

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Introduction

If I cut myself, this causes pain. If we do surgery in a calf this causes pain as well. Pain is increasingly recognised in farm animals (Riebold et al., 2015). Recent advances in understanding the mechanisms of perioperative pain as well as factors influencing persistent pain are important when choosing treatment strategies. More than 30 neurotransmitters acting on over 50 receptors are related to the pain sensation (McKune et al., 2015). Understanding the pathophysiology and neural pathways responsible for pain can help guide the treatment strategy.

Pathophysiology of pain

The following structures are involved in pain transmission and perception:

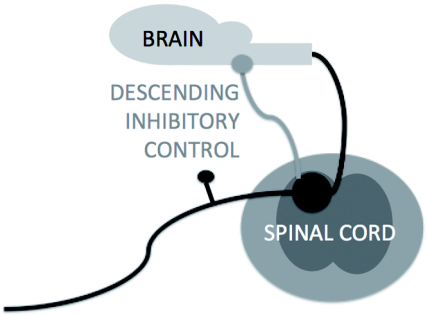

Figure 1: Structures involved in pain transmission and perception

Transduction of a mechanical, thermal or chemical signal into an electric action potential in a nerve fibre: When we perform surgery chemical signals released by destruction of cells trigger an action potential in a free nerve ending (nociceptor).

Transmission of the signal to the spinal cord: The action potential is relayed through a primary nerve fibre to the dorsal horn of the spinal cord.

Transmission to a secondary nerve fibre and modulation of the signal. At the spinal cord the action potential is relayed to a secondary neuron. The spinal cord is a common place where modulation of the incoming signal occurs.

After passing more synapses the action potential travels up to the cortex and the limbic system where pain is perceived (perception).

Descending inhibitory control mechanism are activated mainly in the brain stem to activate a descending inhibitory control system which can influence signal transmission at the level of the spinal cord adding further modulation of the pain signal.

The individual steps are highlighted below (McKune et al., 2015):

Transduction

In the periphery mechanical and thermal stimuli can also trigger the receptor responsible for generation of the action potential. Examples for thermal stimulation are dehorning using electrocautery or branding, mechanical stimuli are rubber bands used for castration and chemical signals are intracellular components of destroyed cells (for example H^+ Ions or ATP), which are not normally found outside of cells (Fig 2a).

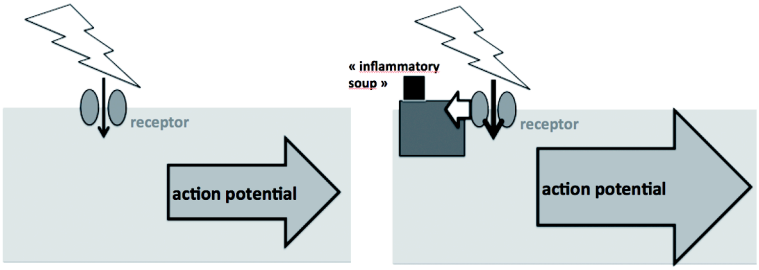

Figure 2a) and 2b): Peripheral mechanisms of pain and peripheral sensitisation

Other chemical substances can modify this peripheral signal generation. This also includes the prostaglandins, the main component of the so called "inflammatory soup"; substances that are present during inflammation after surgery. Inflammatory mediators can bind to different receptors, which facilitate opening of the ion channel receptors, and an action potential is generated more easily. This process is called peripheral sensitisation (Fig 2b).

Transmission

The signal is transmitted along the primary nerve fibre as an action potential. Voltage gated Sodium channels (Na_v) are responsible for this action potential and can be inhibited by local anaesthetics. At the level of the spinal cord the signal is transmitted on to a secondary neuron by means of a "classical" synapse. The neurotransmitter is mainly glutamate, which acts post-synaptically on AMPA Receptors, causing Na^+ influx and the generation of an action potential in the secondary neuron.

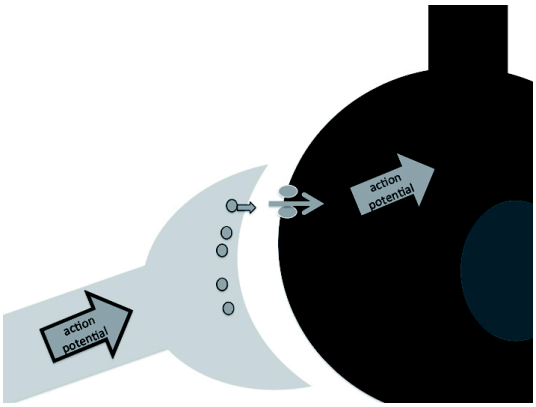

Figure 3: Transmission at the level of the spinal cord to a secondary neuron

Modulation

Modulation at the level of the spinal cord is particularly important. This can be through input from other nerve fibres as well as exogenous drugs, for example opioids.

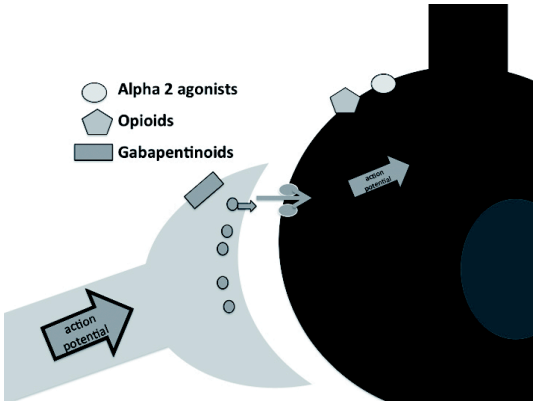

Figure 4: Spinal action of analgesic drugs reducing the action potential in the secondary neuron

After strong stimulation or during chronic pain states plastic changes occur at this level and are responsible for central sensitisation. This process may be responsible for persistence of chronic pain states.

Central sensitisation results in an increase in the "gain of the pain system". One of the responsible mechanisms is the recruitment of NMDA receptors. These receptors are commonly blocked by a Mg^{2+} ion, however with chronic or strong stimulation the Mg^{2+} -block is removed and the NMDA receptor allows influx of Ca^{2+}. This second messenger produces changes in AMPA receptor activation and also causes the production of an increased number of AMPA receptors in the cell nucleus, which are then transported to the synapse. If a normal stimulus now reaches the spinal cord from the periphery and increased signal is sent towards higher centres in the brain.

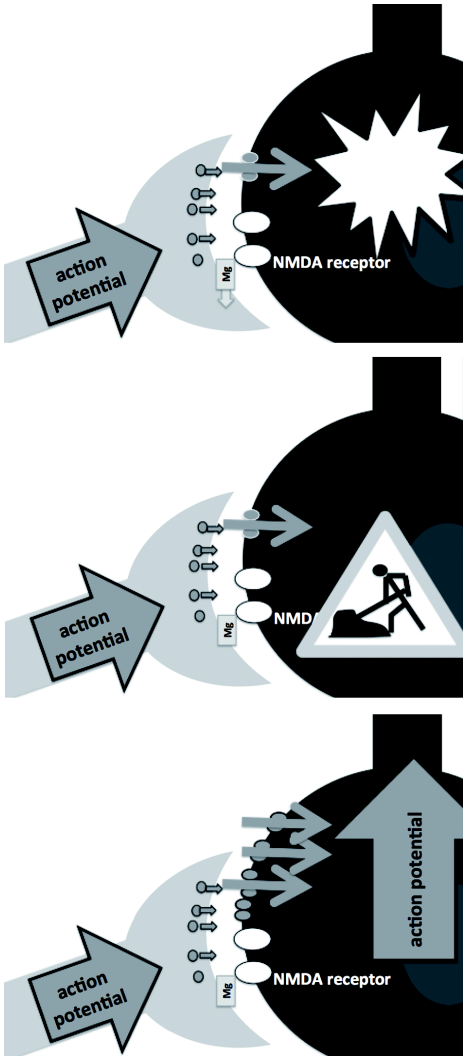

Figure 4a - c: Mechanisms of central sensitisation

This phenomenon is not just a theoretical assumption, but a clinically relevant problem: Sheep with foot rot have increased sensitivity to pressure at the level of the carpus (i.e. not at the claw where lesions exist but more proximal of anatomically changed tissue), representing a sign of central sensitisation. Depending on the severity of the lesions this increased sensitivity (central sensitisation) disappeared after persisted beyond 3 weeks after successful treatment (Ley et al., 1989). Chronic persistent lameness as well as difficulty treating chronic lameness in cattle may have the same mechanisms (Shearer JK et al., 2013).

Other mechanisms resulting in central sensitisation using prostaglandins may also be involved.

Perception

At the level of the brain mainly two different pain areas are responsible for pain perception:

- the somatosensory cortex (sensory –discriminative component of pain) responsible for localisation and magnitude as well as coordinated motor response to pain. This pathway is activated through A δ nerve fibres in the periphery and is responsible for the sharp, acute, localised and quick component of pain.

- the limbic system, responsible for the emotional affective component of pain; incoming signals originating from C-fibres and produce an unpleasant, less localised often throbbing pain which comes a split second after the first "fast" pain. This component is also related to emotion, behaviour changes in response to pain, learning and suffering.

These systems are not strictly separated and they utilise very similar mechanism of signalling as outlined above and can be pharmacologically modified by the same molecules.

By definition perception of pain is not possible when an animal is unconscious (i.e. general anaesthesia), however the above-mentioned pathways are activated up to the level of the brainstem and are called nociception. Plastic changes and central sensitisation may still occur in unconscious patients.

Inhibitory control

The body has also descending inhibitory control mechanisms. Besides the release of endorphins this includes a descending neuronal control system, which originates in the brainstem and is triggered by ascending incoming painful stimuli. Collaterals produce an action potential in descending neurones, which terminate at the level of the spinal cord causing analgesia by activating an inhibitory interneuron. Main neurotransmitters involved are serotonin and noradrenaline. These neurotransmitters are commonly recycled into the descending neuron, however, this reuptake can be influenced by agents reducing the reuptake of serotonin and noradrenaline (i.e. tramadol, antidepressants)

Therapeutic implications

Besides pharmacological management of pain, adjunctive measures like hoof trimming, blocks for lameness, local cooling, reduction of movement, modification of the floor surface and acupuncture can greatly aid analgesic therapy in food animals.

The following will detail pharmacological options to treat pain.

The main drugs used to treat perioperative pain are from the following pharmacological groups (Riebold et al., 2015): NSAID, opioids, α_2-agonists, local anaesthetics and adjuvant medication in Table I.

Table I: Main drugs and their place and mode of action

Place of action	Drug	Mode of action/receptor
Periphery	NSAID Local anaesthetics	Reduce inflammatory soup Na channels on nerve fibre
Spinal cord	Opioids α_2 agonists Ketamine NSAID Gabapentin	Opioid receptors α_2 receptors NMDA Receptor Prostaglandins Calcium channel (?)
Brain	Opioids α_2 agonists Others	Opioid receptors α_2 receptors
Inhibitory control	Tramadol Antidepressants	Serotonin and Noradrenalin (and Opioid) Serotonine and Noradrenaline

Generally speaking, it is useful to combine several drugs, which acts at different levels of the pain "pathway" or on different receptors. Furthermore it is important to understand that different routes of application have different onset and duration of action and combination treatment may be necessary to reduce "gaps" in analgesic coverage.

Practical example:

For a foot surgery ideally an α_2-agonist, an NSAID and local anaesthesia are combined (Figure 5). This combination reduces nociceptive input to the brain at different levels. Additionally it gives maximal analgesia at the time of maximal stimulation (surgery) and guarantees a good transition to the postoperative period.

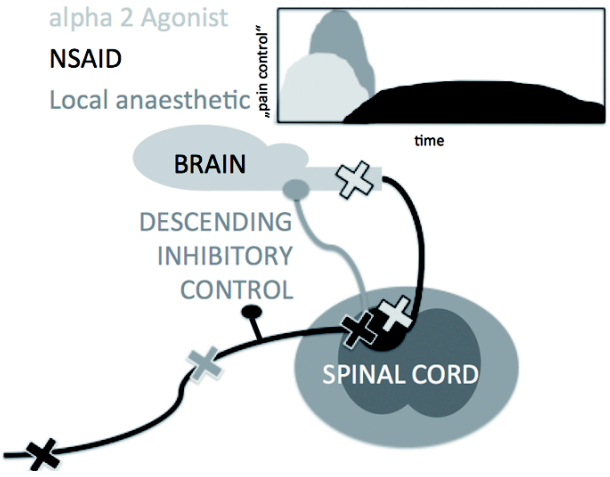

Figure 5: Practical for a balanced analgesic protocol for a foot surgery in a cow

Non steroidal anti-inflammatory Drugs (NSAID)

The most commonly used analgesic drugs are NSAIDs (Coetzee, 2013). They inhibit the formation of the inflammatory soup, mainly prostaglandins. Through inhibition of the cyclooxygenase enzyme the production of prostaglandins is inhibited. Therefore they inhibit peripheral sensitisation of the nociceptor. They have however a second mode of action which is situated in the spinal cord, likely also through inhibition of prostaglandins. Two isoforms of the COX enzyme exist in the body (COX 1 and COX2), which have different homeostatic properties in the body and their inhibition by NSAIDs is to a different degree depending on the drug in question and the species. Prostaglandins are also important in the gastrointestinal tract where they produce vasodilation and proper blood supply to the mucosa and aid in the production of mucus, which also protects the integrity of the gastric mucosa. Through prostaglandin inhibition irritation of the gastric mucosa even ulceration and micro-bleeding may occur. In farm animal medicine abomasal ulceration is particularly feared and recommendations to use COX 2 specific drugs or to use gastro-protectants have been given (Anderson et al., 2013). However scientific evidence regarding the incidence and the preventive measures of GI side effects remain scarce.

In the kidneys prostaglandins are responsible for local vasodilation in case of reduction of renal blood flow, with certain renal disease and low blood flow/pressure (i.e. Anaesthesia, shock, hypovolemia) NSAID should not be used, as there is the danger of kidney damage. After stabilisation of the cardiovascular system NSAID may be used.

Other Analgesics

α_2 Agonists

Xylazine und detomidine can be used for analgesia in cardiovascularly and respiratory stable patients. Sedation usually lasts longer than analgesia and administration of an α_2 -antagonist will reverse sedative AND analgesic effects. They have profound cardiovascular effects and potentiate the respiratory depressant effects of opioids.

Local anaesthetics

Local anaesthesia produces complete abolition of sensory input from a certain area. They are administered locally, perineurally or neuraxially (close to the spinal cord). Lidocaine can also be used intravenously for analgesia (Anderson et al., 2013).

Opioids

Opioids act at Opioid receptors in the spinal cord and the brain. For farm animals most commonly partial μ-agonists and κ-agonist/μ-antagonists are used. The main side effects of opioids are bradycardia and respiratory depression (at clinical doses and low doses often less clinically relevant in animals than in people) (Coetzee, 2013).

Other drugs

Ketamine is a dissociative anaesthetic and an NMDA Antagonist. Therefore it can influence central sensitisation. It is generally less useful as an Analgesic drug but is most commonly used in combination with α_2 -agonists +/- opioids and usually used in very small doses, compared to the ones used for general anaesthesia (Abrahamsen, 2013).

Gabapentin likely acts on Ca^{2+} channels in the spinal cord and reduces release of glutamate. In farm animal practice its use in combination with NSAID of lame calves as been described (Coetzee et al., 2014).

Pain is a common problem in farm animal practice. Knowledge in (patho)physiologic processes leading to pain help setting in place an effective management strategy and give us the "power" to treat pain more effectively .

Table II: Dosages of aforementioned analgesic drugs, which may be used in cattle when licensed products are insufficient

Respective national legislation on the use of these drugs in food producing animals needs to be considered.

Drug	Dosage	Comments
Butorphanol	0.05 mg/kg IV/IM/SC	Commonly used in combination with α2-Agonists
Ketamine	0.01-2 mg/kg IV/IM/SC	Low dose for standing procedures, highest for induction of general anaesthesia
Lidocaine	1 mg/kg IV	WITHOUT ADRENALINE! Slowly IV, may cause sedation
Gabapentin	10 mg/kg q 12 h PO	

IV = Intravenously, IM = intramuscularly, SC = subcutaneously, PO = perorally

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Impact of meloxicam administration in cows prior to caesarean on the efficacy of transfer of passive immunity in calves

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Over the past decade interest in the welfare and pain management of farm animal species has grown substantially But in such experiments, this is mainly the benefit of providing analgesia in cows which is investigated while the calf side is often neglected. Some authors suggest that in case of clinical signs of reduced vitality, it is plausible that providing NSAID to calves could improve the time top standing and increase colostrum intake and therefore decrease the risk of failure of passive immune transfer.

The objective of the current study was to assess the benefit of meloxicam prior to cesarean to cows on the efficacy of transfer of passive immunity under the assumption that providing analgesia on the dam could lead to an earlier or longer colostrum intake by its own calf. The study was performed in Burgundy in France in two veterinary practices. Colostrum quality, delay between the end of the cesarean and the first spontaneous colostrum suckling and the IgG content in the sera of their calf 24 hours after birth of cows treated with meloxicam subcutaneously (0.5 mg/kg) (n = 22) or without analgesia (n = 26) prior to cesarean were compared.

No significant differences were observed in the quality of the colostrum nor in the delay between the end of the surgery and the first spontaneous suckling of the calf between treatment groups. However, the number of calves showing a better transfer of passive immunity (IgG content > 15g/L) was significantly higher among those originating from dams receiving meloxicam prior to cesarean. This effect was notably observed in multiparous cows.

This study confirms that preemptive analgesia in cows prior to cesarean leads also to a benefit for its own calf through an improved colostrum intake ensuring a more efficient transfer of passive immunity crucial for its short and long term survival.

Laparoscopic evaluation of umbilical disorders in calves: description of the technique and comparison with ultrasonography and laparotomy

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Introduction

Umbilical disorders (UDs), such as hernias, abscesses or infection of the umbilical remnants are common in calves. Because of the associated risk of septicemia, septic arthritis, or unthriftiness, surgery is recommended. Despite the advantages of laparoscopy (minimal invasive, excellent intraabdominal observation, low morbidity), only a few studies deals with its use for UDs in large animal neonates.

Our goals were to describe a laparoscopic approach to evaluate UDs and to compare ultrasonography (US), laparoscopy and laparotomy for diagnosing UDs in calves.

Materials and methods

Seventeen calves with an enlarged umbilicus were included. Each calf had an US of the umbilical structures. Subsequently they underwent a laparoscopy under lumbosacral analgesia in dorsal recumbency using two 60 mm-long canulas positioned 10 cm cranial and 5 cm on either side of the umbilicus. Afterwards, an open omphalectomy was performed and infected umbilical structures were removed. If the umbilical vein was infected down to the liver, a marsupialization was performed. When the urachus was infected, a cystoplasty was realized.

Spearman's correlation and Kappa coefficients were used to compare US, laparoscopy and laparotomy.

Results

Seventeen male calves aged 23 +/- 5 days and weighting 44 +/- 6 kg were included. Surgical time was 31 +/- 10 min (7 +/- 3 min of laparoscopy; 24 +/- 8 min of laparotomy).

Laparoscopy allowed an excellent evaluation of the umbilical structures, liver and bladder.

Hernias, omphalophlebitis +/- liver involvement, omphaloarteritis and urachal infection were easily diagnosed, as well as adhesions. Interestingly, "subclinical" thickening of the urachus and arteries were observed.

We found a good to excellent correlation between US, laparoscopy and laparotomy, despite the US inability to diagnose adhesions and focal thickening of umbilical remnants.

Discussion

The described laparoscopic technique allowed thorough evaluation of UDs in calves. Using short trocars with an open insertion limited visceral damage and retroperitoneal insufflation.

The good to excellent correlation between diagnostic techniques confirmed the interest of US in preoperative planning of UDs in calves but laparoscopic findings appeared even superior, giving supplementary prognostic information.

Conclusion

Laparoscopy appears to be an interesting tool to precise the diagnosis and prognosis in cases of UDs in valuable calves.

Automated detection of lame dairy cows

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Introduction

Orthopedic disorders causing lameness belong to the most common and economically most relevant production diseases of dairy cattle worldwide. Detection of lame cows is important to improve animal welfare. Automated methods for lameness detection have the potential to facilitate recognition and monitoring of lame cows in large dairy herds. It was the aim of this study to evaluate the suitability of various automated methods for the assessment of altered behavior in cows, associated with lameness caused by "deep, non perforating, septic pododermatitis" (DNPSP) of one individual hind claw.

Materials and Methods

Thirty-two lame cows (group L) and 10 non lame cows (group C), housed in a commercial loose stall for German Holstein dairy cows, were included in this study. Degree of lameness was scored as previously described by Offinger et al., 2013. Locomotor activity by tridimensional accelerometers (RumiWatch®), weight distribution between hind limbs by the 4-scale weighing platform, feeding behavior by the nose band sensor (RumiWatch®) and heart activity by the Polar® device were assessed.

Results

Neither the evaluated variables of the feeding activity nor of the heart activity revealed significant differences between the two groups. The lying time of cows of group L was significantly longer as compared with cows of group C. Furthermore, cows of group L showed significantly different results concerning the variables derived from the weighing platform. Moderate to high correlations were found between the lameness score and the evaluated variables of behaviour of cows of group L.

Discussion and conclusions

It is concluded from the results of this prospective experimental field study that the 4-scale weighing platform as well as the tridimensional accelerometer (attached to one hind foot) represent the most valuable of the evaluated tools for automated identification of lame cows suffering from a DNPSP of one individual hind limb, when compared with non-lame cows. Variables of feeding and of heart activity are of minor value in this context.

Acknowledgements: This study was generously supported by grants of Boehringer Ingelheim Germany, the Swiss Federal Food Safety and Veterinary Office (FSVO; grant 2.10.04), and the Berne University Research Foundation. RumiWatch® was provided by ITIN+HOCH, Switzerland.

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Significance of infectious agents in bovine respiratory disease complex and the efficacy of novel macrolides in calf feeding

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Bovine respiratory disease complex (BRDC), or "shipping fever" is one of the most serious causes of disease and loss in cattle breeding and in calf feeding in particular. Calves in Switzerland are commingled into to feeding stock as early as at the age of two to four weeks, in which cases it is not possible for the calves to receive prophylactic vaccination against bovine respiratory disease in the original breeding facility. All the more reason the focus is placed on adequate metaphylaxis during housing. In addition to the general significance of the most commonly mentioned bacterial agents *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and Mycoplasma bovis*, it is important to pay attention to their presence during metaphylaxis. In particular, *M. haemolytica* is the most widespread and is found mainly in acute lesions, whereas *M. bovis* is associated with chronic lesions and usually appears in a timeframe later after housing. The antibiotics tildipirosin and tulathromycin of the novel macrolide generation are long-acting formulations, and studies have found them to be effective for metaphylaxis, but their differences are in the details.

Quantification of extended characteristics of locomotor behavior in dairy cows

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Introduction

Change of animal behavior is one of the most important indicators for assessing cattle health and well-being. Parameters of animal behavior can be used to build up an early disease warning system. The objective of this study was to develop and validate a novel algorithm to monitor locomotor behavior of loose-housed dairy cows based on the output of the RumiWatch® pedometer. It was hypothesized that a novel algorithm of the RumiWatch® pedometer device can be developed that provides a high correlation of parameters of behaviour of dairy cows in both upright and lying positions between the output data of the pedometers and the data derived from temporarily staggered video analysis.

Materials and methods

Data of locomotion were acquired by simultaneous pedometer measurements at a sampling rate of 10 Hz and video-recordings for manual observation later. The study consisted of three independent experiments. Experiment I was carried out to develop and validate the algorithm for lying behavior, experiment II for walking and standing behavior and experiment III for stride duration and stride length. The final version was validated, using the raw data, collected from cows not included in the development of the algorithm. Spearman correlation coefficients (r_s) were calculated between accelerometer variables and respective data derived from the video recordings (gold standard). Dichotomous data were expressed as the proportion of correctly detected events, and the overall difference for continuous data was expressed as the relative measurement error (RME).

Results

In all experiments, the mean difference between accelerometer data and respective gold standard was between 0% and 17% (depending on the variable of locomotion), and the correlation between respective data ranged from $r_s = 1$ to $r_s = 0.75$.

Conclusions

The strong to very high correlations of the variables between visual observation and converted pedometer data indicate that the novel RumiWatch® algorithm may markedly improve automated livestock management systems for efficient health monitoring of dairy cows.

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We thank ITIN+HOCH GmbH, Liestal, Switzerland, for providing the RumiWatch® pedometers for this project.

Comparative time, behavior and economic differences associated with calves receiving a pour-on vs an intravenous product

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Intravenous (IV) route of drug administration requires proper animal restrain, familiarity with bovine anatomy, and potentially increases stress, time and costs compared to pour-on administration. The objectives of this study were:

- determine differences in time required to administer a pour-on saline solution compared to inject IV,

- measure behaviors during and immediately post saline administration and evaluate for potential differences,

- determine economic costs associated with both a pour-on saline and an injectable saline solution based on the time and supplies necessary for administration.

One hundred beef crossbred calves were selected and randomized to treatment ((IV) or Pour-On (POUR)) based on chute order prior to the study. Each calf was moved into a processing chute and restrained using a head gate. Calves randomized to POUR received 18 mL of sterile saline on their back to simulate the administration of a pour-on product. Cattle assigned to IV were additionally restrained using a halter to fasten their head to the chute and allow access to the jugular vein prior to saline IV injections. The time calves were restrained in the head gate was recorded and 3 scoring systems (vocalization score, chute score, and exit score) were used to capture potential changes in behavior/stress associated with treatment. Costs associated with labor and materials for each treatment were estimated.

Results showed a reduction in chute time per animal of 36.45 seconds comparing IV injection (53.61 s) vs pour-on (17.16 s). Estimated labor cost per animal were \$0.54 less for POUR vs IV calves. Cost of material needed for IV administration (syringe and needle) was estimated at \$0.70 per animal. The total cost savings achieved by pour-on administration compared to an IV injections was estimated at \$1.24 (\$0.54 [labor] + \$0.70 [material] = \$1.24 [labor + material]). Behavior analysis showed calves receiving a pour-on administration had a higher ($p < 0.05$) probability of not vocalizing while in the chute and having a normal chute score. These data indicate that calves receiving a pour-on administration had a tendency of being less stressed while in the chute compared to those receiving intravenously administered saline.

Comparison of flunixin plasma levels following a single dose of Finadyne® Transdermal to cattle allowed or prevented from licking in a crossover study

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The study was conducted to determine the impact of oral exposure to Finadyne® Transdermal (FTD) via allo-licking or auto-licking on the pharmacokinetic profile. Twenty-four cattle were randomly assigned to two treatment groups; Group-I and Group-II, with 6 males/6 females per group. In Period 1, 12 animals in each group received FTD (0.05 mL/kg [2.5mg/kg]) between the shoulders and along the backbone.

Animals in Group-I (licking-prevented) were dosed while constrained in stanchions and remained in stanchions for 12 hours after dosing; then, animals were released into a pen, so they could freely commingle.

Animals in Group-II (licking-allowed) were dosed while constrained in stanchions but immediately after dosing, they were released into a pen so they could freely commingle.

After 9-day washout period, conditions were reversed for dosing of Group-I and Group-II (Period 2).

Blood samples were collected via jugular venipuncture at 0 (pre-dose), 15 and 30 minutes, and 1, 2, 4, 6, 8, 12, 16, 24, and 36 hours post-dose (licking-prevented) or 0 (pre-dose) 2, 4, 6, 8, 12, 16, 24, and 36 hours post-dose (licking-allowed) and processed for plasma. Liquid chromatography mass spectrometry (LC-MS/MS) assay was used for flunixin quantitation. Matrix-based calibration curves were generated. AUC(2hr-t) was used for treatment comparison.

Flunixin was rapidly absorbed with levels amounting to a large fraction of the Cmax seen only 15 minutes after application for the licking-prevented treatment with complete absorption in1-2 hours. Statistical comparison of AUC(2hr-t) was used to determine relative exposure for licking-prevented vs licking-allowed treatments. The geometric mean AUC(2hr-t) of the licking-allowed group (5,808 hr*ng/mL) was 83% of the licking-prevented group (6,969 hr*ng/mL), and the 90% CI for the ratio of means was (0.715, 0.972). The 90% CI for the ratio did not completely lie within the equivalence bounds of (0.80, 1,25); therefore, equivalence was not confirmed.

While equivalence between the groups could not be statistically confirmed, the small magnitude of the difference between mean AUC(2hr-t) for licking-allowed vs licking-prevented treatment would not be expected to be of toxicological significance. Oral exposure of animals to topically applied FTD by auto-licking does not appear to be an issue on the pharmacokinetic profile.

Profiles of GnRH-induced peak LH following Gonadorelin diacetate vs Buserelin treatment in lactating dairy cows

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The primary objective was to assess the GnRH-induced LH surge profile in dairy cows receiving two GnRH analogs given both at proestrus and diestrus phase. The secondary objective was to investigate whether season could alter LH surge profile.

Lactating Holstein cows at 108.2 ± 2.3 DIM, producing 41.5 ± 0.3 kg/day were randomized to receive, during proestrus and diestrus (7 days after ovulation): Cystoreline/Ovarelin® i.m. (n = 56; 2 mL, 100 mg of Gonadorelin diacetate tetrahydrate; Ceva Animal Health) or 2) Receptal® (n = 52; 2,5 mL, 10 mcg of Buserelin diacetate; MSD).

Data collection was done in two replicates (summer vs. winter) using different cows. Blood samples were collected at hour 0 (just before GnRH treatment), at 30 min, 1 h and then hourly until 5 h post-GnRH. Samples were immediately centrifuged and serum was frozen for later progesterone (P4) and LH measurement. Only cows having complete luteolysis (P4 < 1 ng/mL) during proestrus and with an active CL structure (P4 > 1 ng/mL) during diestrus were included in the analysis.

Statistical analysis was performed with the proc MIXED of SAS. There were no interactions between GnRH type and phase of the estrous cycle or season. Thus, peak LH concentrations (ng/mL) were not affected by type of GnRH (Cystoreline/Ovarelin = 6.2 ± 0.4 vs. Receptal = 6.7 ± 0.4; p = 0.37) or season (winter = 6.8 ± 0.4 vs. summer = 6.1 ± 0.4; p = 0.22), but were largely affected by phase of the cycle (proestrus = 8.2 ± 0.4 vs. diestrus = 4.7 ± 0.4; p < 0.01).

The repeated measures analysis showed a significant interaction (p < 0.01) between treatment and time, indicating that LH profiles were slightly different between GnRH products. Thus, Cystoreline/Ovarelin caused LH concentrations to rise faster, reaching highest concentration sooner (h) than Receptal (1.5 ± 0.1 vs. 2.3 ± 0.1; p < 0.01). As a result, cows receiving Cystoreline/Ovarelin had greater circulating LH concentrations (ng/mL) at 1h after GnRH treatment than cows receiving Receptal (4.2 ± 0.3 vs. 3.1 ± 0.3; p < 0.01). In contrast, cows treated with Receptal had longer (p = 0.01) intervals from peak until return to nadir compared to Cystoreline/Ovarelin. In conclusion, phase of the estrous cycle had a great impact on LH surge, but season and type of GnRH did not influence LH surge profile.

The ability of heterologous electrochemiluminescence immunoassay to detect progesterone in ovine plasma

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The progesterone (P4) is the principal hormone of reproduction secreted by the corpus luteum (CL) and by the placenta during gestation. Measurement of the progesterone concentration in the blood is a reliable indicator of the functional CL The objective of present study is the use of electrochemiluminescence immunoassay (ECL) method with the specific kit human progesterone for measuring progesterone in plasma ovine.

The experiment was conducted during the period of April 2012 in Bass Kabylie, Algeria. Eight females were divided into two groups:

Group 1 (n = 4, pregnant ewes confirmed by ultrasound)
Group 2 (n = 4, non-pubescent lambs and ewe in post-partum)

Blood samples from the jugular vein were collected in tubes EDTA-containing and centrifuged at 1500 rpm for 20 min. Plasma was obtained immediately by centrifugation after collection and stored at -20 °C until assay. The assay of P4 was carried out by a method of competition immunological revealed by electrochemiluminescence (substrate: ruthenium) on the automat Elecsys® 2010 (Roche diagnosis). The antibodies of capture and revelation are monoclonal specific to progesterone of human origin.

The results obtained in pregnant females have revealed high concentrations of P4. Plasma concentrations of P4 were very low in group 2. The progesterone ECL system was very sensitive (0.03 ng/mL). The coefficients of variation intra- and inter-assay calculated were very satisfactory (2.11% and 5.78%, respectively). The results of accuracy and parallelism were acceptable. The specificity shows no cross-reaction with different molecules tested (PMSG, GnRH, Ocytocine, PGF_{2alpha}). In conclusion, the heterologous ECL system could be a tool of progesterone assay for heat detection, monitoring of ovarian activity and even for the complementary pregnancy diagnosis in ewes.

A pre-study: short term effect of dietary boron supplementation at different doses on minerals' distribution of body fluids in dairy cows

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Aim of the pre-study was to obtain knowledge on boron supplemented feed in nutrition of dairy cattle. A total of 24 (6 control, 6 experimental I, 6 experimental II and 6 experimental III) healthy multiparous Holstein dairy cows with 3-3.5 body condition score were used. While the animals being feed with standard ration, boron at three different doses was added to experimentals' feeds (experimental 1: 60 ppm/kg, experimental 2: 120 ppm/kg and experimental 3: 180 ppm/kg) as boron compound: borax, Na-tetra borate, $Na_2B_4O_7.10H_2O$) for 10 days. Boron and other macro and micro minerals (Ca, P, Mg, Na, K, Mo, Co, Cr, Cu, Fe, Mn, Ni, S, Zn) were determined in serum, milk, urine and feces samples on 0 and 10^{th} days by ICP-AES.

In this pre-study, a pivotal knowledge was obtained in dairy cattle fed with boron supplemented feed on boron distribution (absorption and excretion) and its interaction with other minerals. Boron was not completely absorbed in gastrointestinal tract and partly eliminated by feces. Urine was the most important excretion way of boron. More less boron was also eliminated by milk. Boron levels were increasingly changed based on the dose. While these increases in samples of experimentals 1 and 2 were close, the increases in samples of experimental 3 were more dramatic in serum and urine samples. Boron, among other macro and micro minerals, provided an increase for only Ca and Mg levels in serum and urine samples.

Before investigation of the effect of boron on peripartum dairy cows' health, we possessed a knowledge on boron and its interaction with other minerals in the pre-study where dairy cows fed boron supplemented feed.

Recurrent keratoconjunctivitis with corneal abscesses in the sheep herd of an educational farm

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We report a series of cases of keratoconjunctivitis complicated with corneal abscesses on 4 groups of sheep coming from a educational farm situated in the vicinity of Paris (France). Cases appeared during the months of december, january and april on non productive animals of all classes of ages.

The clinical presentation of this ocular affection included severe ocular discharge, conjunctivitis, severe keratitis with corneal vascularisation (*panus crassus*), superficial corneal ulcers often complicated with epithelised linear corneal abscesses and mild to severe anterior uveitis. Lesions were unilateral or mainly bilateral. General clinical examination did not evidence any anomaly, while dysorexia and changes in behaviour was reported by farmers. Suppurative keratitis with superficial ulcers and anterior uvetitis were diagnosed by a portative slip-lamp ophthalmic examination.

The suspected causative organism was *Mycoplasma conjunctivae*, due to the epidemiology and the aspect of the lesions which are associated to this organism in "pink eye". Bacterial isolation (MF dot) and culture on corneal and conjunctival cotton swab failed to identify such a pathogen. Surinfection flora (*Pasteurella spp., Aerococcus urina,*) and a suppurative agent (*Staphylococcus lugdunensis*) were however identified.

Sheep were thus empirically treated by intramuscular injections of long lasting oxytetracycline 20 mg/kg (twice with 3-day interval) and meloxicam 0.5 mg/kg (3 times with one-day interval). Their hospital course revealed clinical improvement with notifiable vascular colonisation of corneal ulcers and abscesses, and improvement in corneal clearness. The therapeutic response obtained was good. Due to the severity of the lesions at admittance, fibrous corneal cicatrisation was inevitable and the corneal abscesses left scars after healing.

Advices were given to farmers concerning introduction of new animals in herd and systematic control procedure, and treatment plan was settled on farm. Empiric therapy is necessary for rapidly progressing suppurative keratitis, but a detailed examination of the causative organism is important for therapeutic planning, taking in account the fragility of the agent and transport media.

The detection of *Mycoplasma bovis* from cases of pneumonia in a cattle herd from the eastern region of Poland

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Mycoplasma bovis is an economically important pathogen for cattle. It causes substantial economic losses in cattle industry. The aim of the study was to evaluate the presence of *M. bovis* infection and concomitant mycoplasmal infections in the cattle herd affected with clinical signs indicating mycoplasma infection in the middle-sized herd from east region of Poland. There were 18 calves introduced to the herd 100 kg each. After seven days some of the animals started to have clinical signs indicating *M. bovis* infection i.e. pneumonia with nasal discharge, cough, dyspnea, apathy, increased body temperature and lack of appetite and swollen carpal and tarsal joints. Five animals have died while five other animals have not shown the signs of infection.

The blood samples from eight animals were tested for *M. bovis* antibodies using the indirect sandwich ELISA. The detection and identification of *M. bovis* was performed using the antigenic direct ELISA from nasal-pharyngeal swabs from all affected animals. *Mycoplasma* spp. were isolated by culturing in Eaton's medium. DNA extracts from swab samples were examined with the *M. bovis* specific PCR for the *uvrC* gene and for *Mycoplasma* spp. identification and differentiation (Subramaniam et al.) denaturing gradient gel electrophoresis (PCR/DGGE) was made.

The serological testing showed the presence of anti-*M. bovis* antibodies among seven of eight animals (87.5%). *Myc. bovis* antigen was detected in five of thirteen nasal swabs (38.5%) by the antigenic direct ELISA. The direct isolation and identification of *M. bovis* by culturing in Eaton's medium, as well as PCR and PCR/DGGE testing gave eight positive results (61.5%) with each method. The positive PCR results were confirmed by the sequencing. PCR/DGGE testing gave the additional information about co-infection with other mycoplasmas - In these animals, four animals calves were co-infected with *M. bovis* and *M. arginini* all had severe clinical findings, one calf had with *M. bovis* and *M. bovirhinis*; two animals had with *M. bovis, M. bovirhinis* and *M. arginini;* Two animals had were infected with *M. bovirhinis.* Only one animal was infected solely with *M. bovis* and two calves were free from *Mycoplasma* sp. infection.

Flow cytometry analysis in the calves experimentally vaccinated against *Mycoplasma bovis* infection

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Mycoplasma bovis is known as a causative agent of pneumonia, keratoconjunctivitis, arthritis and mastitis in cattle. Till now there is a lack of commercial vaccines against *M. bovis* infection in Europe.

The aim of the study was to evaluate the changes in peripheral blood lymphocytes in the calves vaccinated against *M. bovis* infection. The study was performed on twelve calves divided into two equal groups: experimental and control. The experimental calves were subcutaneously injected with the experimental vaccine composed of the field strain of *M. bovis* inactivated by saponin (Sigma, Poole). Lydium KLP (Nika, Health Products) was used as an adjuvant. The blood samples and nasal swabs were collected before the experiment, then in 24 hour intervals up to the 7^{th} day of observation and then each 7 days up to the 84^{th} day of the study. Nasal swabs were examined for *M. bovis* antigen, whereas specific antibodies to *M. bovis* were determined in the serum samples, using two different ELISAs (Bio-X Diagnostics, Belgium). Immunophenotyping of peripheral blood lymphocytes such as T-cells ($CD2^+$), T-helper cells ($CD4^+$), T-cytotoxic suppressor cells ($CD8^+$) and B-cells ($WC4^+$) were performed using flow cytometer (Coulter Epics XL 4C, Beckman Coulter Company, USA).

The results indicated a presence of specific antibodies to *M. bovis* in the vaccinated calves as early as the 14^{th} day post immunisation, whereas no *M. bovis* antigen was shown in this group throughout the study. The lymphocyte immunophenotyping demonstrated the $CD2^+$ cell stimulation in the vaccinated calves during the first days of the study. However the comparable or slight higher values of $CD4^+$ cells than the control were observed post immunisation. In experimental calves the $CD8^+$ cell percent was higher than the control throughout the study, whereas the $WC4^+$ cells were generally stimulated between the 2^{nd} and 84^{th} day post immunisation.

The expeimental vaccine effectively stimulated cellular immune response of the calves, especially dependent on T cytotoxic suppressor and B lymphocytes.

Effect of Flunixin on leather quality of cattle treated with a Finadyne Transdermal solution

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The study was conducted to evaluate the effect of Finadyne® Transdermal (FTD) on leather quality.

Cattle between 16 and 22 months old were treated once with FTD (3.3 mg/kg BW; [n = 20]) or placebo (Saline - NaCl 0.9% with red dye; [n = 10]). Treatments were administered on the dorsum along the backbone. Dandruffs without any local reaction or irritation of the skin were observed for all treated and untreated cattle within the first 6 days post-treatment.

Cattle were slaughtered 2 or 8 weeks post-treatment. Complete raw hides were processed into semi-finished leather using standard tannery procedures. Based on the mean leather thickness (1.52 ± 0.13 mm), ISO specifications for safety shoes, clothing and/or upholstery manufacturing were used for leather samples collected from the standard area (SA) and from leather samples collected from the dosing site (backline area: BLA).

For SA samples, 2 out of 10 (20%) placebo-leathers and 1 out of 20 (5%) FTD-leathers were out of the quality range (40-80%) for elongation at break, and 1 out of 20 (5%) FTD-leathers and no placebo-leathers were below the threshold value of 1.2 daN/mm^2 for tensile strength. For BLA samples, 7 out of 10 (70%) placebo-leathers and 5 out of 20 (25%) FTD-leathers were out of the quality range for elongation at break, and 5 out of 10 (50%) placebo-leathers and 5 out of 20 (25%) FTD-leathers were below the standard for tensile strength. For all SA and BLA samples, tear strength and grain distension were within specified ranges.

Based on results obtained from physico-mechanical characteristics of samples collected from SA and BLA, leathers from cattle treated with FTD were not considered of a lower quality as compared to placebo treated hides. Although a couple of FTD hides had a value from the SA outside the specified ranges, leather from FTD treated cattle would have been considered as suitable for, at least, safety shoes, clothing or upholstery manufacturing. The study showed that FTD administration did not induce local reaction, irritation, swelling or thickening of the skin neither alter its structure, and did not alter the quality of the leather surface and its mechanical resistance.

Respiratory pathogens detected in nasopharyngeal swab samples of Finnish calves

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Respiratory disease is the most common cause of clinical illness, medication, and mortality in growing calves all over the world. Finland is free of important viral respiratory pathogens bovine viral diarrhoea (BVD) and infectious bovine rhinotracheitis (IBR). *Mycoplasma bovis* was detected for the first time in 2012.

The Finnish Food Safety Authority Evira has offered a bovine respiratory disease package for practicing veterinarians since 2006. The package includes bacteriological and virological testing of nasopharyngeal swabs from 1-4 calves per parcel from one herd. The results of 295 parcels, altogether 1238 samples, submitted to Evira during 2006-2014 were analysed. Most of the parcels (58%) were from calf-rearing units, 39% from dairy herds and 2% from cow-calf herds.

Calves with acute respiratory signs and preferably before medication were sampled by vets on farms using a 27 cm long sterile, guarded swab (Medical Wire Equipment Ltd., Corsham, England). Samples were placed immediately in transport media (physiologic saline for bacteria and viruses and liquid mycoplasma D transport medium for mycoplasma and ureaplasma) and sent to the laboratory (Evira, Kuopio) in 24 hours.

Conventional aerobic, microaerophilic and anaerobic bacterial cultivation methods were used, *Histophilus somni* was identified using PCR. BRSV and BCV were detected with PCR. Mycoplasma and ureaplasma were cultivated in specific culture medium (in CO_2) and confirmed with PCR.

Results: A specific pathogen was detected in 77% of the samples and 91% of the parcels.

The most common bacterial pathogen *Pasteurella multocida* was detected in 50% of the samples and in 76% of the parcels. It was followed by *Ureaplasma diversum* in 33% of the samples and 61% of the parcels, BCV 34% and 51%, BRSV 13% and 24%, *H. somni* 6% and 14%, and *Mannheimia haemolytica* 4% and 11%, respectively. *Mycoplasma bovis* has been detected increasingly since its appearance in 2012 (2012: 2%, 2013: 5% and 2014: 7% of samples). *Trueperella pyogenes* and *Fusobacterium* sp. were isolated from less than 2% of samples and 9% and 5% of the parcels, respectively.

Conclusion: The most typical respiratory pathogen detected was *Pasteurella multocida*. The appearance of *Mycoplasma bovis* in Finnish calves is increasing.

Integral management of bovine parasitism in a rural practice

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It is no more possible to expect diagnose or manage parasitism in cattle herds without implementing minimum laboratory analyses. They are the necessary condition to advise customised anthelmintic prescriptions tailored to each herd specific features.

Are available analyses feasible within the veterinary office (mainly coproscopy) or in specialised laboratories (coproscopy, serology). For an infestation diagnosis, coproscopic analysis at office have to be preferred. They regard digestive and respiratory strongylosis and paramphistomosis. Within the framework of a cattle herd follow-up or the establishment of its parasitic status, at the time of a prescription assessment or a global Quality approach for parasitism management, we must resort to serologies (*Ostertagia* ODR [optic density ratio], *Fasciola* Elisa, serum pepsinogen).

Those laboratory analyses allow in the first instance to identify and quantify the parasitic risk in each herd.

Paramphistomum coproscopies, *Fasciola* serologies, *Ostertagia* ODR give a picture of the occurrence, the prevalence and the infestation pressure of those parasites. They also allow, after the implementation of control measures, to objectify their merit and efficiency.

The serum pepsinogen measurement has an incomparable usefulness. It allows to assess the gastro-intestinal strongyles burden at the end of the first grazing season and get a critical approach of the antiparasitic strategies implemented during that one. It is a natural and effective gateway for the practitioner wishing to manage the parasitism of a cattle herd. In our practice, that was the first complementary analysis we promoted. It allows not only to objectify the quality of the strongycid management during the last grazing season but also to define the more adapted housing treatment for the considered situation. The non use of antiparasitic macrolides at housing after the first grazing season allows to strengthen the immunity towards gastro-intestinal strongyles and lighten the necessity of treatments in the second grazing season. The impact towards the environment is strongly reduced.

We systematically suggest all those analyses for the cattle herds for which we globally manage the parasitism. From now on, these are 52 herds who represent a third of the medium and large size herds of our practice.

Bovine respiratory syncytial virus (BRSV) protection provided by maternal antibodies from cows immunized with an inactivated BRSV vaccine

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Bovilis® Bovigrip (MSD Animal Health), against a BRSV challenge in young calves. Briefly, pregnant cows without or with low levels of ELISA and neutralizing BRSV antibodies were allocated in two groups.

The first group was vaccinated twice with Bovilis® Bovigrip according manufacturer's recommendations, the last immunization being at one month prior to calving.

The control group was not vaccinated. Vaccination was followed by a rapid increase of BRSV ELISA antibodies after the first immunization whereas neutralizing antibodies were detected only after the second immunization. At birth, the colostrum of each cow was collected and stored at -20°C.

Then twenty-seven newborn calves were fed individually in the 6 hours following birth, with 4 liters of colostrum sourced from vaccinated cows (vaccinated-colostrum, 14 calves) or not (13 calves). Overall the vaccinated-colostrum intake was followed by detection of high levels of BRSV antibodies in sera of new born calves. At 21 +/- 3 days of age, calves were challenged by intranasal and intratracheal routes with 2 x 10^5 $TCID_{50}$/calf of the BRSV strain 3761.

Clinical and histopathological examinations indicated a partial but significant protection of calves with maternal antibodies. This protection was correlated with reduced BRSV detection in the lower respiratory tract but not in nasal secretions, indicating only a partial virological protection of the calves by vaccination of pregnant cows. Finally interference of maternal antibodies with the active response was assessed by transcriptomic analyses of more than 60 bovine genes involved in both innate and adaptative immune response. This was done at the respiratory level by investigating host response in bronchoalveolar lavages collected before and at days 3, 6, 10 and 15 post-challenge.

Results will be discussed.

Characterization of sensitivity of bovine Endometrial Epithelial Cell (bEEC) to Bovine Herpes Virus type 4 (BoHV4)

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BoHV4 is a double-stranded DNA virus which has been associated with endometritis, metritis, and abortions in cattle. The objective of this study was to characterize the sensitivity of bEEC to BoHV4.

Bovine uteri were collected from slaughter house and bEEC separated and cultivated.

In Experiment 1 bEEC were cultivated for 6 days without virus or following exposure to serial BoHV4 MOI; 0.001, 0.01, 0.1, 1 and 10. Living cells were counted at start of the experiment and by Day 6.

In Experiment 2 cells were challenged with a single BoHV4 MOI (0.1) and cultured for 7 days. Living cells were counted at Day 0 and each day following infection. In both experiments the proliferation profiles of living bEEC were calculated as; number of attached cells for a given condition or day - number of cells on Day 0 / number of cells on day 0. Titration, PCR and Immunofluorescence techniques were developed to evaluate the proliferation of the virus as well.
In Experiment 1 the amount of living cells by Day 6 was significantly increased in controls when compared to Day 0 ($p < 0.0001$). A linear inhibition of cell proliferation was observed with increasing dose of BoHV4.

In Experiment 2 we observed a very strong increase of proliferation from Day 0 to Day 7 in controls ($p < 0.0001$). From Day 1 to 4, the increase in number of cells was similar for infected cells and controls. However, after Day 4, cells exposed to the virus had a limited proliferation and number of living cells were significantly lower in infected group than in controls by Day 7.

Results from titration and PCR showed similar viral replications profile with increase starting by the following day after challenges. Viral particles stained by immunofluorescence increased also from Day 1 to Day 7.

In conclusion both dose of BoHV4 and time affects the proliferation of bEEC in a repeatable way. These results support the implication of BoHV4 in clinical cases of metritis/endometritis and will be used to further characterize the response of bEEC to BoHV4 in molecular studies.

Aborted fetuses and precolostral calves as sentinels for identifying pathogen circulation in bovine herds

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In cattle, many pathogens are able to cross the placenta and infect the fetus. All these infections do not lead to the death of the latter, especially when they occur later in pregnancy, at a time when the fetus is able to develop an immune humoral response.

On the contrary, some pathogens, such as Bovine Respiratory Syncytial virus (BRSv) or Bovine Rotavirus (BRv), widespread in cattle, are deemed not induce viremia and therefore cannot infect the fetus *in utero*. The latter therefore are born without antibodies against these two viruses.

The objective of this work is to demonstrate that the serological analysis of dry blood spots from aborted fetuses and newborn calves before colostrum intake allows to support the existence of such infections while confirming the precolostral status of the sample analyzed.

The blood of 278 aborted bovine fetuses at ninth month of pregnancy (and thus considered as precolostral newborn calves) was analyzed for *Bovine Viral Diarrhea* virus (BVDv), *Schmallenberg* virus (SBv), *Neospora caninum* and *Coxiella burnetii* antibodies detection with commercial ELISA tests. The samples were also submitted to a commercial serological ELISA test for BRSv and BRv to confirm the precolostral status of these aborted fetuses.

Most of samples were unreactive to all tested pathogens while some samples showed a single positive response to *Neospora* or BVD or *Coxiella*. Unexpectedly, some other specimens were simultaneously positive against numerous pathogens, including BRSV and/or BRV. Discrepant results are discussed, based on the anatomical findings at autopsy of the fetus and the determination of IgG1 immunoglobulins in their blood. These results allow to propose a monitoring approach for many pathogens at a herd level.

In conclusion, these inexpensive serological tests can demonstrate the active presence of the pathogen into the herd without necessarily seeking the pathogen itself. The use of serology in precolostral calves and aborted fetuses is an effective means to conduct of epidemiological surveillance applicable at both herd and/or country level.

New horizons in the etiological diagnosis of bovine abortions: the contribution of 16S rRNA sequencing

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Failures in bovine reproduction due to abortions cause heavy economic losses in this sector. Although the determination of the cause of abortion is often difficult, an etiologic diagnosis is necessary to develop appropiate sanitary measures. Since 2010, more than 4000 cases of bovine abortions were submitted annually to the laboratory of the Regional Association of Identification and Animal Health (ARSIA) through the surveillance of bovine brucellosis. These aborted fœtuses were all submitted to a standardized panel of analysis covering a large spectrum of pathogens. In this study, the direct involvement of at least one pathogen was demonstrated in half of analyzed fœtuses, suggesting an important progress marging in the diagnostic efficiency. In this perspective, a method to identifying bacteria that are difficult to isolate by classical culture methods (intracellular or slow growing species) or non-viable germs (due to an antibiotic treatment or a poor sample conservation) has been prospected.

16S rRNA sequencing is an "universal" approach and does not require targeting bacteria with specific primers in order to detect them. Therefore, the application of this technique within the abortion surveillance protocol would increase the sensitivity and thus reduce the proportion of unresolved cases. This analysis was performed on 100 abortions cases in 2014 where the etiology was known or unknown. Indeed, some abortive pathogens that require a particular growing medium were identified by 16S rRNA sequencing in the absence of a positive result on culture. This technique has broadened the etiologic diagnosis and opens new horizons in the diagnosis of abortion cases.

Genetic analysis of Foot-and-Mouth Disease virus serotypes A/O/SAT2 in Egypt during 2013-2014

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Foot-and-mouth disease virus (FMDV) serotypes A, O and SAT-2 was endemic in Egypt. Several focuses were recorded in Egypt during 2013-2014. As FMDV highly mutated virus so it is highly suggestive in endemic countries to follow up the circulating viruses in the field to assure the vaccine efficacy used in Egypt.

In this study epithelial tissue samples were collected from cattle and buffaloes showed clinical signs suspected to be FMD, from six governorates (Cairo, Qaliubia, Giza, Fayoum, sharqeia and Assiut). The collected samples were serotyped by RT-PCR and the complete VP1 coding regions in the PCR products of positive samples were sequenced.

The results confirmed the presence of three serotypes (A, O and SAT-2) of FMDV co-circulating in Egypt. Sequencing and phylogenetic analysis of VP1 further confirmed emergence of the East Africa-3 topotype (EA-3) of serotype O. Serotype O sequence was closely related to O/SUD/8/2008 with identity 93%, but differ from vaccinal strain (O/PanAsia-2) of ME-SA topotype by 14.6%. Mean while Serotype A and SAT-2 were closely related to recent Egyptian isolates and vaccinal strains type A/ EGY/1/2012 (Asia topotype, lineage Iran 2005) with identity 96.4% and vaccinal strain of SAT-2/EGY/9/2012 (topotype VII, lineage SAT-2/VII/Ghb-12) with identity 92% respectively. Emergence of new topotypes of FMDV may require a change of vaccine production strategy.

The present study recommended further studies for serotype O to confirm the immunogenic relationship between the vaccinal strain and the emerging new strains to provide maximum protection against circulating viruses.

Sero-surveillance of Foot-and-Mouth Disease Virus Serotypes A/O/SAT2 in Egypt 2013-2014

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Foot-and-mouth disease (FMD) is a highly contagious disease affecting cloven hoofed animals causing huge economic losses. FMDV serotypes (A, O and SAT-2) are endemic in Egypt. During 2013-2014, several FMD focuses were reported in livestock in different governorates in Egypt.

To investigate the current status of FMDV infection in Egypt, a cross-sectional serological survey was conducted between October-2013 till July-2014 in 10 Egyptian Governorates (Cairo, Qaliubia, Giza, Alexandia, Behaira, Gharbia, Kafer El-Sheikh, Fayoum, Sharquia and Assiut). A total of 321 serum samples were collected from non-vaccinated animals in FMD suspected foci to determine the sero-prevalence and distribution of FMD serotypes. The samples were screened against the three FMDV serotypes circulating in Egypt (A/EGY/1/2012, O/EGY/4/2012 and SAT-2/EGY/9/2012) by using serum Neutralization Test (SNT).

Results revealed that all three serotypes were circulating in all examined Governorates and the more prevalent serotype was SAT-2 (64.1%) followed by serotype O (61.9%) and serotype A (55.8%). The period of the study indicated that serotype A was more prevalent from October to December, SAT-2 more prevalent from January to May while serotype O started to increase till July. In relation to age, both cattle and buffalos less than 2 years old are more susceptible to FMD. Buffaloes showed high sero-positivite than cattle to serotype A, however no significant differences between cattle and buffalos was observed in serotype O and SAT-2.

The study concluded that there is species difference and age susceptibility to different FMDV serotypes in studied groups.

Intrauterine cephapirin infusion is associated with better reproduction performance in cows with purulent vaginal discharge and cytological endometritis

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The objectives of this study were to quantify the impact of an intrauterine infusion of cephapirin on the reproductive performance at first service of *postpartum* dairy cows affected by purulent vaginal discharge (PVD) or cytological endometritis (ENDO) using different diagnostic strategies and to determine if the presence of prolonged anovulation would influence the magnitude of treatment benefit. A total of 2259 Holstein cows in 28 herds were enrolled in a randomized clinical trial.

At 35 (± 7) days in milk (DIM), cows were diagnosed for PVD (purulent vaginal discharge or worse using the metricheck device) and ENDO (≥ 6% polymorphonuclear cells using the cytobrush technique or at least small amounts of leukocytes using the leukocyte esterase colorimetric test). Regardless of reproductive tract disease status, cows were randomly assigned to receive an intrauterine cephapirin infusion or no treatment. Serum progesterone was measured at 35 and 49 (± 7) DIM (14 days apart); cows were considered to have prolonged anovulation if progesterone was < 1 ng/mL at both times.

Statistical analyses were conducted using multivariable mixed logistic regression models adjusted for confounders and herd clustering effect. Intrauterine cephapirin treatment was associated with an increased first service pregnancy risk in cows diagnosed with PVD (no treatment: 15.4%; treatment: 31.4%) and ENDO (cytobrush: no treatment: 16.2%, treatment: 24.4%; leukocyte esterase: no treatment: 15.8%; treatment: 25.1%), but not in cows unaffected by any form of reproductive tract disease (no treatment: 34.8%; treatment: 32.6%). The impact of cephapirin treatment in anovular cows (no treatment: 21.0%; treatment: 26.4%) was numerically lower than in cyclic cows (no treatment: 22.7%; treatment: 34.1%). Overall, an intrauterine infusion of cephapirin improved first service pregnancy risk in cows with *postpartum* reproductive tract disease and this effect was influenced by *postpartum* anovulation status.

Field efficacy of a new ceftiofur plus ketoprofen combination in the treatment of interdigital dermatitis in cattle

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Introduction

A field study has been conducted to assess the efficacy of two 50 mg/mL ceftiofur-based products, Excenel RTU (Zoetis) and Curacef Duo (Virbac), the latter being also composed of 150 mg/mL of ketoprofen, in the treatment of naturally acquired cases of bovine interdigital dermatitis (BIDD).

Materials and methods

181 dairy cattle with BIDD from 12 sites in France and UK were enlisted in this study. A total score (TS : 0 to 18) combining scores of General condition, Lameness, Pain, Inflammation, Lesions and Rectal temperature was assessed twice daily on Day 1 to 3, then daily on Day 4 and 14 (± 1 d). Animals were injected with either ceftiofur 1 mg/kg SC. o.d. for 3 consecutive days (group EXC), or a combination of ceftiofur (1 mg/kg) and ketoprofen (3 mg/kg) IM o.d. for 1 to 3 days (group CUR). If TS ≤ 3 on Day 2 or 3, thus the NSAID was no longer needed and the treatment was substituted by the ceftiofur only-based product, providing a full 3-days treatment course.

On Day 1 before treatment and at study completion (Day 14 ± 1 d), a deep swab of the interdigital lesion was collected from each animal. Samples were cultured for isolation of target pathogens *Fusobacterium necrophorum, Bacteroides melaninogenicus*.

Treatment was a clinical success when animals completed the treatment phase study with TS ≤ 2 on Day 14 (± 1 day).

Results

Reactions at injection site were significantly more severe ($p < 0.05$) in group EXC than in CUR over almost all the study period.

TS tended to be better in CUR than in EXC ($p = 0.068$) at 6 and 24h after treatments started; TS differed significantly at 30 h ($p \leq 0.05$). There was no statistical difference in the odds of clinical success between group CUR and EXC (63.3 vs 57.1%, respectively).

There was no significant difference in the odds of bacterial cure for *F. necrophorum* between the two groups. *B. melaninogenicus* was never found.

Conclusions

The study demonstrated that the ceftiofur + ketoprofen combination induced a significantly faster relief of the clinical signs associated to footrot, as defined by a combination of all scores, and the pain, locomotion and lameness.

Field efficacy of a new ceftiofur plus ketoprofen combination in the treatment of respiratory disease in cattle

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Introduction

A field study has been carried out to assess the efficacy of two 50 mg/mL ceftiofur-based products, Excenel RTU (Zoetis) and Curacef Duo (Virbac), the latter being also composed of 150 mg/mL of ketoprofen, in the treatment of naturally acquired cases of bovine respiratory disease (BRD).

Materials and methods

176 calves with BRD from 12 farms in France and Germany were given either ceftiofur 1 mg/kg SC o.d. for 3 to 5 days (group EXC), or a combination of ceftiofur (1 mg/kg) and ketoprofen (3 mg/kg) IM o.d. for 1 to 5 days (group CUR). A total score (TS : 0 to 7) combining respective scores of General condition and Respiratory score was assessed twice daily as well as Rectal temperature (RT). If RT ≤ 39.4°C and TS ≤ 1, thus the NSAID was no longer needed and the treatment was substituted by the ceftiofur only-based product, providing a 3 full days of antimicrobial treatment being completed at least. The treatment was stopped when RT ≤ 39.4°C and a TS = 0.

The clinical success was declared in animals displaying RT<39.4°C and normal breathing at study completion (5 ± 1 days after the last injection).

Before treatment on Day 1 and at study completion, a deep nasal swab was collected from each animal and processed to isolate target pathogens *Mannheimia haemolytica, Pasteurella multocida*.

Results

Both treatments were well tolerated (mild swelling without pain). The mean treatment duration was 4.25 and 4.36 days for CUR and EXC groups respectively (n.s.).

At time points 12, 24 and 36 h after the onset of treatment, RT was significantly lower ($p < 0.05$) in the CUR group than in the EXC group.

The clinical success rate was significantly higher ($p < 0.01$) in group CUR (67%) than in EXC (48%).

Bacteriological cure was higher (n.s.) in group CUR than in group EXC (75% vs 67% for *M. haemolytica*, 45 vs 33% for *P. multocida* respectively).

Conclusions

This study found that the ceftiofur/ketoprofen fixed combination is more effective than the ceftiofur alone for the control of BRD in calves. The relief of pyrexia comes faster and a higher rate of clinical success can be expected.

Effect of the treatment with Eprinomectin on milk yield in dairy herds

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Infection with gastro-intestinal strongylids (GIS) has been reported to reduce milk yield in dairy cows. Therefore, strategies to treat dairy herds have been established. On the other hand, regular use of medicaments is known to potentially contribute to the development of drug resistance.

The goal of this study was to evaluate the effect of a targeted anthelmintic herd treatment at the beginning of the confinement period on milk yield. Furthermore, a strategy basing the decision of treatment on an *Ostertagia* ELISA and herd data was validated.

Bulk tank milk samples of dairy herds were tested with the Svanovir® *O.ostertagi*-Ab ELISA. A short questionnaire on herd data (average milk yield, duration of pasture, occupation density) was filled. Based on all these criteria, herds were classified into 4 groups: low, medium, high and very high probability of success of the therapy. The access to electronic production data and the precise date of treatment had to be given. The herd had to have at least one milk performance test in the confinement period prior to treatment. Cows which changed lactation during the analysis period were excluded.

A total of 158 herds (77 treated vs 81 non-treated) fulfilled all the inclusion criteria. The average daily milk yield (DMY) of the population was 29.5 kg. In the groups high and very high, treated herds showed an increase of 1.6 kg DMY after treatment, whereas non-treated herds showed a decrease of -0.8 kg DMY during the same period. In the groups low and medium, treated herds showed an increase of 0.3 kg whereas non-treated herds showed a decrease of -0.2 kg DMY.

The results of our study indicate that the targeted treatment of dairy herds with Eprinomectin at the beginning of the confinement period has a beneficial effect on milk yield. Furthermore, the strategy using an ELISA for *O. ostertagi*-Ab in the bulk milk plus herd data is effective for discriminating herds with high and low probability of success of the therapy. Although we did not perform a formal test for resistances, results of this study suggest that Eprinomectin is efficient under Swiss conditions.

A case of pelade or *alopecia areata* in an 4 ½ year-old Holstein cow

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We describe here a case of pelade or *alopecia areata* in a Holstein cow. Pelade is a rare chronic autoimmune dermatitis in cows. It has been described in purebred Holstein, Angus and Eringer cows, but also in humans, dogs, cats and mice. The exact aetiology of this disease is still not well-defined. This condition affects anagen hair follicles. There are no inflammatory clinical signs but the presence of inflammation is noted histologically. In our case, the generalised alopecia occurred following a chronic extension of multiple annular-to-oval areas of alopecia. The primary location was the head without any associated pruritus. The exposed skin appeared normal and the cow was otherwise healthy. These symptoms affected only one cow in the herd. After a few months of evolution, the cow became almost completely "naked", and dandruff appeared on the dorsal line of the back and neck. The differential diagnosis of this condition included black hair follicular dysplasia, effluvium, and *alopecia areata,* less likely copper deficiency. Due to the clinical presentation, dermatophytosis, dermatophilosis, staphylococcal folliculitis, besnoitiosis, were considered but were not likely.

Skin scrapings and bacteriological cultures did not evidence any infectious or parasitic agents. Final diagnosis was given by histopathological examination of skin biopsies. No effective treatment exists. Local treatment using topic corticosteroids has been tried, without any positive result. From the beginning of the case until the end, general condition of the animal remained good. Evolution did not show any hair growth.

Marbofloxacin activity against *Escherichia coli, Staphylococcus aureus* and *Streptococcus uberis* strains isolated from bovine mastitis: *in vitro* Pharmacokinetic/Pharmacodynamic testing of a 10 mg/kg one shot administration

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Background

Mastitis is a leading cause of important economic losses in dairy industry. This disease involves different pathogens of which *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus uberis* are frequently encountered. The aim of the study was to determine the effect of a one shot administration of marbofloxacin, at a dose of 10 mg/kg against the three most prevalent mastitis pathogens using an *in vitro* dynamic system reconstituting the drug concentration-time profile obtained *in vivo* in milk after treatment. The impact of that treatment on the potential development of resistance was also evaluated.

Methods

Pharmacokinetics were determined in ten lactating cows treated by a single intramuscular or intravenous administration of a 16% marbofloxacin solution at a dose of 10 mg/kg (Forcyl®). The concentration-time profile of the drug obtained in milk was then reconstituted in an *in vitro* system. Dynamic killing curves were assessed in milk against two susceptible isolates of *E. coli* with a marbofloxacin MIC of 0.03 (MIC_{90}) and 0.5 µg/mL (close to the susceptibility clinical breakpoint). Two isolates of *S. aureus* with a marbofloxacin MIC of 0.25 (MIC_{50}) and 0.5 µg/mL (MIC_{90}) and one isolate of *S. uberis* with a MIC of 1 µg/mL (MIC_{90}) were also tested.

Results

The time kill curves showed a bactericidal concentration and time dependent activity of marbofloxacin against *E. coli* and *S. aureus* strains with a MIC of 0.03 and 0.25 µg/mL respectively. For both *E. coli* and *S. aureus* isolates with MIC of 0.5 µg/mL, a transitory bactericidal effect was observed for up to 12h depending on the isolate. For *S. uberis* isolate, a bacteriostatic effect was observed up to 12h. None of *E. coli* and *S. aureus* strains developed resistance against marbofloxacin and the proportion of resistant *S. uberis* isolates remained very limited (0.01% at 24 h).

Conclusions

A one shot marbofloxacin treatment at a dose of 10 mg/kg could eradicate *E. coli* and *S. aureus* isolates and limit the growth of *S. uberis* strains in case of mastitis. These results may open new options for treatment adaptation after bacteriology testing and targeted antimicrobial use.

Comparison of two protocols for the treatment of clinical mastitis in dairy cattle

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The aim of this study was to evaluate the efficacy of treatment of clinical mastitis in a herd of dairy cows by comparing the approach historically used in breeding towards an integrated approach in the diagnosis, treatment, recovery and verification of any reprocessing. The study was carried out in a large dairy cow farm located in Rome. Following a 8-months study period, a total of 77 cows with clinical mastitis were enrolled in the study and divided into one of the two groups (Group A and Group B). The animals assigned to Group A were treated according to the traditional approach used in herd, while the animals assigned to Group B were treated in a integrated approach. A pretherapy milk sample was collected from the affected quarter for research of mastitis organisms. At 14/17 and 21/24 days after the end of treatment, milk samples from each cow were analyzed for bacteriological status and somatic cell count (SCC).

Escherichia coli was the predominant microorganism isolated (28/77; 36.4%) followed by environmental streptococci (23/77; 29.9%), mainly *Streptococcus uberis* and *Streptococcus dysgalactiae*.

Staphylococcus aureus was isolated from 7 (9.1%) samples while in 13 (16.9%) samples were isolated other pathogens. The proportion of clinical and bacteriological cure was higher ($p < 0.05$) in Group B (31/38 - 78.9%) than in Group A (24/39, 61.5%). In particular, a better response to treatment of mastitis caused by in *Escherichia coli* was observed in Group B where the rate cure (15/17, 88.2%) was significantly higher ($p < 0.05$) than Group A (6/11, 54.5%). The SCC was lower in Group B than in Group A at 3 wk after treatment (5.852 vs 5.499 $\log_{10}$ SCC; $p = 0.06$).

The results indicate that the use of an integrated therapeutic approach can improve outcomes of clinical mastitis treatment. Moreover, use of on-farm written protocols for mastitis treatment promotes a judicious use of antimicrobials. This study underline the need for a longer period of study to evaluate bacteriological, clinical and SCC outcomes of greater number of pathogen species isolated of clinical mastitis.

Evidence and detection of shedding patterns of *Mycobacterium avium susbspecies paratuberculosis* in dairy cattle

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In herds infected by *Mycobacterium avium subspecies paratuberculosis* (Map), the early detection and culling of animals which are likely to be persistent heavy shedders could be a key factor for limiting Map transmission. The literature provides very few data on shedding by infected cows over a long period.

Therefore, the objective of this study was to describe the heterogeneity of shedding *Map* over time in cattle to characterize different shedding patterns of interest.

To reach this goal, a one year longitudinal follow-up was performed in 22 French dairy herds located in Normandy known to be infected (> 8% of seroprevalence among lactating cows on the previous year). Within each herd, cattle (> 12 months) were sampled four times every 4 months. At each sampling time, feces and blood samples were collected to be tested using respectively qPCR and blood ELISA. A similar approach was conducted on a subsample of 200 cows (selected on the basis of previous PCR results) to describe the shedding pattern on a 7-days period (3 samples within a week). The results were described for each sampling time and over time to identify shedding pattern, based on both qualitative (negative vs positive) and quantitative approaches among positive samples ($Ct < 35$ or $35 \leq Ct < 40$). In a second step, we investigated the possibility to discriminate these longitudinal shedding patterns on a single sampling time based on the combination of both qPCR and serology.

Finally, 1137 Hosltein and Normandes cows were sampled. Among them, 6% were considered as persistent heavy shedder while 18% were considered as frequent heavy shedder. Only 13% of the studied cows never shed (only negative qPCR result) during the study period. Consistent shedding patterns were observed within a 7 days period. To discriminate heavy persistent shedder from intermittent shedder, a combination of a single positive qPCR result on feces ($Ct < 35$) associated with a concomitant blood ELISA positive result (with a high OD) was highly specific and allowed to identify around 50% of heavy persistent shedding cows.

A single injection of BTVPUR ALSAP serotype 4 protects sheep against a virulent BTV-4 challenge for at least one year

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Introduction

Bluetongue virus (BTV) causes an infectious, non-contagious, disease in wild and domestic ruminants. It is transmitted between ruminants through the bites of certain species of Culicoides. Transmission occurs mainly in late summer and fall in Europe, when climatic conditions are favorable and adult insects are active. However, the recent BTV outbreaks in Northern Europe have demonstrated that transmission can occur throughout the year.

Here, we demonstrated with a vaccination/challenge study in sheep that a 1-shot vaccination with an inactivated vaccine containing purified BTV serotype 4 (BTV-4) provided a full protection for a period of 1 year.

Material and methods

Two groups of 8 BTV naïve sheep were maintained in an insect-proof facility throughout the experiment.

- Group A was vaccinated once with 1 mL of a BTV-4 vaccine formulated at low antigen dose,
- Group B was not vaccinated and served as control.

Twelve months after vaccination, Groups A and B underwent a BTV-4 virulent challenge.

After the challenge, all animals were monitored for rectal temperature, clinical signs and viraemia (qRT-PCR) over 14 days.

Results

Two controls did not present any sign of BTV infection while the 6 others were all severely affected. One of them was euthanized on ethical ground. In contrast, none of the vaccinates showed clinical signs.

Maximal rectal temperatures were significantly lower in the vaccinated group (mean: 39.6°C) as compared to the control group (mean: 40.8°C).

Clinical scores were significantly lower in the vaccinated group (mean: 0.0) as compared to the control group (mean: 35.0).

Viraemia was detected in 6/8 controls (high copy numbers) at almost all time points. None of the vaccinated animals was ever detected positive.

Conclusions

These results demonstrated that 1-shot vaccination of sheep with BTVPUR ALSAP serotype 4 provides full clinical and virological protection against a BTV-4 for at least 12 months.

These results are comparable to those obtained with other vaccines of the BTVPUR ALSAP range and show that BTVPUR ALSAP 4 may be used for bluetongue disease prevention (clinical protection) and for epidemiological control (virological protection) of BTV.

A single injection of BTVPUR ALSAP serotype 2 protects sheep against a virulent BTV-2 challenge for at least one year

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Introduction

Bluetongue virus (BTV) causes an infectious, non-contagious, disease in wild and domestic ruminants. It is transmitted between ruminants through the bites of certain Culicoides species. Transmission occurs mainly in late summer and fall in Europe, when climatic conditions are favorable and adult insects are active. However, the recent BTV outbreaks in Northern Europe have demonstrated that transmission can occur throughout the year.

Here, we demonstrated with a vaccination/challenge study in sheep that a 1-shot vaccination with an inactivated vaccine containing purified BTV serotype 2 (BTV-2) provided a full protection for a period of 1 year.

Material and methods

Two groups of BTV naïve sheep (7 vaccinates, 8 controls) were maintained in an insect-proof facility throughout the experiment.

- Group 1 was vaccinated once with 1 mL of a BTV-2 vaccine formulated at low antigen dose,
- Group 2 was not vaccinated and served as control.

Twelve months after vaccination, Groups 1 and 2 underwent a BTV-2 virulent challenge. After the challenge, all animals were monitored for rectal temperature, clinical signs and viraemia (qRT-PCR) over 14 days.

Results

After challenge, specific clinical signs were observed in all controls. One control presenting severe Bluetongue disease was euthanized for welfare reasons.

In contrast, none of the vaccinates showed significant clinical signs.

Maximal rectal temperatures were significantly lower in the vaccinates (mean: 39.6°C) as compared to the controls (mean: 41.7°C).

Clinical scores were significantly lower in the vaccinates (mean: 0.6) as compared to the controls (mean: 32.8).

Viraemia was detected in all controls (high copy numbers), at all time points. None of the vaccinated animals was ever detected positive.

Conclusions

These results demonstrated that 1-shot vaccination of sheep with BTVPUR ALSAP serotype 2 provides full clinical and virological protection against a BTV-2 for at least 12 months.

These results are comparable to those obtained with other vaccines of the BTVPUR ALSAP range and show that BTVPUR ALSAP 2 may be used for bluetongue disease prevention (clinical protection) and for epidemiological control (virological protection) of BTV.

Use of analgesic combination Morphine-Lidocaine-Ketamine in Holstein Calves undergoing ventral midline herniorrhaphy

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Abdominal surgery is commonly performed in cattle for both diagnostic and therapeutic purposes. However, pain evaluation in ruminants is difficult, and recent research suggests that assessment of pain in ruminants requires measurement of both physiologic and behavioral parameters. This study assessed post-operative pain and compared two analgesic protocols in calves undergoing abdominal surgery.

Twenty-one calves presenting for umbilical herniorrhaphy were randomly assigned to one of two treatment groups:

- BAN: Flunixin meglumine 1.1 mg/kg IV following intubation and at 24 hours post-op,

- MLK: Co-infusion of morphine (4.75 mcg/kg/hr), lidocaine (2.11 mg/kg/hr) and ketamine (0.42 mg/kg/hr) for 24 hours beginning immediately following intubation. Co-infusion was discontinued at 24 hours.

A pain scoring system, as well as an algometer to measure incisional pain, were used by one blinded evaluator to assess comfort at 14 time points during the 5 day study period. There were no significant differences in heart rate, respiratory rate, or pain score between groups during the study period or during CRI administration. Incisional algometry scores were significantly different between groups during the CRI administration, with cattle in the MLK group having higher nociceptive thresholds than cattle in the BAN group ($p = 0.019$). During the entire study period, there was not a significant difference between groups, however there was a trend towards higher thresholds in the MLK group ($p = 0.098$).

Serum cortisol values were not significantly different between groups over the study period ($p = 0.390$). However, significant differences were noted between groups during the CRI administration ($p < 0.001$), with MLK animals having higher serum cortisol during this period than BAN animals. Additionally, time is a significant factor in cortisol concentration ($p = 0.001$), with cortisol tending to decrease over time, and increase during periods of more intensive handling.

In conclusion, we found that pain scores were similar among groups both during the CRI administration and during the entire study period, and that cortisol and incisional algometry scores were significantly different between groups during the CRI administration.

Effect of shortening a synchronization protocol with PRID® Delta (PRID-5d vs PRID-7d) for first artificial insemination (AI) in heifers on reproductive performance during the breeding season

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The objective of this study was to compare the use of two different synchronization protocols (PRID for 5d vs 7d) applied at the beginning of the breeding season in Holstein heifers.

A total of 434 Holstein heifers, kept in 4 pasture-herds were randomized to receive two synchronization protocols for 1st AI:

- PRID5d: D0-8:00 am = progesterone releasing intravaginal device insertion (PRID Delta, Ceva Animal Health) plus 100 mcg of GnRH (gonadorelin) i.m. (Ovarelin®/Cystoreline®, Ceva Animal Health); D5-8:00 am = PRID removal and treatment with 25 mg of PGF2a (dinaprost) i.m. (Enzaprost®, Ceva Animal Health); D6-8:00 am = treatment with a second dose of 25 mg of PGF2a i.m.; D8-8:00 am = 100 mcg of GnRH i.m. plus AI,

- PRID7d: D0-8:00 am = PRID insertion plus 100 mcg of GnRH i.m.; D7-8:00 am = PRID removal and treatment with 25mg of PGF2a i.m.; D9-4:00 pm = 100 mcg of GnRH i.m. plus AI.

After this first timed AI, heifers were placed on the same pasture with cleanup Holstein bulls in the estimated ratio of 1 bull to 25 non-pregnant heifers. Pregnancy diagnosis was performed by ultrasound at intervals of 30 days throughout the breeding season that lasted 12 weeks.

Statistical analysis was performed with proc Glimmix and proc Lifetest of SAS. There was a significant effect of herd on proportion of pregnant heifers from weeks 3 to 6 of the breeding season. Thus, heifers that received the PRID5d protocol for 1st AI had an increased proportion of pregnancies at week 3 (PRID5d = 69.9% vs. PRID7d = 57.8%; $p = 0.01$), 6 (PRID5d = 91.4% vs. PRID7d = 81.1%; $p < 0.01$), but not at week 9 (PRID5d = 99.8% vs. PRID7d = 94.7%; $p > 0.10$), or 12 (PRID5d = 100.0% vs. PRID7d = 99.8%; $p > 0.10$).

The survival analysis indicated that heifers on PRID5d attained the 75% pregnancy mark 2 weeks sooner than PRID7d during the breeding season. In conclusion, heifers enrolled in the shorter progesterone-based timed AI protocol for 1st AI (PRID5d) conceived faster during breeding season, anticipating calving and allowing heifers to start milking sooner.

Effectiveness of tildipirosin and tulathromycin in the control of bovine respiratory disease in high risk beef heifers

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Bovine respiratory disease (BRD) is the primary health problem in the beef cattle industry worldwide, and has serious animal welfare impact and causes economic loss. The Italian beef system is based on fattening young calves imported from abroad, especially from France. Cattle are inevitably subject to stressful transport conditions, which last on average 8 to 12 hours. Even under excellent management conditions and administration of well-designed immunization protocols, preventive antimicrobial treatment is often required to reduce morbidity and mortality due to BRD in high-risk cattle.

The purpose of this study was to evaluate the effectiveness of tildipirosin (TIP) and tulathromycin (TUL) administered to newly received beef heifers at high risk to develop BRD for reducing morbidity and mortality under field conditions.

Also, determine whether there are differences in the incidence and severity of lung lesions. A total of 785 Chalolais heifers (Age = 11.1 ± 1.9 months; average body weight = 830.9 ± 78.48 lb (376.9 ± 35.6 kg)) at high risk to develop bovine respiratory disease (BRD) were included in the study.

BRD morbidity was lower in the TIP group (TIP = 6.8%; TUL = 20.9%; $p < 0.01$) over the feeding period. Animals in the TIP group had greater average daily gain (ADG) compared to heifers in the TUL group (TIP = 2.49 lb (1.13 kg); TUL = 2.34 lb (1.06 kg); $p < 0.01$). No differences were observed between groups for number and severity of lung lesions.

In the present study, tildipirosin was more effective than tulathromycin in reducing BRD morbidity and improving growth performance in newly received beef heifers considered at high risk for BRD.

Seroprevalence of bovine leukemia virus in Korean native cattle in South Korea, from 2013-2014

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Bovine leukemia virus (BLV), an oncogenic retrovirus, is the causative agent of enzootic bovine leukosis (EBL), a neoplasm of lymphatic tissue in bovine species. The majority of infected cattle do not display clinical signs of the disease. However, approximately 30% of BLV carriers develop a form of the disease known as persistent lymphocytosis (PL), and only 1-5% of BLV-infected animals develop malignant B-cell lymphosarcomas. No apparent treatment is available for the disease at present. Testing and slaughtering positive animals is one method of control. We conducted this survey to investigate the prevalence of BLV positive cattle in South Korea from 2013-2014.

Blood samples were obtained from Korean native cattle farms in all provinces except Jeju. A commercial indirect enzyme-linked immunosorbent assay (ELISA; SVANOVA, Uppsala, Sweden) with 4,498 samples in 2013 and with 4,905 samples in 2014 to detect antibodies against BLV was conducted in 96-well microplates according to the manufacturer's instructions. On each farm, 10~20 cattle in various ages are sampled and the results of ELISA are analyzed into ages and provinces.

Of a total of 9,403 cattle tested, 7.6% were seropositive for BLV. The prevalence of BLV in 1 year old or younger was found to be 5.0%, and depending on the ages being older the prevalence goes higher approximately by 5 years old. The survey shows our findings indicate that BLV is widespread in Korean native cattle in South Korea. We need to gather additional samples of Jeju and also other provinces to conduct the survey, and then it can be expected to control in organized management.

Antimicrobial susceptibility monitoring of *Mycoplasma bovis* isolated from respiratory tract infections in cattle across Europe during 2010 - 2013

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Introduction

Mycoplasma bovis is often involved in bovine respiratory tract infections. Several antimicrobial agents are licensed and used to control *Mycoplasma* infections but comparatively little is known about their antimicrobial susceptibility. Virtually none of the national resistance monitoring programs has included mycoplasmas, and dedicated ad hoc studies are rare. MycoPath is the first ongoing pan-European resistance monitoring program for *Mycoplasma* pathogens isolated from diseased cattle, pig and poultry. Antimicrobial susceptibilities of *M. bovis* strains from cattle suffering from respiratory disease are presented here.

Methods

Post-mortem lung samples or deep nasopharyngeal swabs were collected from animals with clinical signs of respiratory disease (depression, hyperthermia, polypnea, dyspnea, cough, nasal discharge). All samples were collected from animals not recently treated with antibiotics (> 15 days), in France (F), Hungary (H), Spain (E) and UK. *M. bovis* strains were isolated by national laboratories and only one isolate/outbreak/farm was retained. Susceptibility to nine veterinary-use antibiotics was determined in a central laboratory by broth microdilution methodology. Results are presented as MIC_{50} and MIC_{90} (in µg/mL).

Results and discussion

Overall, 156 *M. bovis* isolates were obtained: 43 from France, 37 in Hungary, 37 in Spain and 39 in UK. Similar MIC ranges were determined for danofloxacin (0.06->64), enrofloxacin (0.12->64) and marbofloxacin (0.25->64) with $MIC_{50/90}$ values of 0.25/1, 0.25/4 and 1/4, respectively. Country-specific differences of the MIC_{90} values for the fluoroquinolones of 0.5-2.0 (F/H/UK) and 1.0-16 (E) were determined. Generally, the macrolide antibiotics displayed higher MIC values: $MIC_{50/90}$ 4/16 for spiramycin and $MIC_{50/90}$ 32->64/>64 for tylosin, gamithromycin and tulathromycin. MIC ranges were as follows: 0.12->64 (spiramycin and tylosin), 1->64 (gamithromycin), and 0.03->64 (tulathromycin). Country-specific differences of $MIC_{50/90}$ values for the macrolides were absent. The florfenicol $MIC_{50/90}$ values (2/4) were the same in the four countries. Nevertheless, differences in the MIC range between F/E (0.5->64) and H/UK (0.5-8) were determined. MIC range of oxytetracycline was 0.25->64 with $MIC_{50/90}$ 4/>64. MIC_{90} data for oxytetracycline indicated differences between H/E/UK (> 64) and F (16). In the absence of validated standards and clinical breakpoints, it is currently not possible to reliably extrapolate these *in vitro* results towards *in vivo* efficacy. This is problematic for veterinary practitioners who need to decide on the most appropriate treatment of diseased animals.

Conclusions

This project provides valuable information on *in vitro* antimicrobial susceptibility of European *M. bovis* isolates, but underlines at the same time the urgent need for *Mycoplasma*-specific laboratory standards and interpretive criteria.

Indicators of lipid mobilization and their relation to variables of protein profile in dairy cows after calving

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In dairy cows, metabolic status can influence the incidence of peripartum diseases. The mechanism of association between metabolic changes and inflammatory diseases around calving is not entirely clear, but may be mediated through diminished immune-cell functions.

The aim of this study was to determine the concentrations of the main indicators of lipomobilization and selected variables of protein profile in dairy cows after calving, including immunoglobulins and acute phase proteins, as well as to evaluate the relationships between the altered lipid metabolism and changes in protein profile.

Into the evaluation we included fifty four dairy cows of a Slovak spotted breed, low-land black spotted breed and their crossbreeds in the period from one to two weeks after parturition. The animals were housed in free-stalls, and fed twice a day diets for lactating cows. The evaluated cows were without health disorders during the observation. Blood samples were analyzed for non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBl), total proteins, albumin, immunoglobulin G (IgG), haptoglobin and serum amyloid A. Unpaired Student´s test was used to evaluate the significance of differences in means between the groups of cows. Relationships between the concentrations of the evaluated variables in the monitored cows were calculated by linear regression and Pearson correlation test. In cows with higher concentrations of NEFA (Group B) we found significantly lower mean serum concentrations of total proteins, albumin and IgG than in cows with lower serum NEFA concentrations (Group A). On the other hand, cows with higher values of NEFA showed significantly higher mean concentrations of haptoglobin and serum amyloid A. Trend of significantly higher values in cows with higher NEFA concentrations was found also in the concentrations of BHB. The concentrations of NEFA significantly negatively correlated with the values of IgG, total proteins and albumin. Between the concentrations of NEFA and haptoglobin, as well as NEFA and serum amyloid A significant positive correlations were found.

This study indicates strong relationships between the concentrations of NEFA and selected variables of protein profile in cows after parturition.

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Risk factors of calf omphalitis: a clinical study

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Newborn calf omphalitis, following navel infection by fecal bacteria after calving, ranks third of neonatal pathology. The mortality rate of this affection is close to 8% in French herds and can affect herds in an enzootic way. The infection is multifactorial. In this trial, 128 calves coming from 15 dairy farms, born in autumn, were examined and followed for 3 months to assess risk factors for omphalitis. 65 were Montbeliard breed calves, 35 were Prim Holstein, and others were crossbred. Colostrum quality and colostral transfer, calving management, calf housing and many other factors were evaluated in this study. 13 omphalitis were detected and the results confirmed that male are more at risk to develop omphalitis than female calves. Montbeliard breed and cow parity above 2 are also significant risk factors of omphalitis ($p < 0.05$). This last result is surprising but may be the consequence of farmers increased attention at first calving. Calf intensive care at birth and housing conditions immediately after birth require further assessment as results display a tendancy. These results need further development to establish some factors to reduce navel disease.

Impact of low and high antimicrobial regimen on selection of resistant bacteria in commensal gut flora of young bulls treated for bovine respiratory disease

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Objective

In the context of requested decrease of antimicrobial use in human and veterinary medicine, the aim of our study was to assess the impact on commensal gut flora of a fivefold decreased antimicrobial regimen administered to young bulls (YBs) to treat bovine respiratory disease (BRD).

Materials and methods

Twenty eight YBs from 6 commercial farms presenting signs of bronchopneumonia were randomly assigned to one of the two experiment group L (low) and H (high) based on treatment regimen. In L group, 14 YBs were given a single intramuscular injection of 2 mg/kg marbofloxacin, a short acting fluoroquinolone commonly used in the treatment of BRD. In H group, 14 YBs were given a single intramuscular injection of 10 mg/kg marbofloxacin. Faeces were sampled on day 0 just before treatment, and on D 3, 7, 10, 21, 40 after treatment. Total and resistant *Enterobacteriaceae* counting was performed by plating dilutions of faecal samples on Mac Conkey agar plates without or with marbofloxacin supplementation at 0.12 and 4 mg/L.

Results and conclusions

Before treatment, total *Enterobacteriaceae* counts were similar in the two groups and no clinical resistance to marbofloxacin was observed. At D3, a transient decrease in total *Enterobacteriaceae* count was observed in the two groups. This decrease was higher in group H (- 2 $\log_{10}$) than in group L (- 1 $\log_{10}$). Total *Enterobacteriaceae* count returned to baseline at D7 in both groups. During the study, bacteria with high level resistance to marbofloxacin were isolated from D7 to D40 in one YB from group H; bacteria with decreased susceptibility to marbofloxacin were isolated from D10 to D21 in one YB of group H, and from D3 to D40 in two YBs of group L.

Our results suggest that a unique treatment with 2 or 10 mg/kg marbofloxacin exerts a low selective pressure on commensal *Enterobacteriaceae* in YBs. A decrease in antibiotic regimen allowed to limit the decrease of total *Enterobacteriaceae* count. Such findings enlightens that protocols using short acting fluoroquinolones may limit selection of resistant bacteria among commensal flora possibly transferred to humans.

Validation of *Mannheimia haemolytica* and *Pasteurella multocida* challenge models in calves using recent field isolates

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Mannheimia haemolytica is a causative agent of pneumonic pasteurellosis, a disease of newly weaned calves. Calves are particularly susceptible during transport to market or when held at high stocking density, hence the disease is often referred to as "Shipping Fever or Crowding Disease". *Pasteurella multocida* is an important veterinary and opportunistic human pathogen with a diverse and complex structure, host range and virulence, that causes pneumonic and systemic disease in livestock as well as fowl cholera in chickens and turkeys, atrophic rhinitis in pigs, and dog and cat bite infections in humans.

This study optimised challenge models for experimental infection of calves with either *M. haemolytica* or *P. multocida* based on Moredun Scientific's existing models but using more recent field isolates of the organisms to produce mild to moderate clinical signs of disease.

Twenty calves at approximately six weeks of age were allocated to two groups of seven (*M. haemolytica* and *P. multocida*) and one group of six (saline control). Challenge material consisted of log-phase cultures diluted in phosphate buffered saline and administered as 300 mL volumes via the intra-tracheal route using an endoscope. Calves were clinically observed for a period of four days post-challenge and then necropsies performed with lungs assessed for lesion formation and bacterial recovery.

Both models resulted in clear clinical signs with pyrexia, increased respiratory effort and rate, mild to moderate depression, sporadic coughing and occasional nasal discharge. At necropsy, the mean percentage of lung with lesion was 25.13% in the *M.haemolytica* animals, 20.35% in the *P. multocida* group and 6.71% in the controls. The relevant challenge isolates were recovered from lung tissue samples from all *M. haemolytica* and *P. multocida* challenged animals. These models will provide stable platforms for prophylactic, metaphylactic and therapeutic efficacy testing for the control of bovine respiratory disease.

Improving colostrum management by IgG measurement combined with recording colostrum administration data

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Objectives

Neonatal Calf Diarrhea (NCD) is a common disease affecting newborn calves. To reduce the incidence of NCD it is important to use a preventive approach. Vaccinating the dam is a proven preventive method to increase the specific "protection" and good colostrum management is a crucial element to get a good start as calf. More insight in the colostrum management can be obtained by determine the IgG levels of calves. The aim of this study is to find out reasons why IgG's are low.

Materials and methods

On 13 Dutch dairy farms the colostrum management of 58 calves was recorded in the first 24 hours. These calves were blood sampled and examined on IgG level between the 2nd and the 5th day after birth.

Results

62% of the examined calves had a low IgG level (IgG < 15 g/L). Most calves with a low IgG level received 5 liters or lesser colostrum in the first 24 hours (odds ratio (OR) = 5.2, p = 0.0175, 95% confidential interval (ci) 1.3-21.5). A trend was seen that the amount of colostrum in the first feeding was too low (OR = 2.8, p = 0.07, 95% ci 0.9-8.8) and the interval between first-second feeding too long (OR = 2.6, p = 0.09, 95% ci 0.9 -7.9). There were no differences seen between calves with high and low IgG in the interval birth-first feeding (OR = 1.2, p = 0.78, 95% ci 0.3-4.9), the interval second-third feeding (OR = 1.3, p = 0.7, 95% ci 0.4-4.6) and the amount of colostrum fed in the second and third feeding (OR = 1.1, p = 0.85, 95% ci 0.4-3.4), (OR = 1.2, p = 0.75, 95% ci 0.3-4.5).

Conclusions

Conclusion of this study is that most calves still don't receive enough colostrum. Most calves with a low IgG received colostrum directly after birth, but a strong trend was seen that the amount of colostrum in the first feeding was often not enough and the second feeding was offered too late. Not all farmers milked out the dam completely directly after birth, which raises concerns. This study emphasized that by recording the colostrum management in combination with determining the IgG level a customized advice can be given.

Using the doppler index as a method to characterize ovarian physiological changes in response to optimal cooling

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Heat load may have a negative effect on the production and welfare of the high-yielding dairy cow. The main mean to relief heat stress is by various cooling regime. The use of ultrasonography in recent years has demonstrated that exposing dairy cows to elevated temperatures alters reproduction system function. Transrectal color doppler sonography is a useful technique for the investigation of the ovary blood flow and provides new information about physiological changes of the ovary.

Material and methods

The aim of the selected study was to evaluate the blood flow in multipouros dairy cows' follicle through estrous cycle during the hot summer season. The observed group was randomly divided in to two groups. The first group was exposed to 8 cooling sessions per day, while the second group was treated with 5 cooling sessions per day. Then we compared the follicle's blood flow during the first and second follicular wave until ovulation.

Results

The group that was exposed to 8 cooling sessions had a shorter estrus cycle, significantly, in comparison to the group that was exposed to 5 cooling sessions (21.75 ± 0.68 days & 23.17 ± 0.54 days, respectively). There was no difference between the two groups in the aspect of dominant follicle diameter throughout the estrous cycle. Nevertheless, the dominant follicles blood flow in the last 3 days before ovulation was higher in the 8 cooling sessions group as compared to the 5 cooling sessions group.

Conclusions

Based on these results we assume that intensive cooling program can improve the blood flow to the ovary and in particular to the dominant follicle, thus, reducing the effect of heat stress. Improving the blood flow to the ovary might enhance its function and elevate the quality of the ovum, leading to surpass dairy cow fertility.

Studies on the curative and prophylactic concentration of isometamedium chloride in the treatment of experimental *Trypanosoma congolense* infection in Red Sokoto Bucks

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The effectiveness of therapeutic and prophylactic drug concentrations of isometamidium chloride was studied in *Trypanosoma congolense* infected Red Sokoto bucks (RSB). Twelve RSB were divided into three groups of four animals each:

- Group I (1 % Isometamidium treated),
- Group II (2 % Isometamidium treated),
- Group III (uninfected and untreated-Control).

Groups I and II bucks were each inoculated intravenously with approximately $1x10^6$ *T. congolense*. Parasitemia was monitored daily post-infection and post-treatment on wet-mount and micro hematocrit centrifugation technique (HCT) till the end of the study. Groups I and II were treated with 1% and 2% isometamidium chloride at the dose rate of 0.5 mg/kg body weight intramuscularly. Seven days post-treatment the blood from the treated groups were sub-inoculated into mice.

Clinical signs, recorded were, rectal temperature, body weight, total plasma protein, packed cell volume (PCV), total white blood cell count (TWBCC) and differential leucocyte counts.

Analysis of variance (ANOVA) with Turkiy's multiple comparison post-hoc test using Graph Pad Prism® version 5.0 was used to compare the level of significance between the test groups.

The pre-patent period of the infection in the two groups was three to four days. The Group I had relapse of the infection two weeks post-treatment while no relapse of the infection was observed in the Group II. Pale mucous membranes, pyrexia, inappetance, rough hair coat, depression, epiphora, nasal discharges and pre-scapular lymph nodes enlargement were among the clinical signs observed post-infection.

There was a significant decrease ($p < 0.001$) in the daily mean rectal temperature post-infection and post-treatment between the treated groups and the control group. The weekly mean body weights significantly decreased in both the two groups ($p < 0.001$) post-infection and post-treatment but the decrease in total plasma proteins observed were not significant ($p > 0.05$). A significant decrease ($p < 0.001$) in the PCV was also noted in both the treated groups post-infection and post-treatment. Also a significant decrease ($p < 0.01$) in the PCV following relapse was observed in Group I.

The total white blood cell counts (TWBCC) in the two treated groups decreased significantly ($p < 0.001$) post-infection and post-treatment compared with the control group. A significant decrease in TWBCC ($p < 0.001$) was observed in Group I following relapse. A significant decrease ($p < 0.01$) in neutrophils and lymphocyte was noted post-infection and post-treatment respectively. The difference in absolute eosinophil, band cells, monocyte and basophil counts were statistically insignificant ($p > 0.05$).

This study showed that treatment with 1% isometamidiuim chloride lead to relapse infection two weeks post-treatment. The 2% isometamidium chloride was however curative and no relapse infection was observed during the period of observation.

Antibacterial sensitivity of bacterial isolates in calf scours of various breeds of cattle in Nigeria

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Diarrhoea in young pre-weaned calves is one of the most important causes of calf morbidity and mortality, calf diarrhea has an adverse effect on the calves'immediate health status, longevity in the herd and productivity performance thus causes great economic losses. In order to minimize this losses, we studied the prevalenth bacterial, the role of the system of management in the perpetuation of the condition, variation in breeds susceptibility and antibacterial susceptibility to various bacterial isolate from the suspected cases of calf scours.

One hundred and twenty faecal samples (120) from various breeds of calves showing clinical manifestation of scours were screened for bacterial agents in farms in Oyo and Ogun States in South Western Nigeria. *Escherichia coli, Salmonella spp*. and *Campylobacter spp*. were identified. Out of all the calves, 19 calves (15.8%) were positive for bacterial agents, *E. coli* was isolated from 12 calves (10%), *Salmonella spp.* was isolated in 10 calves (8.3%) and *Campylobacter spp*. was isolated in 1 calf (0.8%).

The study revealed that White Fulani was the least susceptible breed (8.9%) while the most susceptible breed was Jersey breed (50%) to bacterial scours. The severity of diarrhea showed that calves having severe diarrhea had highest percentage positivity (26.09%) for bacterial isolates while the calf with moderate diarrhea had the least percentage positivity (13.16%) for bacterial scours.

Calves reared with semi intensive system of management were the most susceptible (18.03%) while those reared under extensive system were least susceptible (13.56%). Antibiotic Sensitivity test revealed that the most efficacious antibiotics were Meropenem (91.3%) and Amikacin (82.6%) while the least was Ampicillin (21.7%).

Therefore age, breeds, sex, severity of diarrhea, hygiene of calves and system of management have some important roles in the outbreak of calf scours.

Evolution of seroprevalence for BHV-1 gE in a French herd vaccinated with a live bovine herpesvirus type 1 marker vaccine

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A herd vaccination programme was started in November 2008 in a large beef herd in Normandy with a high seroprevalence of IBR (Infectious Bovine Rhinotracheitis, due to Bovine Herpesvirus type 1 or BoHV-1): 74% of heifers aged from 14 to 30 months and vaccinated with a live marker vaccine (Bovilis® IBR Marker Live, MSD Santé Animale) had BoHV-1 gE antibodies before beginning the herd protocol.

All cattle within this herd, i.e. four hundred animals including calves, were vaccinated with the same live marker vaccine. Calves were vaccinated intranasally at two weeks of age, revaccinated at three months and thereafter boosted every six months. Cattle aged three months or more were vaccinated once intramuscularly and revaccinated every six months. All the animals aged between 12 and 48 months were sampled every six months to evaluate their BoHV-1 gE antibody status. Biosecurity measures were also applied and every positive animal was removed.

The incidence of new seroconversion (seroincidence) decreased from 5% in November 2009 to 0% in November 2012. As a result, the herd seroprevalence decreased from 50% in May 2009 to 0% in May 2014.

It can be concluded that vaccination with a live IBR marker vaccine could significantly contribute, if associated to hygienic measures and to a specific herd management, to the eradication of BoHV1 in a large herd with a high seroprevalence under field conditions.

Congenital abnormalities in the offspring of a Montbeliard bull

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At the end of 2012, feedbacks from the field describe severe abnormalities and a high mortimortality rate around 10% in the offspring of a Montbeliard bull. Face anomalies such as cleft lips or palate and nervous troubles were reported. A genetic disease was suspected. The aims of the study were to identify the subjects affected among the offspring and to describe the phenotype associated to the genetic anomaly.

1057 calves were born. The bull and 74 individuals out of 453 alive subjects were examined between March 2013 and November 2014. Complete clinical examination including neurological and ophtalmic exams was carried out. Echocardiography was undertaken on 3 affected patients. Necropsy was performed on 4 of them. A wide range of phenotypes were observed, from mild to severe affection including orofacial clefts, heart defects, growth retardation, nervous symptoms and many other signs. Examined animals were sampled after being classified into 3 groups: affected, doubtful, healthy. A dominant mutation was found on gene CHD-7. Among the 75 examined animals, 28 were carrying the mutation.

In Human medicine, this mutation is known as responsible for CHARGE syndrome. CHARGE is an acronym standing for Coloboma, Heart defects, choanal Atresia, Retarded growth and development, Genital abnormalities, and Ear anomalies and deafness. All of these symptoms have been found in the offspring. Cattle appear to be a great model to study this neurocristopathy. Further assessment is needed to understand the gene expression mechanisms.

The risk factors, prevalence and prophylaxis of BRDC in Hungarian large-scale cattle herds

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The authors surveyed 15 large-scale Hungarian cattle herds including 13 dairies (9,326 cows and 7,864 calves and heifers) and 2 beef herds (1,155 cows and their progeny) between February and April 2013 in terms of environment, management and housing of calves, and the prevalence and prophylaxis (vaccination and treatment) of Bovine Respiratory Disease Complex (BRDC) with the ResCalf Farm Audit Tool™ (MSD AH).

In all the surveyed dairy herds the following risk factors were identified; overpopulation, dehorning, previous BRDC cases, and lack of the all in/all out system and grazing. On most farms (92.3%) the implementation of the biosecurity measures (e.g. quarantine) was not proper. At the time of the survey in 80% of the herds the BRDC was present with different severity, mainly in the calves aged from 3 to 6 month (83.3% prevalence amongst the dairy calves). The most prevalent clinical symptoms of the BRDC were cough (in 100% of the herds), nasal discharge (73.3%), ocular discharge (66.7%), fever (60.0%), heavy breathing (53.3%) and decreased appetite (53.3%). The BRDC caused decreased growth rate (in 92.3% of the herds) and mortality (69.2%) of the calves and reproductive disorders of the heifers (61.5%).

From more than half of the herds *Mannheimia haemolytica* (75.0%), BRSV (58.3%), PI3 (58.3%) and *Pasteurella multocida* (58.3%) were identified. In the majority of dairy herds vaccinations were applied against BRSV (66.7%), PI3 (66.7%), BVD (60.0%) and *Mannheimia* (60.0%), besides the mandatory IBR marker vaccination. The macrolids were the mostly used antibiotics against BRDC (in 92.9% of the herds), followed by amoxicillin, enfrofloxacin and marbofloxacin (on 50.0% of the farms each). Metaphylaxis was practised on 28.6% of the farms.

The BRDC is a multifactorial disease, thus, besides a well-scheduled vaccination program the success of its prevention also depends on the environmental, housing conditions and the management of the farm.

Risk factors associated with mortality in dairy calves of the Vosges department: a study in 62 farms in 2012

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The purpose of this case-control study was to determine the risk factors for mortality of zero to six months old dairy calves in 62 farms in the Vosges department. This department is indeed nationally ranked 83 regarding mortality rates of cattle.

At first, in order to build the questionnaire, mortality risk factors of interest had to be identified thanks to a review of literature. These risk factors may be involved in different aspects of farming and influence expression of diseases: the sectors that may be influenced by these risk factors include feeding management, housing management, animals themselves, herd management, microbism and finally the farmer.

Ninety four farms were selected on the basis of 2010-2011 mortality rates. Fourty seven farms with a mortality rate of dairy calves less than 5% were considered as controls while 47 farms presenting a mortality rate over 15% were considered as cases. Sixty two farmers agreed to participate to the study. After completion of the questionnaires, statistical analysis of the data was carried out: univariate analysis followed by a multivariate analysis using logistic regression was performed. Five risk factors were found significantly associated with mortality of dairy calves: the purchase of adult cows, the recent completion of an audit, the distribution to calves of colostrums from mastitis cow, the housing of calves on slats, and the non-fulfillment of a three weeks fallowing. We also stressed in our study the importance of the sociological aspect on calves' management.

In vitro activity of tylosin against *Staphylococcus aureus* and *Streptococcus uberis* isolated from bovine mastitis during an eleven-year period

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The macrolide tylosin is commonly indicated to treat bovine mastitis in many European countries (France, Italy, Spain, United Kingdom). The objective of this study is to determine the Minimum Inhibitory Concentrations (MIC) of tylosin against *Streptococcus uberis* and *Staphylococcus aureus* isolated from bovine mastitis over eleven years in France.

Materials and methods

Between 2001 and 2012, 320 strains of *Staphylococcus aureus* and 320 strains of *Streptococcus uberis* isolated from dairy cows with clinical mastitis were tested. For each bacterial species, only one strain per year was included from each herd sampled, in order to test epidemiologically unrelated strains. Strains were isolated and identified in mastitis milks in accordance with the French Guideline CNEVA PR 116/00/BA 140/00. The reference agar dilution method was used to determine MIC. Susceptibility testing was realized in accordance with the Clinical and Laboratory Standards Institute M31-A3 Guideline. Tylosin was tested at concentrations that ranged between 0.06 and 256 µg/mL. Logistic regression was performed to determine the probability of antimicrobial resistance by year using the package Glm2 of R (version 3.0.2; R Foundation for Statistical Computing, Vienna, Austria). The logistic regression model for the percentage of resistant isolates by year included resistance as a response variable (yes vs no) and year as a continuous variable (0 to 7). For all analyses, values of $p < 0.05$ were considered significant.

Results

Both *Staphylococcus aureus* and *Streptococcus uberis* showed bimodal distributions of MICs which allowed distinguishing between the susceptible strains and the resistance strains. For the 320 strains of *S. uberis*, 87,5% (280/320) strains were classified as tylosin-susceptible by their tylosin MICs < 8 µg/mL. Among this tylosin-susceptible isolates, a high proportion of strains had tylosin MICs between 0.25 and 2 µg/mL (273/320 strains, 85.3%). For the 320 strains of *S. aureus*, 96.5% (309/320) strains were classified as tylosin-susceptible by their MICs between 0.25 and 4 µg/mL.

During this eleven-year period, the percentage of *S. aureus* strains resistant to tylosin described a decreasing trend. *S. aureus* strains were 0.82 less likely to be resistance at the end of the period compared to the start of the period (confidence interval = 0.67 - 0.96). No change in the trend of resistance was observed for *S. uberis* during the study period.

Conclusions

We have shown that there were no consistent trends among *S. aureus* and *S. uberis* isolates from bovine mastitis towards increased resistance to tylosin.

Failure of passive immune transfer in calves: total cost and economic strategies

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Colostrum intake is reported as a key factor of the newborn calf health. Failure of passive immune transfer (FIT) is linked to no or low colostrum intake and is defined as low plasma IgG or total proteins at 24 to 48 hours of age. FIT is associated with an increased risk of mortality and morbidity, and is consequently not directly seen by farmers. In spite FIT stakes are consensual, no economic assessment has been performed up to now.

This work aims to: estimate the total cost of FIT and to define the resources to be allocated to FIT, in the French context.

To define the total cost of FIT a stochastic two-step economic method was developed. A meta-analysis was firstly performed to clarify the adjusted links between FIT and morbidity or mortality. The economic model was secondly run, considering costs of extra diseases and mortality in case of FIT.

To define the resources to be allocated for FIP management, the net value at the farm level was calculated accounting for different scenarios (various levels of resources used).

The total cost of FIT was € 60 (95% prediction interval = € 10-140) and € 80 (95pcPI = € 20-200) per calf with FIT, for dairy and beef calves, respectively. This result can be easily used in the field to calculate the avoidable costs of FIT for a given farm, thanks to comparison of the initial prevalence and the expected prevalence after changes in practices, multiplied by the unit total cost (€ 60-80 or values in the PIs range). This may help making decision at farm level.

The optimal time to feed colostrum was around 15 min per calf, whatever the total cost of labor. Resources to be allocated for FIT management did not show important changes between scenarios. Importantly, increasing this duration led to small decrease in the farm net value, but decreasing this duration led to high decrease in the farm net value. As conclusion, there is an economic justification to spend at least 15 min per calf to make colostrum be ingested.

Increased cerebrospinal fluid sodium concentration in a diarrheic calf

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A 14-day-old Holstein dairy calf is referred because of a diarrhea of 3 days' duration with recumbency. A diagnostic test made on the farm to identify the causative agents of diarrhea revealed a positive result for cryptosporidiosium. The calf was treated with an oral electrolyte solution (of an undetermined quantity) and antibiotics (trimethoprime suldadoxine then marbofloxacin), without clinic amelioration.

At admission, the calf was hypothermic, dehydrated, in a comatose state (lateral recumbency and no sucking reflex) and with scours. Initial differential diagnoses include neonatal diarrhea with acidosis, meningoencephalitis or septicemia. First ancillary diagnostic tests included venous blood gas analysis, measurement of serum electrolytes concentration and feces examination for parasite. A marked metabolic acidosis with a loss of bicarbonates (pH = 7.1, base excess = -18 mmol/L, bicarbonate = 18 mmol/L), a severe hypernatremia (178 mmol/L) and a hyperchloremia (129 mmol/L) were found. No parasite was identified in the feces. Alkalinizing and hypertonic intravenous fluid with bicarbonate was administrated to correct acidosis, followed by an isotonic glucose solution to rehydrate, as well as an antibiotic (marbofloxacin) and a non-steroidal inflammatory drug (flunixin meglumine) to prevent a septicemia.

The following morning, the calf's clinical condition was critical with persistence of the comatose state and hemorrhagic diarrhea. Two successive blood analysis showed no modification of electrolytes concentration (natremia = 179 mmol/L, chloremia = 129 mmol/L) while the blood pH was less acidic (pH = 7.3) after a new supplementation with bicarbonate. A severe increase of sodium concentration (178 mmol/L) was surprisingly measured in cerebrospinal fluid (CSF). An intravenous 5% glucose solution was continuously administrated to reduce hypernatremia. The calf was found dead a few hours later.

The very high sodium concentrations in CSF and blood (with a ratio equal to 1) are suggestive of salt intoxication and are probably the result of feeding with an improperly formulated oral electrolyte solution and an impaired water intake on the farm. Fluid therapy, in this calf, has not resulted in a reduction of the sodium concentrations in blood and CSF. The present case shows that the combination of acidosis, dehydration and hypernatremia presents a therapeutic dilemma.

Efficacy of Hiprabovis Somni/Lkt in young calves under field conditions

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The aim of this study was to demonstrate the efficacy under field conditions of HIPRABOVIS® SOMNI/Lkt for reducing pneumonic problems caused by *Mannheimia haemolytica* and *Histophilus somni* in cattle and the consequent reduction in the number of antibiotic treatments.

One-hundred-and-thirty two-month-old calves, apparently or visibly healthy, not showing severe clinical signs during the first days of life and presenting a good weight in proportion to their age, were randomly assigned to group A (n = 64) and group B (n = 66).

Animals in group A were vaccinated subcutaneously two times three weeks apart: with 2 mL/calf on day 0 and 21 of HIPRABOVIS® SOMNI/Lkt containing *M. haemolytica* Biotype A serotype A1, inactivated cell free suspension with leukotoxoid (ELISA > 2.8/dose) and inactivated *H. somni* Bailie strain (MAT > 3.3/dose).

Animals in group B (non-vaccinated) received PBS (Phosphate Buffer Saline, pH 7.4) following the same schedule as group A.

The main variables observed, recorded and compared to the control group were: clinical signs according to the Canadian Council on Animal Care Guidelines, number of antibiotic treatments, and lung damage. The level of significance used was 95%.

Both clinical respiratory signs and general symptoms did appear during the trial, and were significantly higher in the control calves than in the vaccinated ones. In the non-vaccinated group, 44% of the calves, as opposed to a 20% of the vaccinated ones, showed respiratory signs ($p < 0.01$).

The mean percentage of pneumonic tissue in the vaccinated group was lower than that for the control group (20.4% vs 41.3% respectively). Chemotherapeutical treatments were concomitant with the presentation of clinical respiratory signs, and so were significantly higher in the control calves than in the vaccinated ones. A total of 26 treatments as opposed to 144 were applied to vaccinated and control calves respectively.

The animals vaccinated with HIPRABOVIS® SOMNI/Lkt presented significantly fewer respiratory problems than the non-vaccinated ones ($p < 0.01$) and significantly reduced pneumonic lung lesions caused by *M. haemolytica* and *H. somni* ($p < 0.05$). Consequently, a significant reduction in the number of treatments was achieved.

Possible involvement of *Leptospira* in an emergent syndrome of bovine congenital jaundice: about a clinical case

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In September 2014, Belgium faced an increase of icteric aborted fetuses. Despite a wide range of analyses, no definitive cause of abortion was established. Some further analysis results however support a leptospirosis hypothesis. Indeed, antibodies directed against *Leptospira* serogroups Australis and Grippotyphosa were evidenced at high titers in some of the aborting cows. In the meantime, two similar cases were discovered in France. The first one that we deal with in this paper occurred in the Ardennes, a French province situated along the border of Belgium.

We describe the case of a calf that was born during the night. Less than 12 hours later, he was found recumbent, hypothermic, and icteric. Immediately after its clinical management, he deceased consecutively to an important epistaxis. Whole blood was tested for leptospiral DNA by a polymerase chain reaction which led to a positive result. The histological examination conducted on the liver and the kidneys was consistent with a leptospirosis hypothesis. A microagglutination test (MAT) was performed on the dam's serum. The higher MAT titer was directed against serovar Australis. All three results suggest that *Leptospira* serovar australis was involved in the icteric syndrome observed on the calf.

Efficacy of flunixin meglumine pour-on administration in a tissue cage model of inflammation

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We evaluated in cattle the effect of flunixin meglumine in reducing PGE2 concentration in exudates after induction of subcutis inflammation using a tissue cage model.

Six healthy not-lactating dairy cows (514 ± 42 kg) and six healthy grass calves (369 ± 49 kg) were included in the study and randomly assigned to two treatment sequences. Sterile hollow perforated polyethylene balls were surgically embedded in the subcutis at four distinct sites per animal three weeks prior to D0. On D0 and D14, an aseptic inflammation of the subcutis was induced by injecting in three balls per animal 0.5 mL of a 2% carrageenan solution. Carrageenan challenge was immediately followed by the topical treatment on the dorsal midline area: NaCl or flunixin meglumine (3.33 mg flunixin meglumine / kg body weight). Exudate was collected prior to challenge and 2, 4, 8, 12, 24, 36, and 48 hours after. Cages were emptied after each collection. PGE2 concentrations were measured in exudate using a validated liquid chromatography coupled with mass spectrometry method. Each animal received the two items as treatment sequentially on D0 and D14 according to group assignment.

In NaCl-treated animals, PGE2 concentration levels displayed a sharp increase, peaked 8 hours after challenge, and gradually decreased over time (PGE2 concentration levels were still elevated 48 hours after challenge). In flunixin meglumine treated animals, PGE2 peak occurred later (12 hours after challenge) and was strongly reduced compared to that in NaCl treated animals: PGE2 concentrations were consistently lower than those measured after NaCl administration. Percent of inhibition was close to or over 90% at the peaks of PGE2 concentrations (8 and 12 hours after challenge) after flunixin meglumine treatment, and inhibition lasted until the end of the animal phase. The log ratio of concentrations for the two treatment groups are significantly different (linear mixed model, p values ≤ 0.05) at hours 8, 12, 24, 36 and 48, but are not significantly different at hours 2 and 4.

This study shows that flunixin meglumine applied topically inhibits carrageenan-induced subcutis inflammation in cattle. Anti-inflammatory effects occurred as soon as 2 hours and lasted at least for 48 hours post-administration.

Effect of decreasing the duration of a synchronization protocol with PRID® Delta and addition of a second prostaglandin F_{2alpha} treatment on fertility after resynchronization of ovulation in lactating Holstein cows

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During a resynchronization protocol for timed artificial insemination (TAI), our objective was to evaluate the effects of:

- decreasing the interval between the first GnRH injection (G1; Cystoreline®/Ovarelin® - CEVA Santé Animale) and the $PGF_{2\alpha}$ (PGF; Enzaprost® T - CEVA Santé Animale) injection,

- a second PGF treatment 24 h after the first one.

Lactating Holstein cows (n = 795) from 3 commercial dairy farms were randomly assigned at a nonpregnancy diagnosis to one of three hormonal protocols for resynchronization of ovulation:

- a 7-d PRID-synch protocol with 1 PGF injection (7D1P: day 0; GnRH+PRID; day 7, PGF-PRID; 56-h, GnRH; 16-h, TAI),

- a 7-d PRID-synch protocol with 2 PGF injections (7D2P: day 0, GnRH+PRID; day 7, PGF-PRID; 24-h, PGF; 32-h, GnRH; 16 h TAI),

- a 5-d PRID-synch protocol with 2 PGF injections (5D2P: day 0, GnRH+PRID; day 5, PGF-PRID; 24-h, PGF; 32-h, GnRH; 16-h, TAI).

Ovaries of all cows were examined at G1 using transrectal ultrasonography, and cows were classified as either having or lacking a CL. Data were analyzed by logistic regression using PROC GLIMMIX of SAS. Pregnancy diagnosis was conducted at 32 and 60 d after TAI using transrectal ultrasonography.

At 32 d after TAI, pregnancies per AI (P/AI) tended to differ ($p = 0.08$) among treatments and was least for 7D1P cows, and greater for 7D2P and 5D2P cows [37% vs 43% vs 44%, respectively]. Pregnancy loss from 32 to 60 d after TAI did not differ ($p = 0.73$) among treatments (7% vs 11% vs 10% for 7D1P, 7D2P, and 5D2P). At 32 d after TAI, primiparous cows had more ($p = 0.04$) P/AI compared to multiparous cows [47% vs 39%, respectively].

In conclusion, addition of a second PGF injection to a 7-d PRID-synch protocol increased P/AI 32 d after TAI by 6 percentage points (7D2P vs 7D1P cows). Whereas, when the 5-d PRID-synch and 7-d PRID-synch with the 2 PGF injections are compared, decreasing the interval between G1 and the first PGF injection no difference in P/AI 32 d were found after TAI in resynchronized Holstein cows.

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Assesment of bovine tuberculosis with risk analysis in Turkey

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Many developed country eradicated or reduced bovine tuberculosis disease from farm animals but the disease is problematic on some developed and developing countries. Scientific studies results come fore that Bovine tuberculosis disease recurred in the same area and transport of animals from these areas play a critic role in the spread of disease. The light of these data, this study aim at to evaluate the epidemiological risk of the disease. This study, proposed a risk-based management strategy for combating the disease of bovine tuberculosis in Turkey field condition.

Firstly Turkey's official strategy on disease were investigated and deficiencies of the disease control were detected. Than, International Office of Epizootie data were used for epidemiologic analysis at provincial level and the distribution of the disease outbreak were determined by region. It is recomended a new approch of disease control by the risk level. Turkey's regional high risk areas were identified as the Marmara and Aegean region. Disease-specific risk criteria, methods of selection and evaluation criteria was determined in the study. Some risk factors awarenes, such as, Tuberculosis in goats, wild life risks and the time of the disease were evaluated in this study. Study results show that the disease is mostly seen in Turkey in summer, There is no data is available on the level of the other risks.

Due to the insufficent financial support of controlling the disease and continuing to increase compensation payments, risk based control starategies needs is increasing in recent years. In conclusison, this study recommended the use of this risk based methodology on bovine tuberculosis disease control in Turkey. In order to ensure economic efficiency in disease control.

International harmonization of the nomenclature of foot and claw disorders in cattle - ICAR Claw Health Atlas

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A survey of the International Committee for Animal Recording (ICAR) Working Group Functional Traits (WGFT) in 2014 showed that several countries recently introduced systems to routinely record foot and claw disorders, and many more are planning or have committed to begin recording in the near future. The broad range of recording practices and documentation schemes with mixture of descriptive and etiological codes suggested a need for a standardized, practice-oriented approach that accommodates most common circumstances in the field. This motivated the ICAR WGFT to prioritize foot and claw health and to invite internationally recognized claw experts to collaborate in the development of best practices for data recording. This collaboration intended to complement existing research on claws and feet of dairy cattle, focusing solely on the standardization and harmonization of data recording. This fruitful interdisciplinary collaboration among experts from different backgrounds (claw health experts, claw trimmers, bovine practitioners, geneticists) resulted in harmonized descriptions of 27 different claw disorders, providing comprehensive coverage of theoretical and applied needs. It is designed to provide a universal tool for claw trimmers and practitioners and presents guidelines for the recording of important conditions affecting the claw health of cattle. Descriptive trait definitions ensure accurate classifications, which will support the collection of comparable and high-quality data within and across countries to support many activities.

Harmonized descriptions of foot and claw disorders were agreed upon by the international experts and members of the ICAR WGFT, and representative photographs of each disorder were collected. Claw experts, trimmers, veterinarians, and other contributors submitted photographs, and the most representative examples were selected by voting. The results were discussed and the final selections made by the working group. Harmonized descriptions, photographs, and other descriptive information were assembled to create the first ICAR Claw Health Atlas (Egger-Danner et al., 2015). This Atlas, published in the official ICAR language (English), is available for translation by any country that would like to distribute it to its professionals and/or farmers. Information about translation and access to a print-quality version will be provided by ICAR.

An on-line English-language version is now available on the ICAR website:
http://www.icar.org/Documents/ICAR_Claw_Health_Atlas.pdf.

Field prevalence of bovine respiratory pathogens by PCR technique in France

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Bovine Respiratory Diseases (BRD) are a major pathological complex in cattle, with significant impact on farm economical results. The pathogen agents responsible for these disorders are essentially viruses and bacteria, often associated on the field. Polymerase Chain Reaction (PCR) is a major technique of diagnosis, due to its better detection performances in comparison with traditional bacteriological methods.

Since December 2012, 2 major laboratories for diagnosis located respectively in Nièvre and Ile et Vilaine departments participate in a field BRD epidemiological survey, with support from MSD Animal Health. The sampled animals, from a few days to several months old, had generally clinical symptoms of respiratory disease in early evolution.

After sampling by the vets either from deep nasal swabs (3 swabs per farm) (80% of all samples) or trans-tracheal fluids (< 10%) or pulmonary tissues of animals, PCR was performed after mixing maximum 3 samples from the same BRD outbreak in the same farm. A total of 648 individual viral and bacterial diagnosis were analyzed.

A majority of analysis showed the presence of bacteria with more than 80% of samples positive for *Pasteurella multocida* (up to 85% associated with another pathogen) and 46% of samples positive for *Mannheimia haemolytica*. *Haemophilus somni* and *Mycoplasma bovis* were found to a lesser degree. Regarding viruses, Bovine Respiratory Syncytial Virus (BRSV) was detected in less than one third of all cases, half in association withDans presque moitié des cas (47%), les résultats montrent une association du VRSB avec M. haemolytica. *M. haemolytica*.

This prevalence study in French field farms confirmed the presence of the two major BRD pathogens:

- *M. haemolytica,* for which one could think that it could be overestimated because of the sampling technique (nasal swabs) and the testing method (PCR). But former studies showed a correlation between nasal swabs and trans-tracheal aspiration on pooled samples (3 particularly),

- BRSV, for which the prevalence of 30% could seem to be low.

Is it because of the sampling technique in relation to the PCR testing? Some studies set the transtracheal aspiration as the most sensitive sampling method to isolate BRSV when PCR is performed. Or is it an effect of usual BRSV vaccination in farms?

Treatment of naturally occurring bovine respiratory disease in juvenile calves with a single administration of a combination of florfenicol plus flunixin meglumine

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Whether BRD causing factor is physical, environmental, or infectious, a sequence of events occurs resulting in inflammation and ultimately activation of the innate and adaptive immune systems. It is advisable that NSAID and anti-infective agents are used concurrently.

Florfenicol (Nuflor®) and flunixin meglumine (Finadyne®) are frequently administered concurrently for the treatment of BRD. A new therapeutic product, combining both actives, was developed and is currently marketed as Resflor®. It has been shown to have similar efficacy as florfenicol in the treatment of the BRD and flunixin in the reduction of pyrexia, while having the advantage of eliminating the need for separate injections.

The present study intended to confirm the efficacy and the safety of the florfenicol-flunixin combination formulation for the treatment of BRD in juvenile calves less than 6 weeks of age in comparison to an approved positive control, florfenicol, that represented a negative control in regards to flunixin meglumine.

A total of 210 calves of less than 6 weeks of age, showing severe signs of respiratory disease, were randomly assigned to treatment with either the test product, florfenicol plus flunixin meglumine, or the control product, florfenicol, both administered subcutaneously once, on day 0. The animals were observed for clinical signs of disease at 6 hours post-treatment and daily for 10 days.

The predominant respiratory pathogens were *Pasteurella multocida*, *Mycoplasma bovis*, *Mannheimia haemolytica* and *Histophilus somni*. All isolates were found susceptible to florfenicol.

In both groups, rectal temperature significantly dropped and clinical index (depression and respiratory signs) significantly improved after treatment. Specifically, for the change in rectal temperature from pre-treatment to 6 hours post-treatment, the florfenicol-flunixin formulation was found significantly superior to florfenicol ($p < 0.0001$). Moreover, the florfenicol-flunixin formulation alleviated the clinical signs of disease more rapidly, and was demonstrated non-inferior to florfenicol on days 4 and 10 success rates.

Neither florfenicol-flunixin formulation nor florfenicol had a negative influence on the health status including appetite and faecal consistency, confirming that both products are safe.

The use of the product combining florfenicol and flunixin in juvenile calves less than 6 weeks of age is safe and efficacious in the treatment of outbreaks of BRD.

Effectiveness and safety of a novel flunixin meglumine transdermal pour-on solution in the treatment of bovine respiratory disease

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Whether BRD causing factor is physical, environmental, or infectious, a sequence of events occurs resulting in inflammation and ultimately activation of the innate and adaptive immune systems. It is advisable that NSAID and anti-infective agents are used concurrently.

Flunixin is a NSAID commonly used for the relief of pain and control of inflammation and pyrexia associated with diseases of different origin and nature. A novel 50 mg/mL flunixin transdermal formulation was developed by MSD Animal Health (Finadyne® Transdermal) and is now the first NSAID registered to be administered as a pour-on product along the dorsal midline in cattle.

This study intended to demonstrate the safety and effectiveness of flunixin transdermal in the treatment of BRD. A total of 206 animals, showing severe signs of respiratory disease, were randomly assigned to treatment with either the test product, flunixin transdermal, administered topically once, or the control product, carprofen, administered by injection once, on day 0. All animals received cefquinome on days 0 and 2. The animals were observed for clinical signs of disease at 6 hours post-treatment and daily for 5 consecutive days.

The decrease in rectal temperature 6 hours post-treatment was greater in the flunixin group (-1.30°C) compared to the control group (-0.96°C). This difference was statistically significant ($p < 0.0001$) and the superiority of the flunixin transdermal to the control was demonstrated.

In the following days, rectal temperature and clinical index (depression and respiratory signs) improved similarly over time in both treatment groups.

Neither flunixin nor control had a negative influence on the health status including appetite and faecal consistency, confirming that both products are safe.

The new 50 mg/mL flunixin transdermal demonstrated to be safe and to provide strong anti-pyretic effect and anti-inflammatory properties, providing a convenient and suitable adjunct therapy to anti-infectives in cases of respiratory infections in cattle.

Efficacy of Resflor injectable against an experimentally induced *Mycoplasma bovis* infection in calves

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The objective of this blinded, randomised study was to demonstrate the efficacy of Resflor®, a combination of florfenicol and flunixin, against an experimental *Mycoplasma bovis* challenge in calves, in comparison to a negative control saline and a positive control tulathromycin associated with flunixin.

A total of 230 calves between 4 and 8 weeks of age, confirmed to be *M. bovis* antibody and antigen negative, were challenged daily for 3 consecutive days by intratracheal deposition of 12 mL pure *M. bovis* culture (5.14×10^9 cfu, European isolate 208/B01).

For 4 days after challenge, calves meeting enrolment criteria (pyrexia > 39.5°C and abnormal respiration and/or depressed demeanour) were enrolled for treatment with either saline (n = 21), tulathromycin plus flunixin (n = 64) or florfenicol-flunixin combination (n = 64). After treatment, animals were observed daily for seven days for clinical recovery (rectal temperature, respiration and demeanour). On day 7 post-treatment, animals were euthanized, lungs collected for lesions scoring and lung lavage fluid collected for *M. bovis* bacteriology.

After treatment, rectal temperature values, respiration and demeanour scores significantly decreased within 24 hours post-treatment with florfenicol-flunixin combination and tulathromycin plus flunixin, while they remained high for 7 days post-treatment with saline ($p < 0.0001$). At both day 4 and day 7 post-treatment, clinical recovery with florfenicol-flunixin combination was significantly superior to saline ($p < 0.0001$) and non-inferior to tulathromycin plus flunixin.

The proportion of lungs with *M. bovis* lesions was 6.6% for florfenicol-flunixin combination, 5.25% for tulathromycin plus flunixin and 19.2% for saline.

Recovery of *M. bovis* from lung fluid was significantly lower for florfenicol-flunixin combination than for saline (2.27×10^5 cfu/mL vs 1.33×10^7 cfu/mL - $p = 0.0020$) and for tulathromycin plus flunixin (2.27×10^5 cfu/mL vs 2.53×10^6 cfu/mL - $p = 0.0120$).

A significant difference in daily weight gain over the 7-day study was observed in florfenicol-flunixin combination and tulathromycin plus flunixin (mean 0.40 kg/day - $p < 0.0001$), compared to saline (0.13 kg per day - $p = 1.0000$).

The combination of florfenicol and flunixin provided an efficient clinical and microbiological cure of *M. bovis* pneumonia in calves.

Effectiveness and safety of a novel flunixin meglumine transdermal pour-on solution in the treatment of bovine mastitis

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Anti-inflammatory drugs are commonly used as adjunct therapy to antibiotics to treat clinical mastitis, since many of the physiological and pathological changes associated with clinical mastitis are a result of the inflammatory response to infection.

Flunixin is a NSAID commonly used for the relief of pain and control of inflammation and pyrexia associated with diseases of different origin and nature. A novel 50 mg/mL flunixin transdermal formulation was developed by MSD Animal Health (Finadyne® Transdermal) and is now the first NSAID registered to be administered as a pour-on product along the dorsal midline in cattle.

The objective of the study was to demonstrate the safety and effectiveness of flunixin transdermal in the treatment of clinical mastitis in dairy cows. A total of 133 cows, showing severe signs of mastitis, were randomly assigned to treatment with either the test product, flunixin transdermal or the negative control product, a red dye saline solution (to preserve masking), both administered topically once. All animals received intramammary antibiotics and systemic antibiotics starting at 6 hours post-treatment. The animals were observed for clinical signs of disease for 6 hours post-treatment and daily for 5 consecutive days.

The decrease in rectal temperature 6 hours post-treatment was greater in the flunixin group (-1.8°C) compared to the control group (-1°C). This difference was statistically significant ($p < 0.0001$) and the superiority of the flunixin transdermal to the control was confirmed.

The alleviation of pain, firmness and swelling of the udder was also significantly greater at 6 hours ($p < 0.0001$) and at 24 hours ($p < 0.05$) after treatment initiation in the flunixin group compared to the negative control group. Rectal temperature and clinical index (general attitude and udder clinical signs) improved over time.

Neither flunixin nor control had a negative influence on the health status, confirming that flunixin transdermal is safe.

The new 50 mg/mL flunixin transdermal demonstrated strong anti-pyretic effect and anti-inflammatory properties, providing a convenient and suitable adjunct therapy to anti-infectives used in cases of mastitis infections in cattle to reduce pyrexia and to alleviate clinical signs of pain, firmness and swelling of the udder.

Flunixin meglumine transdermal pour-on solution as adjunct therapy in the treatment of bovine respiratory disease in calves less than 8 weeks of age

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Whether BRD causing factor is physical, environmental, or infectious, a sequence of events occurs resulting in inflammation and ultimately activation of the innate and adaptive immune systems. It is advisable that NSAID and anti-infective agents are used concurrently.

Flunixin is a NSAID commonly used for the relief of pain and control of inflammation and pyrexia associated with diseases of different origin and nature. A novel 50 mg/mL flunixin transdermal formulation was developed by MSD Animal Health (Finadyne® Transdermal) and is now the first NSAID registered to be administered as a pour-on product along the dorsal midline in cattle.

The objective of the study was to demonstrate the safety and effectiveness of flunixin transdermal in the treatment of BRD in juvenile calves. A total of 49 calves of less than 8 weeks of age, showing severe signs of respiratory disease, were randomly assigned to treatment with either the test product, flunixin transdermal, administered topically once, or the control product, carprofen, administered by injection once, on day 0. All animals received cefquinome on days 0 and 2. The animals were observed for clinical signs of disease for 6 hours post-treatment and daily for 5 consecutive days.

The decrease in rectal temperature 6 hours post-treatment was greater in the flunixin group (-1.7°C) compared to the control group (-1°C). This difference was statistically significant ($p < 0.0001$) and the superiority of the flunixin transdermal to the control was confirmed.

In the following days, rectal temperature and clinical index (depression and respiratory signs) improved similarly over time in both treatment groups.

Neither flunixin nor control had a negative influence on the health status including appetite and faecal consistency, confirming that both products are safe.

The new 50 mg/mL flunixin transdermal was shown to be safe and to have a strong anti-pyretic effect and anti-inflammatory properties, and makes it a very convenient and suitable adjunct therapy to anti-infective therapy used in cases of respiratory infections in juvenile calves less than 8 weeks of age.

Evaluation of the efficacy and performance of preweaned Holstein calves treated with either Resflor Gold or Baytril for bovine respiratory disease

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The study was conducted to compare the therapeutic response, milk replacer and calf starter intake, growth, treatment costs and performance of calves with bovine respiratory disease (BRD) treated with Resflor-Gold or Baytril and compared to calves not treated for BRD.

Holstein bull calves identified with BRD were randomly assigned to treatment to receive either: Resflor-Gold (n = 104, Florfenicol (40 mg/kg) and flunixin-meglumine [2.3 mg/kg]) or Baytril (n = 110, Enrofloxacine, 12.5 mg/kg). Before treatment administration, deep nasal-pharyngeal swab samples were collected from calves with a respiratory score ≥.

Samples were tested for BRSV, BVDV, IBRV and BoCV using multiplex real-time PCR and cultured for Mycoplasma. Calves were re-evaluated 72 hours post-treatment, and if they were not clinically normal, they received a second dose treatment with the same antimicrobial drug. Total weight gain, calf starter and milk replacer intake (dry matter basis), treatment costs, feed to gain ratios and first treatment success rate were evaluated. Performance data was compared to calves that were not treated for BRD (n = 249).

There was no difference in the number of respiratory pathogens found between treatment groups The most common respiratory pathogens found were *Mycoplasma bovis* (77.8%) and BoCV (68.4%). Before treatment administration, there was no difference in the respiratory rate, total respiratory score and rectal temperature between treatment groups. There was a significant difference ($p < 0.01$) in first treatment success rate between calves receiving Resflor-Gold (50.4%) or Baytril (33.3%). There was no difference in fecal score, electrolyte and antimicrobial drug costs between treatments. Performance data showed that dairy calves with BRD are adversely affected. Calves not treated for BRD had higher calf starter intake (13.5 kg vs 10.5 kg, $P< 0.01$), better feed to gain ratios (2.08 vs 2.42, $p < 0.01$) and weighed more (66.9 kg vs 63.8 kg, $p < 0.01$) than calves that were treated for BRD. However, there was no difference in milk replacer intake between calves that were treated for BRD and calves that were not treated for BRD ($p > 0.05$). Resflor-Gold treated calves trended to have a better feed-to- gain ratio than calves that were treated with Baytril ($p = 0.07$).

Effects of dietary omega-3 fatty acids on the mammogenesis pattern of Holstein dairy cows

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The number and secretion activity of the epithelial cells in the mammary gland determines milk production potential in dairy cows, so for dairy industries higher milk yield and longer persistency have economical importance. A dry period is a magic time course because in this short period, mammary gland cells incurred apoptosis, proliferation and differentiation.

Ten multiparous Holstein dairy cows in late pregnancy were individually fed one of two diets: palm oil (PO) and fish oil (FO). Diets were consumed for 45 days before expected calving up to 60 days after parturition. Two biopsy samples took from rear quarters on day 7 and 60 after their calving and prepared for histological. Six fields for each section have photographed and analyzed by using a digital image analysis program, Image J software.

The epithelial percentage in the first biopsy for FO was significantly greater than PO whereas, stroma percentage in FO was significantly ($p < 0.01$) less than PO. Also, mean number of epithelial cell count per each alveolus for FO mammary tissue was significantly ($p < 0.05$) further than PO. In second biopsy, the epithelial percentage of FO was significantly ($p < 0.01$) greater than PO but there were no significant differences between stromal percentages of PO and FO mammary tissues. Mean number of epithelial cell count per each alveolus of FO mammary tissue was significantly ($p < 0.05$) lower than PO. Because for PO's tissue slices it can be ascertained that the alveoli were non-uniform in their size and many numbers of huge alveoli surrounded a few small alveoli, however for FO most of alveoli in each area of tissue slides were isodiametric. Expression of gene IGF-I was significantly decrease in FO vs PO ($p < 0.05$).

Results showed supplemental fish oil had beneficial effects on the modulation of proliferation pattern of cells in mammary gland tissue of dairy cows, which would be directed to a more uniform mammary texture.

Clinical efficacy of a novel Flunixin Transdermal formulation for the control of pyrexia associated with naturally-occurring bovine respiratory disease: a multi-center field trial

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The objective of this prospective, randomized, masked, multi-center field study was to demonstrate the effectiveness and safety of Finadyne Transdermal® (FTD) in cattle suffering from fever associated with bovine respiratory disease (BRD).

The multicenter study was conducted between April and May of 2011 at four different geographic locations in the USA (Kansas, Texas, Nebraska, and California). Calves from auction markets arrived at the study sites, were identified with ear-tags, weighed, vaccinated (viral respiratory and 7-way Clostridial), and dewormed (only 2 sites). Enrollment criteria was a Respiratory Score of 2 or 3 (0 = normal; 1 = mild respiratory distress; 2 = moderate respiratory distress; 3 = severe respiratory distress), an Attitude Score of 2 or 3 (0 = normal; 1 = mildly depressed; 2 = moderately depressed; 3 = severely depressed; 4 = moribund), and a rectal temperature ≥ 40.3˚C. A randomized complete-block design was used at each location. Qualifying animals received either FTD at 3.33 mg/kg (1 mL/15 kg) or placebo at 1 mL/15 kg administered topically along the dorsal midline. At 6 hours ± 45 minutes after dosing (H+6), rectal temperature was measured and dosing site was examined.

A total of 251 calves (156 males, 95 females) predominantly Angus, Charolais, Hereford, and Simmental were enrolled. Body weights ranged from 102-281 kg. Rectal temperatures at enrollment ranged from 40.3-42.1°C. On one day at one study site, enrolled animals (16 calves) were exposed to hide-wetting rain between dosing and the H+6 observations; by protocol, these animals were excluded from the effectiveness analysis leaving 235 calves eligible for analysis. The mean (± SD) temperature decrease was 1.28 (± 0.59) °C in the FTD-treated group and 0.17 (± 0.61) °F in the control group ($p < 0.0001$). The proportion of calves with at least 1°F temperature decrease was 90.0% in the FTD-treated group and 24.3% in the control group ($p < 0.0001$). The proportion of calves with at least 1.1°C temperature decrease was 58.3% in the FTD-treated group and 6.1% in the control group ($p < 0.0001$). No abnormalities were reported at the dosing site in the FTD-treated animals.

This study demonstrated that FTD is safe and efficacious for the control of pyrexia associated with BRD in a variety of cattle in a broad range of environmental conditions.

Post-treatment dosing site assessment following topical administration of a novel formulation of Flunixin Transdermal solution: summary of seven studies

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Seven prospective, randomized, masked studies were conducted to examine hide response following administration of various formulations of Flunixin Transdermal (FTD).

Studies (numbered chronologically as Studies-1 through 7) were conducted between October 2008 and August 2010. A total of 632 animals received a single dose of FTD at either 2.5 or 5.0 mg/kg, which brackets the label dose of 3.33 mg/kg. There were 141 negative control animals. FTD was administered on the dorsal midline either from withers to tailhead (Studies 1, 2, 6, and 7) or just in the area of the withers so that hide reaction observations could be focused on one area (Studies 3-5). Studies were conducted at three locations in the USA (location/Study: Idaho/3, 4, 6; Texas/1, 7; Wisconsin/2, 5). Treatments were administered in in warm and cold environmental temperatures ranging from -9˚C to 35˚C. Animals were healthy bulls, steers, cows, and heifers. Breeds included purebred and crossbred Angus, Charolais, Hereford, Holstein, Wagyu, and Bos indicus. Animal body weights ranged from 145 to 699 kg. Topical dosing with FTD was done on Day 0. Each animal was examined at multiple times after dosing (ranging from Day 2 to Day 42).

Results indicated that FTD was well tolerated in most animals. Examination of the animals showed that dosing with FTD at 2.5 or 5 mg/kg had no or only mild reactions at any time. The mild skin abnormalities observed on FTD-treated and untreated control animals were considered acceptable for normal cattle handling practices. The number of treated animals exhibiting abnormal observations increased over time after dosing, peaking between Days 10 and 28. Only when observed, signs of irritation included skin flaking, dandruff, broken/brittle hair, thickened skin without signs of inflammation, and alopecia (thinning or bald spots). Dosing site reactions were not permanent and resolved within 6-7 weeks after dosing.

These studies indicated that FTD is safe to use in a broad range of ambient temperatures and in a wide range of cattle breeds. FTD causes mild to no irritation at the administration site. When observed, signs of irritation resolved without treatment and were acceptable in typical cattle handling conditions.

The efficacy of a single treatment with Cydectin® 10% Long Acting Injection at housing in the control of psoroptic mange in Belgian beef cattle

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Bovine mange may be caused by several mite species. *Psoroptes ovis* affects a large proportion of Belgian Blue cattle. This study aimed to evaluate the benefit of a single treatment with Cydectin® 10% Long Acting Injection (CLA: 1 mg moxidectin /kg bodyweight) early in the housing period, combined with re-treatment using Cydectin® 1% Injection (two treatments 7 to 10 days apart) of those animals that were positive for live mites at any follow-up visit, in order to manage the infestation and clinical disease at group level.

Two farms were selected and on each farm, 15 animals, positive for live mites, were enrolled. Animals were treated at housing (Day 0) and were examined for the presence of live mites at fortnightly intervals throughout the housing period. Also an animal based Clinical Index (CI) and a group based Scratching Index (SI) were recorded at each of these time-points. Before treatment the average mite count on farm 1 was 465 (range 1 - 3156), with an average CI of 10.2 (range 2.3 - 20,6) and a SI of 3.2. On Farm 2, the average mite count was 953 (6 - 5590), with an average CI of 10.3 (3.1 - 6.6) and a SI of 3.7. On Day 14, 8 and 11 out of the 15 animals still had positive mite counts on Farm 1 and 2 respectively. The average mite count in these positive animals was 26 and 102 and the average CI had decreased to 4.2 and 6.2, on Farm 1 and 2 respectively. These positive animals were re-treated. In the follow-up, 2 animals were still positive on Farm 1 and treated again. On Farm 2, 6 and 3 animals were positive and treated at subsequent time-points. On Farm 1, all animals were negative by Day 42 and on Farm 2 by Day 56. All animals remained negative for mite counts thereafter with low CI and SI.

This study demonstrates that a single administration of CLA provides a rapid decrease in number of mites and clinical symptoms, requiring only targeted follow-up treatments to provide adequate control of psoroptic mange in Belgian Blue cattle.

Influence of dipyrone on nociception of calves undergoing umbilical surgery

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Objective

In preventing nociception of calves undergoing surgeries, the commonly used anesthesia regimes (xylazine, ketamine, isoflurane, meloxicam) may be insufficient. Under the NSAIDs admitted for cattle in Germany, only dipyrone has an indication for pre-emptive analgesia. In the present literature no information is available regarding the analgesic potency of metamizole in calves. For this reason a double blind study in calves was conducted, in order to examine if additional application of dipyrone prior to an umbilical surgery reduces the operation induced cortisol release, as a possible sign of less nociception.

Methods

26 calves with uncomplicated umbilical hernia and unobtrusive general condition were randomly divided into a metamizole group (MG) and a control group (KG). The test procedure was strictly standardised. The groups differed only in the presurgical regime of analgesia. All calves received meloxicam (0.5 mg/kg IV) preoperatively. One hour before skin incision, the MG got 40 mg/kg dipyrone i.v. applied and the KG the equal volume of sterile sodium chloride solution. The calves were sedated with Xylazine (0.2 mg/kg IM), the anesthesia was induced with ketamine (2 mg/kg IV) and maintained with isoflurane (average of 1.4% etIso). Artificial respiration was given to all animals. For the analysis of plasma cortisol concentration, blood samples were taken 60 minutes before and 5, 30, 60, 150 and 510 minutes after skin incision. For the statistical analysis multiple linear models, mixed linear models and the Fisher's exact test were used.

Results

Considering the plasma cortisol concentration course, the cortisol level rose significantly higher in the KG (+11. 9 nmol/L, SD 5.09) than in the MG. 1.5 hours after the end of the operation, the cortisol concentrations differed on average 37.8 nmol/L ($p < 0.01$) between groups. While 92.3% of the MG reached the maximal cortisol concentration intraoperatively, 53.8% of the KG recorded it postsurcically. This variation was significant ($p = 0.03$).

Conclusions

The additional application of metamizole presurgically results in a significantly lower increase of the plasma cortisol concentration in the course of a umbilical surgery and to a quicker decrease of the cortisol level after the painful procedure. This could be a sign of decreased nociception.

Seasonal and environmental comparisons of red blood cell indices and Theileria infections from Holstein calves

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The purpose of this study is to compare seasonal changes of general CBC and the infection rates of *Theileria* for Holstein calves that are raised in pasture vs bred in crowded housings.

Holstein calf farm having the housings and the grazing land in Korea was selected as the target farm for this study. Blood samples were collected from 28 Holsteins in spring (March, before pasturing), 28 cows in summer (August, n = 10 in housing vs n = 18 in pasturing), and 30 cows in fall (November, n = 15 in housing vs n = 15 in pasturing). CBC and conventional PCR targeting *Theileria* were performed for all samples.

As results, RBC counts and hematocrit (Hct) values were lower in pastured group compared to housed group, and also lower after pasturing compared before pasturing ($p < 0.01$). *Theileria* infection rates were 11% in March but to 22% and 60% in Aug and Nov, respectively. Particularly, as compared to the *Theileria* negative cows in Mar, the RBC and HCT in pastured *Theileria* positive cows in Nov were remarkably decreased ($p < 0.01$).

A remarkable increase of vectors in summer may increase the infection rates of *Theileria*, which is the vector borne pathogen, so that RBC profiles of the cows pastured in summer and August were significantly different from cows in spring and raised in house.

Red blood cell indices were significantly affected by season, and environmental factors such as grazing in the field. Further studies regarding other vector borne pathogens and healthy status should be performed in the future.