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Postpartum uterine disease and immunity in cattle

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Introduction

Feeding the ever expanding population of the world is the grand challenge facing plant and animal scientists (Campbell, 2010). Dairy cows help to feed the world by converting low quality protein from plants to higher value proteins in milk, which have a more appropriate essential amino acid profile for human consumption and are more readily digestible; milk has at least twice the value compared with plant proteins in cereal-based diets (Schaafsma, 2000). Furthermore, milk is readily processed into many foods and products. Lactation depends on fertility and pregnancy but dairy production is facing a major obstacle because the fertility of dairy cows is at an all time low, due in a large part to post partum uterine disease (Sheldon et al., 2009). Uterine disease causes infertility, not just sub-fertility, and is therefore a significant burden on the dairy industry. The cost of replacing infertile animals, reduced milk yields and treatments for uterine disease is €1.4 billion each year in the EU (Sheldon et al., 2009).

Uterine disease

Bacteria contaminate the uterine lumen of about 90% of dairy cattle after parturition and half of these animals subsequently develop clinical disease. Uterine disease ranges from metritis within the first two weeks post partum to endometritis that can persist for several weeks. These clinical diseases affect about half of all dairy cows in some countries. An emerging challenge is the recognition that there are also many animals that have subclinical endometritis; although, the extent of the problem is not obvious to most clinicians or farmers because there is now cow-side diagnostic test.

Dairy cows are usually treated with parenteral antimicrobials for metritis and intra-uterine antimicrobials for endometritis. In addition, hormones such as prostaglandin F are used to induce luteolysis and oestrus to treat endometritis. The success rates for treatments are higher for less severe disease, and grading systems for metritis and endometritis have been devised to assist veterinary surgeons with prognosis as well as add value to their clinical evaluation of disease (Sheldon et al., 2009). Overall clinical cure rates for endometritis over a 2-week period are about 65% but many of these animals remain sub-fertile even though they appear healthy. Compromised uterine function is only part of the problem as uterine disease also perturbs ovarian function. Cows with uterine disease have smaller and less functional ovarian follicles, and are less likely to conceive (Sheldon et al., 2002).

The present article will discuss the mechanisms of uterine disease, and further detail is available in multiple reviews (Gilbert, 2011; LeBlanc et al., 2011; Sheldon et al., 2009).

Microbes cause postpartum uterine disease

The bacteria that are consistently associated with uterine disease are *Escherichia coli*, *Trueperella pyogenes* (formerly *Arcanobacterium pyogenes* and before that *Corynebacterium pyogenes*) and a range of anaerobic bacteria such as *Fusobacterium pyogenes*, *Bacteroides* and *Prevotella* species. Uterine disease is associated with specific strains of *E. coli*, called endometrial pathogenic *E. coli* (EnPEC) that were identified by molecular typing techniques, and are distinct from strains of *E. coli* that cause diarrhoea or mastitis (Sheldon et al., 2010). These EnPEC strains are more adherent and invasive to bovine endometrial cells than other strains of *E. coli*. As well as the role of EnPEC in disease, it is well established that the presence of *T. pyogenes* is correlated with the extent of endometrial pathology (Bonnett et al., 1991). Both *E. coli* and *T. pyogenes* stimulate inflammatory responses in the endometrium (Borges et al., 2012; Herath et al., 2009b). The genotyping and phenotyping of bacteria that contribute to uterine disease remains an area of active investigation. In particular, a multitude of anaerobic bacteria have been identified in the endometrium using molecular biology rather than standard microbiology techniques. There is also an emerging role for viruses that contribute to uterine disease with bovine herpesvirus-4 the main candidate because the virus is tropic for bovine endometrial cells (Donofrio et al., 2007).

Immunity and inflammation in the uterus

The two main arms of immunity are innate and adaptive immunity. Innate immunity provides non-specific, immediate alarm-type responses to microbes, initiates inflammation and directs the adaptive immune response. Adaptive immunity provides a slower but more sophisticated and antigen specific response against matter recognised as “non-self”. However, the recognition of non-self in the endometrium is a problem after conception because the embryo is semi-

allogeneic being derived from the father as well as the mother. So, the adaptive immune response is highly regulated in the uterus to tolerate the semi-allogeneic embryo. On the other hand, innate immunity rapidly responds to pathogens without being triggered by the embryo, fetus or placenta.

Innate immunity has multiple systems for the non-specific defence of the host against microbes, including antimicrobial peptides, the epithelial barrier, and the complement cascade. Impetus for the expansion of research in innate immunity came from seminal discoveries made in the laboratories of Jules Hoffmann and Bruce Beutler of specific cellular "pattern recognition receptors" that detect pathogen-associated molecular patterns (PAMPs) commonly associated with microbes (Ferrandon et al., 2007; Ronald and Beutler, 2010), which led to the award of the 2011 Nobel Prize in Physiology or Medicine. The first discovery was that the "Toll" protein involved in embryonic development of the fruitfly *Drosophila melanogaster,* was also required for an effective immune response to the fungus *Aspergillus* (Ronald and Beutler, 2010). The next major advance was the discovery that Toll-like receptor 4 (TLR4) in mammals was necessary for the inflammatory response to the lipopolysaccharide (LPS) cell wall component of bacteria, which acts as a PAMP (Ferrandon et al., 2007). Interestingly, whilst TLR4 binding to LPS should lead to an appropriate immune response to defend against bacteria, in some situations over exuberant TLR4-dependent inflammation exacerbates disease, which is why LPS is also commonly called "endotoxin".

The pattern recognition receptors of the innate immune system appear to have some importance for the detection of microbial infection and PAMPs in the uterus of cattle. Using endometrial biopsies from postpartum dairy cows we confirmed that the Toll-like receptors (TLRs) were expressed in the bovine endometrium and that genes encoding inflammatory mediators were activated during bacterial infection post partum (Herath et al., 2006; Herath et al., 2009b). Surprisingly TLRs were not only expressed by immune cells such as neutrophils but were also present in purified populations of epithelial and stromal cells isolated from the endometrium of dairy cattle (Davies et al., 2008; Herath et al., 2009b). Furthermore TLR4 is functionally active in endometrial epithelial and stromal cells from many species and is able to detect LPS from *E. coli* (Cronin et al., 2012; Sheldon and Bromfield, 2011; Sheldon and Roberts, 2010). To investigate the importance of TLR4 expression in endometrial cells, the production of the TLR4 protein was inhibited at the cellular level using short interfering RNA (siRNA) technology (Mello and Conte, 2004). Indeed, the inhibition of TLR4 in endometrial cells ameliorated the effects of LPS, confirming the essential role of TLRs in the recognition and response to microbes in the bovine endometrium (Cronin et al., 2012).

Exploring the interactions between immunity and endocrine function further extended the clinical relevance of the discovery that endometrial epithelial cells have roles in innate immunity. The TLR4-mediated response to LPS in the endometrium also switches prostaglandin production by the epithelial cells from the luteolytic F series to the luteotrophic E series (Herath et al., 2009a). The increased prostaglandin E_2 produced in response to LPS from *E. coli* may help explain the established clinical observation that uterine disease can delay luteolysis. Conversely, if animals ovulate but still have microbes in the uterus, then progesterone perturbs the TLR4 mediated response to LPS. Perhaps removing the negative impact of progesterone on immunity, when animals are treated with prostaglandin $F_{2\alpha}$, helps to resolve clinical endometritis. Beyond the existing treatments, researchers are now targeting the components of the TLR cell signalling pathway using small molecules to regulate the inflammatory response (Cronin et al., 2012).

Immunity and inflammation in the ovary

A novel aspect of our work was the discovery that bacterial infection of the uterus perturbs postpartum ovarian function. Specifically, the presence of bacterial infection in the uterine lumen reduced the rate of ovarian follicle growth and perturbed follicle function as determined by reduced oestradiol secretion (Sheldon et al., 2002). Furthermore, the peripheral plasma concentrations of progesterone are lower in cows with uterine disease than those in normal fertile animals (Williams et al., 2007). The mechanisms linking uterine disease to ovarian function were not obvious because bacteria are rarely found in the ovary and ovarian follicles are devoid of immune cells such as macrophages. However, LPS was found in the follicular fluid of diseased cows, perhaps reaching the ovary by the same vascular mechanisms used by prostaglandin. Furthermore, the granulosa cells that line ovarian follicles expressed TLRs, and PAMPs perturb their endocrine function as well as causing inflammation (Bromfield and Sheldon, 2011; Herath et al., 2007). The essential role of granulosa cells in innate immunity was confirmed by targeting TLR4 using siRNA, and this reduced TLR4 expression limited the expected cytokine response to LPS (Bromfield and Sheldon, 2011).

A further question was whether perturbations of the ovarian antral follicle were the only mechanism linking uterine disease to lower ovarian fertility, or whether there may also be a direct impact on the egg. Mammalian oocyte growth and maturation from the primordial follicle until ovulation is dictated by a highly ordered cascade of hormones, growth factors, nutrients, and signalling molecules from the surrounding environment (Albertini et al., 2001; Matzuk et al., 2002). *Ex vivo*, LPS increases primordial follicle activation in the ovarian cortex, which reduces the primordial follicle pool (Bromfield and Sheldon, 2013). In addition, ovarian cortex cultures produce the inflammatory mediators IL-1β, IL-6 and IL-8 in a LPS concentration-dependent manner and modulated typical intracellular regulators of follicle activation (Bromfield and Sheldon, 2013). The reduction in the primordial ovarian follicle pool in the bovine ovarian cortex could help explain why infections around calving have longer term effects on fertility.

Oocytes must undergo nuclear and cytoplasmic maturation for successful fertilization and embryonic development, and oocytes progress from the germinal vesicle stage until pausing at the M-phase of meiosis II. The oocyte is nurtured by the surrounding cumulus granulosa cells via trans-zona projections from the cumulus cells which cross the zona pellucida and synapse on the oolema, allowing bidirectional communication between the granulosa and oocyte (Albertini et al., 2001; Matzuk et al., 2002). These intimate interactions expose mammalian oocytes to exogenous factors more than eggs enclosed in an impermeable shell. We therefore considered whether in the absence of immune cells, cumulus granulosa cells may play a role in protecting mammalian oocytes against PAMPs. Indeed, the cumulus granulosa cells mount inflammatory cytokine responses to PAMPs (Bromfield and Sheldon, 2011). Furthermore, we were the first to show in any species that the meiotic spindle, containing the chromosomes that should form the embryo, was disrupted in oocytes treated with LPS (Bromfield and Sheldon, 2011). This damage of oocytes by PAMPs may help explain how infertility persists beyond the duration of clinical uterine disease in dairy cows.

Conclusions

Uterine disease is a common and important cause of infertility in cattle, which compromises global food security. The current treatments and veterinary management of uterine disease leaves considerable room for improvement, yet little progress has been made through applied research. An alternative approach to developing new therapeutics is to understand the fundamental biology of the host-pathogen interactions that lead to uterine disease in postpartum cattle. Bacterial diseases are common and an important cause of infertility in cattle, which includes perturbation of ovarian function. Endometrial cells and ovarian granulosa cells have roles in innate immunity and respond to PAMPs via TLR pathways. Furthermore, even the oocyte is susceptible to damage by PAMPs. These mechanistic findings are providing new target therapeutic pathways, which the pharmaceutical industry is exploring toward smarter ways of treating or preventing uterine disease.

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Differential Immunoglobulin G glycosylation in postpartum dairy cows with uterine disease: potential for a predictive test

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Uterine disease in dairy cattle is a significant inflammatory condition of the uterus, resulting in reduced fertility. It has been suggested that the immune alterations to pregnancy and the reversion to normal during the peri-partum period are important in post partum uterine health. Immunoglobulin G (IgG) is the predominant immunoglobulin in the bovine uterus and is an important immune mediator of pathogen defence. During pregnancy, the immune actions of IgG are modulated by glycosylation and this is reversed after birth. Differences in IgG glycosylation are associated with chronic inflammatory disease. Therefore, we hypothesized that IgG is differentially glycosylated in healthy cows and cows with postpartum uterine disease.

Serum samples were collected from 96 dairy cattle approximately 10 days before calving and on days 7, 14 and 21 postpartum. Uterine health was monitored by regular vaginal mucus assessment and animals were retrospectively diagnosed based on standard definitions. IgG glycans were isolated and fluorescently labeled using an automated glycomics platform, followed by exact quantification by ultra performance liquid chromatography with fluorescence detection.

Thirty-one glycan peaks, each representing a different glycan structure, were identified on the bovine IgGs. In cows with uterine disease, there was an increase ($P < 0.05$) in the percentage of IgG fucosylation as identified by higher quantity of fucosylated glycans. The level of IgG fucosylation in diseased cows was significantly increased on day 10 pre-calving and on days 7, 14 and 21 post-calving ($P < 0.001$) compared to cows without uterine disease. Using a data classification and regression training model we were able to correctly predict the disease phenotype based on the IgG fucosylation ratio with an accuracy of 71% on day 10 pre-partum, 82% on day 7 postpartum and 90% on day 14 postpartum ($P < 0.001$).

In conclusion, fucosylation of IgG is increased in cows that suffer from postpartum uterine disease possibly indicating reduced immune function in these animals. Furthermore, these differences are detectable before calving and in the first week postpartum, offering the potential of using IgG fucosylation analysis as a predictive biomarker of disease. (United Kingdom Patent Application No. 1315705.2).

Validation of two diagnostic methods for endometritis in postpartum dairy cows

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The objectives of this observational study were to determine diagnostic criteria for two endometritis diagnostic methods and to quantify their impact on subsequent reproductive performance in postpartum dairy cows. Data from 558 untreated Holstein cows (25 herds) enrolled in a randomized clinical trial were used. Cows were examined 34 (± 7) days after parturition for endometritis using a standard cytobrush technique. After collection of the endometrial sample, the cytobrush was rolled on a microscope slide for subsequent cytological evaluation using a microscope and was dipped into 1 ml of sterile water for leukocyte esterase (LE) colorimetric testing (Multistix®, Bayer Corporation, Elkhart, IN). The voluntary waiting period for breeding was 50 days. Subsequent reproductive events were recorded up to 250 days after parturition. Diagnostic criteria for endometritis were determined based on the maximal sum of sensitivity and specificity for predicting the risk of pregnancy at 120 days after parturition. The impact of these diagnostic criteria on reproductive performance were quantified using logistic regression and Cox proportional hazard models adjusted for herd clustering effect. Cytological endometritis (CYTO) was defined as ≥ 6% polymorphonuclear cells in endometrial cytology. Endometritis determined by LE testing was defined as ≥ ++. Prevalence of endometritis based on CYTO and LE disease definitions were 41 and 32%, respectively. Both diagnostic methods were associated with a detrimental impact on first service conception risk (CYTO: Yes = 19.3%, No = 35.4%, $P < 0.01$; LE: Yes = 24.5%, No = 35.9%, $P < 0.01$) and on median time to pregnancy (CYTO: Yes = 158 days, No = 113 days, Hazard Ratio = 1.27, $P < 0.01$; LE: Yes = 136 days, No = 105 days, Hazard Ratio = 1.28, $P < 0.01$). These findings suggest that CYTO and LE results can be used to diagnose endometritis in postpartum dairy cows.

Immune response profile of a standardized *E. coli* challenge in late gestation versus mid lactation

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Coliform mastitis that presents itself at parturition or in the early weeks of bovine lactation is often characterized by severe inflammation and impaired milk production and can lead to death of the animal. Intramammary infections caused by persistent strains of *Escherichia coli* may result in high production losses. The aim of this study was to determine the difference in inflammatory response profile to an intramammary challenge of bovine mammary glands in either late gestation or mid lactation with a persistent strain of *E. coli*. In 10 cows, approximately two weeks before parturition and in six cows in mid lactation, animals were challenged in 2 quarters with 30 cfu of a persistent strain of *E. coli*; control quarters were vehicle-infused and not infused, respectively. Samples of dry cow secretions and milk were taken from all quarters before challenge and at 6, 12, 18, 24, 48, 72, 96, and 120 h following challenge. Bacterial culture, combined with random amplified polymorphic DNA genetic strain-typing analysis, indicated recovery of the bacterial challenge strain in the majority of challenged quarters. Clinical signs were virtually absent after challenge in late gestation cows, while cows in mid-lacation showed signs of a more typical coliform mastitis in mid-lactation with abnormal milk, serum-like milk and moderate systemic signs. Cytokine profiles in cows that were challenged in late gestation were dramatically different from cytokine profiles in cows that were challenge in mid-lactation. Cytokine analysis indicated a minimal proinflammatory cytokine response, including interleukin-1β and tumor necrosis factor-α in late gestation challenged cows, while a standard pro-inflammatory response was observed in cows that were challenged in mid-lactation. This was the first study comparing a fully identical challenge protocol in cows in late gestation versus cows in mid-lactation. The late gestation challenge model turned out to be a reliable and repeatable. The results indicate that proinflammatory signaling after intramammary bacterial infection may be actively suppressed during late gestation while present in mid-lactation. We hypothesize that this immune-inhibitory response allows intramammary infections to become persistent in the dry period and cause clinical signs immediately after parturition.

Modulating mammary immunity in the postpartum cow: a bold challenge for the future

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Postpartum cows carry the highest risk of severe systemic response syndrome caused by intra mammary infection. Impaired function of innate defense mechanisms allow uncontrolled bacterial growth in milk setting off a cytokine storm. Several reasons for this inadequate response have been analyzed during the last decades highlighting parity and metabolic/endocrine changes around parturition. However the exact mechanisms of this compromised immune response remain unsolved. Recently we could show for primiparous midlactating cows, that intramammary stimulation with pathogen associated molecular patterns increased bacterial clearance and prevented acute clinical mastitis. This immunomodulatory concept aimed at inducing a transient refractory state to pathogen threats in mammary tissue and simultaneously improving endogenous antimicrobial actions. We adapted this concept to freshly calved multiparous cows. Three days after receiving intramammary LPS pretreatment, cows were challenged in one quarter with 500 cfu *Escherichia coli*. Compared to control animals, intramammary LPS pretreatment resulted in a significant reduction of mastitis severity after *E. coli* challenge, however it did not completely prevent the disease. The pathogen load in milk was reduced faster and stronger in pretreated animals demonstrating improved antimicrobial activity. The initial response to intramammary LPS treatment was less marked in postpartum compared to midlactating animals. The data indicate that cows postpartum initially show a reduced intramammary tissue reactivity to pathogen threats which may be the underlying reason for excessive bacterial growth during postpartum mastitis. Immunohistological analysis revealed a differential composition of S100A8/A9 and CD163 positive macrophages in the teat, dependent on the lactation stage. This may have contributed to the repeatedly observed different response towards intramammary LPS and *E. coli* challenge. Acute clinical mastitis may not be eradicated in future, however the reduction of disease severity may decide the future productivity of the individual. Thus immunomodulatory concepts for mastitis prevention should aim at the alternation of the tissue or local responsiveness in a defined time period. This can basically be achieved by the selective attraction of innate immune cells, their biased in situ differentiation, the selective inhibition of immune cell attraction, or the induction of a transient refractory state of the tissue.

Improving udder health in Swiss dairy herds: a one-year randomized field trial

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Mastitis remains one of the most important cattle diseases in Switzerland. Many farm-specific risk factors are known but farmers do not always implement the specialist advice. Several strategies exist to support farmers to improve udder health but the most effective has not been identified. The aims of this study were (i) to decrease the proportion of cows with high somatic cell count (SCC) by 20% within one year and (ii) to identify the most effective support strategy to achieve this.

One hundred Swiss dairy farms with a bulk milk SCC between 200,000 and 300,000 cells/ml in 2010 were recruited for a one-year randomized field trial. The herds were visited between September and December 2011 to evaluate their udder health management and were randomly allocated to four intervention groups. Group 1 formed the control group and received neither recommendations nor any active support. The remaining 75 farmers received a farm-specific report with recommendations in order to improve the udder health. Group 2 received no further active support during 2012. Group 3 additionally got support in the form of monthly visit by their private practitioner. Group 4 got support in the form of bimonthly study group meetings where different issues concerning udder health were discussed. One year later, the implementation of the recommended management changes was evaluated.

875 recommendations selected from a list of 77 different recommendations were given. Most recommendations were given in the topic “milking” (n = 419), followed by “milking machine” (n = 223), “environment/housing” (n = 116), “cow specific” (n = 73) and “dry period” (n = 44). Each farm received between seven and seventeen recommendations. Of the farms that received a report, 67.3% of the recommendations were partially or fully implemented. The three support strategies did not show any significant differences in the degree of recommendation implementation. However, the farmers randomly assigned to their preferred group before enrollment did significantly implement 8% more than farmers assigned to an intervention group they initially preferred not to belong to. A farmer-preferred support strategy may therefore result in a greater udder health improvement. Further multivariable analyses will evaluate the effects of the farm-specific report on the udder health improvement.

Bovine Respiratory Disease: diagnosis and prevention

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DIAGNOSIS OF BOVINE RESPIRATORY DISEASE

Introduction

Bovine respiratory diseases (BRD) have a major impact on the feedlot industry in North America (Fulton et al., 2009; Griffin, 1997). Economic losses are due to mortality, cost of therapy and prophylaxis, and reduced performance. Veterinarians and animal owners are faced with challenges of accurate and timely diagnosis of ill and dying cattle to implement intervention strategies to control or minimize BRD, the clinician and those managing the animals are the first line of defense in disease control. Observation of the animals to detect clinically affected animals is important. However, clinically ill animals often do not exhibit signs or lesions which are diagnostic for a specific etiology.

There are several questions facing animal owners and veterinarians with respect to BRD diagnostics:

- sick calves with BRD signs recover from illness. What caused the disease?
- calves dying after acute illness. What caused the disease?
- cattle dying after prolonged disease and treatment. What caused the disease initially, why did the treatment fail, and what was the cause of death?
- above cattle were “well-vaccinated”. What went wrong with the vaccinations?
- whenever an agent is found, is it an “infection looking for a disease”?
- experimental infections and disease? How to prove the infecting agents cause disease?

In veterinary medicine, particularly food animal production, diagnostic testing is used for prevention and control measures. Four disease intervention areas are:

- vaccination programs,
- selection of antimicrobial treatment for infected animals,
- attempts to remove the cause of disease, usually infectious, but metabolic and toxicity causes are often involved,
- implementation of a biosecurity plan.

Veterinary medicine and the animal industry also have a definite role in animal health regulations, both federal and state, such as reporting potential foreign animal diseases, as well as those North American agents regulated for cattle movement.

Diagnostic support for the veterinarian and cattle production system has been by three areas:

- state and provincial diagnostic laboratories, with most accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD),
- the Canadian Food Inspection Agency (CFIA) diagnostic laboratories and USDA National Veterinary Services Laboratory,
- a growing number of private laboratories offering a variety of tests.

Diagnostics in veterinary medicine

In addition to the initial examination of live affected animals and obtaining a history, important areas for the veterinarian to gain information include:

- assessment of involved organ system(s) by gross and microscopic lesions (histopathology),
- identification of the etiologic agents(s),

- contributing factors such as metabolic defects and altered nutrition. An example of the contributing factor and the role of the diagnostic laboratory regarding the identification of the etiology/diagnosis might be the effect of mineral deficiencies interacting in the pathogenesis of disease.

All three areas might require tests by the diagnostic laboratory for veterinarians providing prevention and control recommendations.

Diagnosis of BRD pathogens utilizes detection of lesions of involved organ systems plus detection of etiology. What are the "Gold Standards for diagnosis"? Usually there is a request to identify the infectious etiologic agents. These agents include numerous viruses [bovine herpesvirus-1 (BoHV-1), parainfluenza- 3 virus (PI-3V), bovine viral diarrhea viruses (BVDV), bovine respiratory syncytial virus (BRSV), bovine adenoviruses (BAV), and bovine coronavirus (BoCV)], and several bacteria (*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma* spp.) (Fulton et al., 2009; Van Donkersgoed et al., 1994). It is important to address the infection versus disease issue. In the fall 2010, a post on the AAVLD LISTSERVE (August 13, 2010) concerning diagnostic tests and diagnostic criteria, "Gold standard relative to what diagnostic question you are attempting to answer? Clinical disease? Subclinical disease? Infection without disease? The best diagnostic test may vary according to the question asked:

- organism identification (ID) without lesions is confirmation of infection,
- organism ID + lesion = proves disease (may be clinical or subclinical),
- organism ID + lesion + clinical signs explained by the lesions = proves clinical disease,
- serology = provides a nice history of exposure, but often fails to shed significant light on disease status".

There are instances in which evidence of infection is identified without concurrent disease manifestations; this can still be of diagnostic significance because of their regulatory significance. These include brucellosis, trichomoniasis, and anaplasmosis. Each of these diseases is characterized by a persistent infection and measurable immune response with little chance of natural clearance of the infectious agent.

Glossary of terms related to laboratory diagnostic testing and epidemiology

Seroconversion

This is production of increased concentrations of antibodies capable of binding the antigen in a defined assay system (Tyler and Cullor, 1989). It is assumed that the initial sampling is performed shortly after antigenic challenge and that the 10 to 14 d between sample collections permits the detection of an increasing titer to the agent (Tyler and Cullor, 1989). Seroconversions are used for the detection of active infection with resulting antibody increase.

Sensitivity

Sensitivity is used to help rule out a disease (Drobratz, 2009). If a test is 100% sensitive, then all animals with disease test positive. Therefore, if the test is negative, then the animal does not have the disease and it can be ruled out. There can be false positives, however.

Specificity

Specificity is used to rule in a disease (Drobratz, 2009). If the specificity of disease is 100%, then all of the animals without disease test negative. Similarly, if the animal tests positive, it has the disease and can be ruled in for the disease. There can, however, be false negatives.

Positive predictive value

Predictive values are more intuitive than sensitivity and specificity (Drobratz, 2009). Positive predictive values (PPV) answer the question, "if an animal tests positive, does it have disease?" The PPV is the ratio of the number of test positives to the number of true positive tests. Negative predictive value (NPV) answers the question, "of the animals testing negative, how many actually do not have the disease?" The NPV is the ratio of number of test negatives to the number of true negatives.

Prevalence

Prevalence is the number of cases that are present in a population at a given time (Drobratz, 2009). This is opposed to the often-misused term, *incidence,* which is a measure of the risk of developing disease and is a fraction of a population developing disease during a given period time, i.e., the rate of new cases.

Diagnostic tests

Serology

Laboratories providing antibody assays have mainly focused on viruses.

Virus neutralization tests (VNT) involve neutralization of a fixed amount of virus by the serum containing antibodies. The titer is the inverse of the highest dilution causing neutralization (endpoint). The term seroconversion for VNT is indicated when there is a 4-fold increase in VNT titers between the acute and convalescent sera, which may take 10 to 14 d to develop (20).

Enzyme-linked immunosorbent assays (ELISA) measure primary binding of antigen to antibody. Results of ELISA are often reported as the optical density of resulting reaction of the second antibody tagged with an enzyme reacting with its substrate. The second antibody in this case would be antibody to the Ig subclass reacting to the antigen. Potentially, reactions to the different Ig subclasses (IgM, IgA, and IgG) can be determined using appropriate reagents. In addition to reporting results as optical density values, sometimes serum dilutions are made, and endpoint ELISA titers are reported. The laboratory may provide information on interpretation of titers relating to seroconversion. The ELISA for antibody determination can be reversed with assays detecting antigen, such as the antigen capture ELISA (ACE) for BVDV antigen detection and the ELISA for detection of human RSV antigen used by some diagnostic laboratories to detect BRSV. VNT measures antibodies that interfere with viral infection and, therefore, provide a functional indication of antibody efficacy against the virus. ELISA results, in contrast, provide only indication that antibodies bind to the virus, and those antibodies may bind portions of the virion that are not essential for viral invasion of host cells. Detection of antibodies to bacteria, including *Rickettsia* spp., *Mycoplasma spp*., chlamydia, and protozoa varies widely with the agent. The methods of detection may include ELISA, as described, complement fixation tests (CF), and/or agglutination tests. Often testing for shipment of semen, fertilized ova, and animals for international movement requires special testing, and must be performed by certain accredited laboratories. In addition to the assays described, toxin-neutralization assays can be used for certain bacteria that secrete toxins. Detection of antibodies to *Mannheimia haemolytica* leukotoxin in cattle is a good example of this. Agglutination or ELISA tests have been used to measure the antibody responses to bacterial somatic antigens, whereas leukotoxin (LKT)-neutralization (LN) assays and ELISA have been reported for measuring antibody responses to the LKT. As with VNT and ELISA for viral infections, an LN assay indicates a functional antibody that interferes with the toxin-induced cytolytic process. In contrast, anti-LKT ELISA only measures binding of antibodies to all parts of the molecule. Although there is good statistical correlation between anti-LKT ELISA and LKT-neutralization assays when numerous sera are tested, LN may be a better indicator of possible protective immunity than would anti-LKT ELISA.

Viral isolation

Viral isolation (VI) tests by diagnostic laboratories have utilized attempts to grow virus in cell cultures from diagnostic samples (Jerome, 2010). These have been used traditionally until recent use of molecular diagnostics (discussion follows). VI tests use susceptible cell cultures for inoculation. During incubation, evidence of virus replication may be observed visually under the microscope as cytopathic effects (CP). However, not all viruses show cytopathology, such as noncytopathic (NCP) BVDV, and other detection methods are used confirming virus presence such as antigen detection by fluorescent antibody, ELISA, or mmunohistochemistry (IHC). For VI, initial incubation usually is 7 d, and if no CP or antigen is detected, a second incubation is used. A downside to VI in cell cultures is that 2 passages are used, taking at least 2 wk before a test is called negative. Secondly, there may be a lack of antibody reagents for identifying "new or re-emerging viruses." Thirdly, some bovine viruses may require a special cell line for the virus to be identified, such as bovine coronavirus-susceptible human rectal tumor cells (HRT), which was not readily available to many laboratories for several years after their susceptibility to bovine coronavirus was demonstrated. Thus, there is growing use of molecular diagnostic tests such as polymerase chain reaction (PCR).

Electron microscopy (EM)

Electron microscopy is used primarily for identification of enteric viruses; however, viruses from respiratory swabs can also be identified. Samples, often from nasal swabs and fecal swabs, are prepared for EM for visualization for viral morphology in negatively stained samples (Jerome, 2010). In addition, ultra thin sections from fixed tissue may be examined for viral morphology.

Bacterial isolation

Isolation of bacteria from cases of respiratory disease can be done from nasal, nasopharyngeal, or tracheal swabs; transtracheal wash or bronchoalveolar lavage fluids; or lungs at necropsy. These samples should be maintained cold but not frozen when submitted to the laboratory. Lung specimens should be large enough where the surface of the lung specimen can be seared without killing the bacteria within the tissue. Bacterial growth is usually attempted on blood

agar plates with selection of colonies for further testing. If the specimen yields multiple colony types, which is the norm for nasal and nasopharyngeal specimens, a trained technician must select colonies that appear similar to those that are known to be from pathogenic bacteria. This selection is only as good as the technician doing the selection.

Use of nasal, nasopharyngeal, transtracheal, and bronchoalveolar samples to determine the cause of pneumonia in a living animal can provide data that can be helpful for the clinician. However, several considerations must be kept in mind. First, as described, if multiple bacteria grow on the plate, the selection and identification of the one(s) responsible for pneumonia can be missed by an inexperienced technician. Secondly, all bovine respiratory bacterial pathogens can be members of the normal upper respiratory flora, and identification of one or more of such bacteria does not necessarily indicate that an organism is the cause of the disease or lesion. A laboratory indication of large numbers of colonies of one or more of those bacteria does strongly infer a cause and effect relationship. Thirdly, one of the most frustrating aspects for a pathologist is when bacterial isolation from an obvious case of bacterial pneumonia is negative due to antimicrobial therapy. Finally, use of nasal, nasopharyngeal, transtracheal, or BAL cultures in a living animal as a surrogate for lung culture is less than perfect. For example, when bacterial isolates from nasal and tracheal samples from cattle were compared, there was 96% correlation between the bacterial species recovered from the 2 sites (DeRosa et al., 2000). When isolates were genetically tested with ribotyping and antimicrobial sensitivity, only 70% of the nasal and tracheal samples were similar, indicating that multiple strains of individual bacterial species are carried in the respiratory tract of cattle.

In another study, *M. haemolytica* and *Mycoplasma bovis* were isolated from deep nasopharyngeal swabs collected prior to euthanasia and from the lungs collected by lung lavage or from tissue samples at necropsy and were compared genetically (Godinho et al., 2007). There was 86% correlation between upper and lower respiratory isolates of *M. haemolytica* and 100% correlation for *M. bovis* isolates; however, this study only represents isolates from 10 calves.

Fluorescent antibody tests

The direct fluorescent antibody test (FA) is a laboratory test that uses fluorescent dye-tagged, agent-specific antibodies to detect the presence of infectious agents (Jerome et al., 2010). Fresh/frozen tissue sections, cytology preparations, or tissue touch preparations are fixed with acetone or alcohol, and reacted with the fluorescently labeled antigen-specific antibody. If fluorescence is observed microscopically, this is a positive test for antigen. Another form of the FA test is the indirect FA test. Untagged antigen-specific serum is reacted with the tissue, and a second fluorescein-labeled anti-immunoglobulin is reacted as well. The direct FA test has been widely used by veterinary diagnostic laboratories since the days of hog cholera control programs. Most BRD viruses may be identified in tissues by FA testing. A drawback to the FA test is that samples cannot be stored or archived for long due to rapid decay of the dye activity. Therefore, one cannot send a slide of an FA test to another laboratory for consultation. This difficulty is overcome by using an immunohistochemistry test (description follows).

Immunohistochemistry

Immunohistochemistry (IHC) has become a much used diagnostic tool in veterinary diagnostic laboratories. It is used when infected tissues embedded in paraffin are available as starter material (Jerome et al., 2010). The potential use of IHC for identification of viruses in cells obtained from bovine BAL has been demonstrated (Narita et al., 2000). In the case of tissues, the paraffin block is sectioned and mounted on slides as done for routine histopathology preparations. The sections are deparaffinized, hydrolyzed, digested with proteinase, and incubated with monospecific immune serum or an antigen specific monoclonal antibody. The sections are then reacted with an-immunoglobulin-specific antiserum tagged with an enzyme such as horseradish peroxidase. The enzyme reacts with a color substrate and the localized color reaction identifies the antigen in the tissue section. This permits detection of antigen within a lesion or within specific cells by light microscopy. The positive IHC test along with the presence of microscopic lesions offers the strongest evidence for an infectious agent being associated with lesions. The accuracy of the IHC test depends on the specificity of the monoclonal antibody or hyperimmune serum for the infectious agent. The limitation to this test is often the lack of reactive monoclonal antibodies to selected agents, especially newly recognized ones.

Molecular amplification methods in the diagnostic laboratory

Several molecular based assays are used in veterinary diagnostic laboratories to detect pathogen DNA or RNA (26). Users should consult with their laboratory for available tests and the interpretation of test results. There is often not a standard protocol, and thus each laboratory should provide information for the interpretation of results. The critical information required for this testing is the genetic sequence information for the agent so that oligonucleotide primers can be produced and used for testing. These PCR-based tests are widely used in veterinary medicine; however, strict adherence to good laboratory practice must be observed to obtain consistent and credible results. Methods of reporting data and quality control standards can vary from laboratory-to-laboratory. Veir and Lappin (2010) recently recommended to small animal veterinarians, but the same would apply to large animal veterinarians, that if a new molecular diagnostic test is published; the originating laboratory should be used, because they will be more familiar

with the nuances and have more experience with the largest number of samples. Users should consult with the Web sites for the diagnostic laboratories using PCR and IHC. Two Web sites have comprehensive lists of molecular tests available to veterinarians: http://ihc.sdstate.org and http://pcr.sdtate.org

Descriptions of molecular tests follow (Jerome et al., 2010). One of the most common uses of molecular technology in microbiology is for identification of bacterial genera, species, and subspecies as well as viral genotyping. These technologies allow laboratories to rapidly identify bacteria without the requirements of additional time-consuming biochemical tests. In the case of *P. multocida,* capsular type of A, B, D, E, or F can be determined without cumbersome enzymatic digestion techniques. Similarly, molecular techniques are used for identifying
BVDV types 1 and 2.

Conventional PCR detection

A known genetic region is amplified in a thermocycler using polymerase to produce an amplified segment of nucleic acid. Those products are then compared to known positive controls using gel electrophoresis or sequenced and compared to published sequence for the specific agent. For RNA viruses, a reverse transcriptase enzyme reaction is required. The PCR product is directly visualized using agarose gel electrophoresis with dyes such as ethidium bromide. These gel-based PCR assays are qualitative, indicating only presence or absence of visualized product of the amplification. Some tests use a "nested "format, wherein a second round of amplification is used with another set of primers. Most laboratories have moved away from gel-based PCR to the real-time PCR formats.

Multiplex PCR formats

An advancement of PCR testing has been multiplex PCR, wherein multiple viruses and/or bacteria may be detected with one test. This greatly decreases costs to the veterinarian when compared to the one PCR assay/one pathogen approach described. It is also valuable for diseases such as BRD when multiple viral and bacterial pathogens can be involved. Primers producing different sized fragments permit the simultaneous detection of nucleic acids from various infectious agents based on the size of the product. These multiplex PCR tests are often used for human virus detection, especially for multiple human respiratory viruses in the differential diagnosis including influenza, coronaviruses, parainfluenza viruses, and respiratory syncytial virus. These multiplex PCR tests have been attempted for veterinary medicine, mostly for research purposes including typing of *Salmonella* spp., *P. multocida, Haemophilus parasuis,* and toxigenic *Escherichia coli* from pigs and *Clostridium* spp. in meat. Recently, a multiplex PCR test for BHV-1, BVDV, and PI-3 viruses was described (Horwood and Mahony, 2011). Several veterinary diagnostic laboratories are now offering the multiplex PCR for bovine infectious agents including BRD agents.

Real-time PCR

Real-time PCR has largely taken over molecular diagnostics, including molecular tests used in veterinary diagnostic laboratories. The most common sequence-specific oligonucleotide probe format used in diagnostic real-time PCR is the dual-labeled *Taq*Man probe consisting of a fluorescent reporter dye coupled at the 5'- end and a quenching dye at the 3'-end (Jerome et al., 2010). When the probe is intact, the close proximity of the quenching dye prevents the emission of the fluorescent dye. However, during PCR primer extension, the DNA polymerase enzyme digests any bound *Taq*Man probe, separating the 2 dyes. The reporter dye is no longer suppressed by the quencher dye and may now emit a fluorescent signal. The principle behind the quantitative real-time PCR (qPCR) is that during thermocycling PCR amplification will begin sooner in specimens containing a higher infectious agent nucleic acid load compared to a specimen with a lower infectious agent load. This will be observed as earlier generation of fluorescent signal [or earlier cycle threshold values (Ct)]. Conventionally, qPCR protocols are set for 40 cycles, which yield in theory, a trillion amplicons. Thus, the lower the Ct the more infectious agent. Another method for reporting results is copies per mL. The specificity of the primers and length of the amplified PCR product may alter the efficiency of the reaction and alter the Ct value. Therefore, one should be aware of each laboratory's interpretation for positive or negative qPCR results.

Microarrays

In human medical diagnostics, broad microarrays are used for diagnosis of many pathogens from patients, and in veterinary medicine attempts are underway to develop broad-spectrum microarrays for diagnostic testing. This is a multiplex technology consisting of thousands of microscopic spots of DNA oligonucleotides for specific DNA sequences known as probes. Probe-specific hybridization is detected and quantitated by detection of a fluorophore, silver, or chemiluminescence to reveal abundance of nucleic acid sequences in the target (Jerome et al., 2010). Microarrays have been used to study pathogenesis and host-pathogen interactions as well as to detect, and type various animal pathogens (Oiha and Kostrzynska, 2008). Correctly conducted microarray analyses require strict adherence to proper controls and substantial experience on the part of those designing the arrays and conducting the assays.

In-situ hybridization

This is a relatively simple molecular probe assay that, unlike PCR, measures direct binding of a complementary nucleic acid probe to the nucleic acid of an infectious agent within a tissue specimen (Narita et al., 2000). The complementary probe is labeled with fluorescein or an enzyme for detection purposes and is a molecular-based counterpart to the FA and IHC tests. *In-situ* hybridization can potentially identify small numbers of organisms within a specific lesion, and the pathologist can visualize whether they are associated with foci of inflammation, within macrophages, or other lesion components. Compared to conventional IHC, *in-situ* hybridization is potentially more sensitive and can identify low numbers of copies of the infectious agent nucleic acid. It is a test that can be developed and applied when specific antibodies are not available, but the gene sequence for the infectious agent is known. Sensitivity of *in-situ* hybridization is not as great as for PCRbased tests, which amplify the copies of nucleic acid.

Sequencing of infectious agent genomes

A few veterinary diagnostic laboratories provide sequencing of selected regions of infectious agents' genomes for determination of bacterial species, genotyping, subtyping, or identifying toxin or antimicrobial resistance genes. It is likely that, with new rapid sequencing regimens and lower costs for the technique, gene sequencing will be offered more in the future for diagnostic testing.

Benefits and limitations to diagnostic tests

Serology

Serotesting for naive animals (susceptible and without prior exposure) and absence of maternally derived antibodies is often performed using acute (early as possible after exposure) and convalescent (collected 3 to 4 wk later) serum samples. Seroconversion could follow either vaccinal or natural exposure (with or without disease) to the test antigen or to another antigen with similar binding properties (Tyler and Cullor, 1989). Seroconversion suggests exposure and is not diagnostic for disease. Potentially, use of gene-deletion mutants in vaccines or highly purified antigens can permit differentiation of types of exposure, such as selected vaccines versus natural infection. Serology is best used to monitor for active infections in a group of cattle, rather than specific disease in a single animal. There are published studies where there were seroconversions to BVDV, PI3V, BRSV, and/or BoCV in animals without clinical signs of BRD and no history of vaccinations against these viruses (Fulton et al., 2000; Fulton et al., 2011). However, there were clinically ill animals seroconverting to these agents as well. Those studies support the use of serology to detect active infections on a group or herd basis. Some may try to equate magnitude of antibody levels as means to differentiate vaccine-induced antibodies from those naturally induced. Yet there are studies, using BVDV antibody levels, where this concept was not confirmed (Fulton et al., 2005; Fulton et al., 2006). Variations in antibody responses can be great with some animals being high antibody responders and some low responders, and there are variations in vaccine potency. It is also noted that an animal with active immunity induced by an MLV BVDV vaccine, may have a high response upon exposure to a persistently infected BVDV animal (Fulton et al., 2005; Fulton et al., 2006). Practitioners may want to know what is the "protective titer" that a vaccine must induce. Unfortunately, that is often not known. Under experimental conditions, a protective titer may be determined for a specific vaccine and pathogen; however, under field conditions with multiple infectious agents, stressed cattle from various sources and genetic pools, and pathogens with varying virulence, the statistically calculated "protective titer" may not hold true. Antibodies represent one of many adaptive immune effector mechanisms. It would also not be possible to determine a protective titer when the role of the T-cells (cell mediated immunity) such as CD8 positive cells is important for host protection. In fact, although VNTs are important for anti-viral immunity, protection against viruses and other intracellular pathogens requires cell-mediated immunity. Therefore, it is not possible to determine a protective antibody titer even under experimental conditions. In addition, individual animal variation with respect to intensity of immune responses, specificity of immune response and susceptibility to pathogens come into play. We have experienced the occasional individual cattle that have high antibodies to viral or bacterial agents and yet are highly susceptible to experimental BRD challenge. Conversely, individual cattle with low antibody levels can occasionally be highly resistant to challenge.

Molecular-based tests

Nucleic acid-based assays are usually not broad spectrum as compared to culture of viruses and bacteria. The adage, "You only find what you are looking for" applies to PCR and other nucleic acid-based tests (Chiu and Miller, 2011). Molecular tests identify nucleic acid sequences specific for the agent. A positive result may indicate either infectious or noninfectious material. For instance, it has been shown that DNA from inactivated organisms injected into the bloodstream of laboratory animals can be detected using PCR assay for more than a week after injection (Veir and Lappin, 2010). This demonstrates the high sensitivity of the test and indicates that demonstration of nucleic acid does not guarantee infection is occurring. In addition, subtle genetic variations in infectious agent strains may cause the agent to be not detected by a highly specific molecular technique. The design of PCR probes may target sequences that are

highly conserved among a broad based agent such as viral family, genus, or species, yet it is also possible to include multiple sequences for a specific agent. Thus the design of the probes may enhance the testing.

Nucleic acid-based tests offer rapid turnaround time with high throughput, permitting large number of tests to be performed in hours compared to culture requiring several days to weeks. Improper controls, variation among RNA/DNA extraction methods, and contamination can confound test results. Other issues include the lack of standardized criteria among laboratories for interpretation of positive or negative. Each laboratory has the criteria that they use for reporting results. Another critical issue is whether a positive nucleic acid-based test equates in all instances to the detection of infectious virus. A case in point is reflected in a paper on bluetongue virus (BTV) (Chatzinasiou et al., 2010). Inoculation of embryonated chicken eggs has been the standard method for the titration of infectious BTV. Egg inoculation for detection of BTV was compared to real-time PCR in blood samples collected from experimentally infected sheep. There was positive correlation for the first 28 d post-infection, but not thereafter as there were positive PCR results in later samples. Thus the implication is that PCR positive results may be found in samples in which infectious virus may no longer be detected. Another consideration is that a PCR test may be applied to a situation for which it was not developed and validated. In a recent exchange of letters in Applied and Environmental Microbiology (2011, 77:1923-1924), a reader questioned the authors of an article in which a specific test for *Mycobacterium avium* subsp. *paratuberculosis* that is valid for detection of the bacterial infection in cattle detected the bacterial DNA in 81% of drinking water sampled by PCR. The letter's authors logically attacked the use of that assay under conditions for which it has not been validated and argued strongly that the results of the manuscript under attack do not make biological sense. In the case of BRD pathogens, therefore, we must diligently fight the urge to ask a diagnostic laboratory to test water or feed samples for pathogens using their PCR tests designed for use on tissue or excretions, because it would likely be using that test under conditions for which it has not been validated, and therefore, finding of positive results would be invalid and meaningless. Another disadvantage of identification of pathogenic bacteria in specimens using molecular techniques is that antimicrobial susceptibility and resistance information is not readily ascertained. Once a bacterium is identified, resistance to some antimicrobials may be subsequently tested using molecular probes for certain resistance genes. For example a microarray was used to test *E. coli* DNA for several tetracycline resistances and ß-lactamase genes (Oiha and Kostrzynska, 2008). Antimicrobial resistance, however, is not always linked to 1 specific gene and may involve multiple genes, gene complexes and plasmid-based genes. Therefore, for now, antimicrobial resistance and susceptibility are best determined on isolates. In some instances, the availability of more tests complicates the diagnostic process (Tegmeier et al., 2000). Lungs were examined for *H. somni* from 65 cases of BRD pneumonia using conventional and molecular tests. The bacterium was cultured from 10 cases, identified by IHC in 17, and found by *in-situ* hybridization in 19 cases. Using PCR, they found 21 positives using material washed from the lung culture plates, 29 positive from lung swabs from lung cut surface and 30 positive from bronchial swabs. The authors recommended that, due to its rapidity and sensitivity, PCR should be considered for use as a supplemental tool for diagnosis of specific pathogens in bovine lungs, but they acknowledged that IHC and *in-situ* hybridization may give the most accurate and useful results. It is our opinion that direct culture, IHC and *in-situ* hybridization can be more readily interpretable with respect to cause and effect of a specific pathogen in BRD. PCR tests could be detecting only small amounts of bacterial DNA inhaled from nasal or tracheal normal flora, residual DNA from bacteria or viruses killed by host immunity, or incidental secondary agents. An important issue facing those receiving diagnostic laboratory results from molecular testing is the interpretation of positive results in the absence of lesions or appropriate clinical signs confirmed by VI, FA, or IHC. One must ask, "Does PCR positive carry the day for the causative agent without other criteria?" Many times submitted tissues are unsuitable for traditional testing, yet a tissue homogenate can be tested by nucleic acid tests, and a positive result reported without recovery of the infectious agent. There are differences in professional opinions among diagnosticians and clinicians as to the etiologic agent when infectious material is not isolated or agent specific lesions are not observed, yet a molecular test is positive. For example, there is a report of an experimental challenge study of heifers exposed to BoHV-1, in which abortions occurred post-exposure with a small group of fetuses that had no histologic lesions, yet were VI positive (Smith et al., 1978). Studies need to be performed using known experimental challenge experiments and a complete battery of tests available including VI, FA, IHC, and PCR. Some of these are in progress, or at least archived tissues need to be examined

Conclusions

In recent years, a battery of diagnostic tests has become available; the extent of which was unthinkable 25 years ago. With modern molecular tests, the presence of infectious agents or their nucleic acids can be rapidly identified in samples from BRD cases. Unfortunately, the high sensitivity of molecular tests is such that positive results must be carefully considered with respect to their validity. The rate of development and use of molecular diagnostic tests have outpaced validation, standardization, and standards for interpretation relative to their use in BRD diagnostics. Finally, each veterinarian upon receipt of molecular test results should ask the question, "Does the result make biologic sense?"

Table I: Comparisons of uses of diagnostic tests along with their strengths and weaknesses

Test	Use	Positives	Negatives
Serology	Antibody detection	Detect vaccine responses and past infections	Titers do not necessarily infer resistance and are not able to differentiate vaccine-induced antibodies from infection-acquired antibodies
Culture nasal, nasopharynx, trachea, BAL	Detect bacteria and viruses	Demonstrates the presence of colonization or active infection	Positive culture does not necessarily mean lung infection or causative for disease. Time for results to be obtained are days to weeks
Culture lung lesion	Detect bacteria and viruses	Requires active replication of the agent in the tissue at time of death, so isolation usually indicates that high concentrations are in tissue. Antimicrobial resistance can be determined	Sensitivity is not great and may miss true positives due to concurrent infections and antimicrobial therapy. Time for results to be obtained are days to week
Immunohistochemistry lung lesion	Detects antigen in lung lesion	One can localize the infectious agent within the lesion. Strong evidence that infectious agent is related to disease	Sensitivity and specificity depend on available monospecific immune serum or monoclonal antibodies to specific infectious agent
In situ hybridization lung lesion	Detects region of genome of agent in lesion	One can localize the infectious agent within the lesion. Strong evidence that infectious agent is related to disease. Monospecific antiserum or monoclonal antibodies not needed	Depends on known, pathogen-specific genomic region for development of specific oligonucleotide primers
Single PCR nasal, nasopharynx, trachea, BAL swabs or collection	Detects genetic material of agent in sample	Provides specific evidence that infectious agent is in or recently been in a sample	Cannot differentiate subclinical or incidental concurrent infection from natural exposure or vaccination, Does not always detect infectious material. Cannot determine antimicrobial resistance
Single PCR lung lesion from supernatant of tissue homogenate	Detects region of agent genome	Potential evidence of specific infectious agent is associated with disease	May not represent causative infectious agent within diseased tissue or differentiate natural infection versus MLV vaccine
Multiplex PCR nasal, nasopharynx, tracheal, BAL swab or collection	Detects region of several agents genomes	With a single test, potential evidence of one or more infectious agent associated with disease can be determined. Test provides more information than single PCR	May not represent causative agents within diseased tissue or differentiate natural infection versus MLV vaccine
Multiplex PCR lung lesion from supernatant of tissue homogenate	Detects region of several agents genomes	With a single test, potential evidence of one or more infectious agent associated with disease can be determined. Test provides more information than single PCR	May not represent causative agents within diseased tissue or differentiate natural infection versus MLV vaccine

PREVENTION OF BOVINE RESPIRATORY DISEASE

Introduction

Prevention of BRD has in most circles, focused on immunogens/vaccines to cattle at various critical control points in the animal's life. Ideally vaccines should provide protection against challenge with the pathogens. Whether the vaccines work is paramount to protection (efficacy). Vaccines may be used in neonates, calves 2-3 months of age at branding, pre-weaning, weaning, and post-weaning prior to delivery. Cattle marketed in our region of the U.S. may go to stocker operations as post weaned calves on either native grasses as forages in the summer or to wheat pasture in the fall. Sometimes calves may go directly to the feedlot as post weaned calves dependent on the price of grain in the rations. Stockers reaching yearling age after forage go to the feedlot. At the feedlot cattle at processing/entry receive viral vaccines consisting of BoHV-1, PI3V, BVDV1 and 2, BRSV MLV (5-way) strains and often selected bacterial vaccines for *Clostridium spp.* (USDA APHHIS, 2013). In the feedlot it is common to revaccinate at time of growth promotant reimplant with a monovalent BoHV-1 MLV vaccine or the 5-way MLV. Thus there are numerous times in the animal's life when they receive vaccines. In our region, auction markets serve as major delivery points for cattle of mixed origins that are commingled as sold and delivered. Unfortunately the vaccination/immune status is not known. Since this may be the first collection point, it is quite common to vaccinate with MLV 5-way viral vaccines. The question is whether the potential pathogen wins the race in the commingled, stressed calves, from multiple sources or does the vaccine "work" and provides protection against newly acquired pathogens. Our studies at Oklahoma State University have focused on the single sourced animals from specific ranches where the calf is born and raised and later weaned.
Infectious agents associated wth BRD include: viruses, BoHV-1, PI3V, BVDV1 and 2, BRSV, and BoCV; bacteria, *M. haemolytica, P. multocida, Histophilus somni*, and *Mycoplasma ssp*. (Fulton et al., 2009). There are licensed and commercially available vaccines in the U.S. for these pathogens (Compendium of Veterinary Products, 2010). An important management system for the beef industry is the use of "preconditioning programs". These programs are instituted in the beef breeding herd at the earliest intervention. These programs generally prepare the weaned calf to meet the "stresses" occurring when:

- the calf comes in contact with other cattle of unknown vaccination status that are potentially shedding numerous infectious agents,
- the calf is placed in an environment facilitating transmission, such as trucking over long distances,
- there is overcrowding in the markets and shipping,
- the calf develops a compromised immune system,
- the calf is exposed to environmental conditions such as dust, humidity, and environmental temperature changes,
- nutrition changes predispose the calf to increased BRD risk.

Preconditioning programs often require weaning of calves 30 to 45 days prior to shipment and commingling, and include dehorning, castration of bulls; anthelmintic treatment, vaccinations, and good nutritional status (some suggest or require calves to be bunkfed). Vaccination requirements are often prevalent in preconditioning programs, yet unfortunately documentation of efficacy of viral and bacterial vaccines under field conditions is limited (Perino and Hunsaker, 1997). Our investigations were to determine if cattle in a preconditioning program with better or increased humoral immunity (antibody levels) to certain BRD pathogens had better feedlot performance than those with lower levels of immunity.

Current cattle marketing often involves sale of recently weaned calves through auction markets or order buyers, wherein calves are purchased from many farms/ranches and. /or auction markets, commingled, and shipped to stocker operations or feedlots. This sale of recently weaned calves through auctions is more common than "retained ownership programs" (ROP), whereby the breeding herd owner maintains ownership through the feeding period. These ROP are often associated both as an educational tool and as an economic benefit to the owner of calves. By retaining ownership, breeding cow-herd owners may potentially increase economic return if they have better genetic potential in their cattle to capture increased feedlot performance and carcass value at harvest processing. Also by retaining ownership, owners could benefit with healthier cattle with a return on their preconditioning costs. The Noble Foundation (NF) Agricultural Division, Ardmore, Oklahoma (OK), provided educational programs to cooperating ranches in southern Oklahoma and northern Texas. Ranchers receive programs and consultation regarding nutrition for cattle, forage management, and breeding, including selection of heifers, cows, and bulls for a variety of traits. With educational programs above, attention is given to the economic impact of decisions. The NF ROP permits cooperators to select representative cattle for delivery to the NF ranch where they are processed, weighed, and shipped to a feedlot. The ROP ranchers learn how their cattle perform compared to industry norms, which allows them to see how their health programs prepare cattle for the feedlot as well as how the decisions on breeding females and bull selection impact feedlot performance. The NF ROP recommends that cattle be weaned 45 days prior to shipment, males be castrated, and all cattle dehorned. Calves also are to receive anthelmintic treatment. Recommended vaccines include clostridial vaccines and two doses of viral

vaccine. Use of *M. haemolytica* and *P. multocida* vaccines is highly recommended. Choice of vaccines, including modified-live virus (MLV) or killed viral (KV), is left to the rancher based on their management strategy and veterinarian's counsel.

The objectives of these studies were to determine predictors of health status in the feedlot, including treatment costs, morbidity, mortality, and other economic findings by collecting samples at processing for viral and bacterial serology, and detection of viruses and bacteria present in nasal swabs or blood samples. Results of these studies are included in references (Fulton et al., 2002; Fulton et al., 2011).

Materials and Methods

Cattle and sample collection

There were two studies performed in the ROP, 2000-2001 and 2001-2002. These cattle were from southern Oklahoma and north-central Texas. Guidelines for the program including vaccinations, weaning, and anthelmintic treatment, plus dehorning and castration of males, had to be completed prior to delivery. Calves were delivered in November of the initial year for processing, which included weighing, identification, and sample collection. Samples included nasal swabs for viral and bacterial isolation, an EDTA blood sample for BVDV isolation from peripheral blood leukocytes (PBL), and serums to be tested for viral and bacterial antibodies. Owners provided a herd health history, including weaning date, specific vaccines used, vaccination dates, anthelmintic used, and annual herd vaccinations. The calves were then shipped to a western Oklahoma feedlot near Guymon, a distance of approximately 380 miles (about 600 km). To assign a value to the calves at processing, the price for steers and heifers based on prices for that day at markets was used. During processing at the feedlot, calves received a vaccine containing: 2000-2001 study, MLV BoHV-l, BVDVla, PI3V, and killed BRSV, and the next year, MLV BoHV-l, BVDVla, PI3V, and BRSV.The normal pull-and-treat regimen for the feedlot was followed.li An animal was pulled from the pen for respiratory disease when one or more of the following signs were present: depression, nasal discharge, lack of rumen fiil, and lethargy. If the rectal temperature was less than 104°F (40°C), the calf was called a “respiratory observe”, given an MLV BIIV-I vaccine, and sent to a hospital observation pen. Calves that died during the study were necropsied, and tissues were collected for histopathologic study and viral/bacterial isolation. The diagnosis of enterotoxemia was based on clinical signs and necropsy. Numerous performance data were obtained from the cattle at delivery and during the feeding period. Carcass data were obtained at processing

Virologic, Bacteriologic, and Serologic Studies

Blood samples and nasal swabs were submitted for viral and bacterial isoIation. A microtiter VNT assayed for BVDV1a, BVDV2a, PI3V, and BRSV antibodies: a plaque reduction assay was used for BoHV-1 antibodies. Antibodies to *M. haemolytica* whole cell (WC) antigen, *M. haemolytica* leukotoxin (LKT) and *P. multocida* outer membrane proteins were measured by ELISA (Fulton et al., 2002; Fulton et al., 2011).

Statisitical analysis

All data were analyzed using software for statistical analysis as described (Fulton et al., 2002; Fulton et al., 2011).

Results and Discussion

Clinical Data and Laboratory Testing

In the 2000-2001 (Study A), there were 24 herd with 417 calves. Of the 417 calves, 114 (27.3%) were treated and 4 died (0.96%) with 3 died with BRD and one with enterotoxemia. Of the calves at entry, 115 (27.6%) bacteria from the nasal swabs (*M. haemolytica, P. multocida*, or *H. somni*). Nasal swabs at entry had 7.9% with PI3V. No other viruses were identified by culture, and no persistently infected BVDV calves were found. The presence of viruses or bacteria at entry in nasal swabs did not predict illness. In the second study in 2001-2002, there were 201 head from 18 herds (Study B). There were 126/291 (43.3%) treated for BRD. Nine calves died (3.1%) with 7 due to BRD and two to entertoxemia. In the second study 8.0% (23) had *M. haemolytica* in the nasal swabs but no *H. somni* or *P. multocida*. No viruses were found in the nasal swabs or the PBL. No PI BVDV were found. The morbidity rates among the herds in each study varied with statistical differences.

Vaccine use varied among the two studies. In Study A, for 24 herds, 10 received killed virus vaccines (including chemically altered MLV for BoHV-1 and PI3V), 9 received MLV vaccines, and 5 received a combination of MLV and killed. Seven herds received vaccines containing BVDV2a. Ten herds used *M. haemolytica* and/or *P. multocida* vaccines. Table II for example lists the vaccine viral, MLV or killed, strains and for bacterial, product descriptions in the Study B. In the next year’s study, Study B, there was a move to more MLV vaccines (almost 20% increase) and BVDV2a, and also more to *M. haemolytica* and *P. multocida* (an increase of 30%). Of the 18 herds, 6 received kill

vaccines (including the chemically altered BoHV-1 and PI3V), 10 received MLV vaccines, and two used a killed followed by MLV vaccine. Seven herds used BVDV2a (six with MLV and one with killed). There were 13 of the 18 herd receiving *M. haemolytica* alone or a *M. haemolytica/P. multocida* and five herds received no *M. haemolytica* or *P. multocida* vaccines. The antibody levels five viral immungens and the three bacterial antigens are listed in Table III are represented by mean antibody levels for animals for each of the 18 herds. There were significant differences for a specific antigen among the herds.

Table II: Summary of vaccination histories (study B)

Herd	Viral vaccine and date of vaccination	Bacterial vaccine and date of vaccination
1	1- Killed BoHV-1, BVDV1a, BVDV2a, PI3V, BRSV 2- MLV BoHV-1, BVDV1a, PI3v, BRSV 3- MLV BoHV-1, BVDV1a, PI3v, BRSV	2- *M. haemolytica* bacterin-toxoid 3- *M. haemolytica* bacterin-toxoid
2	1- Chem altered ML BoHV-1, PI3V nasal 1- MLV BoHV-1, BVDV1a, PI3v, BRSV 2- MLV BoHV-1, BVDV1a, PI3v, BRSV	 3- *M. haemolytica* bacterin-toxoid
3	1- MLV BoHV-1, BVDV1a, PI3v, BRSV 2- MLV BoHV-1, BVDV1a, PI3v, BRSV	1- *M. haemolytica* toxoid 2- *M. haemolytica* toxoid
4	1- Chem altered ML BoHV-1, PI3v, killed BVD1a, BVD1 2- Chem altered ML BoHV-1, PI3v, killed BVD1a, BVD1	None recorded
5	1- MLV BoHV-1, BVDV1a, BVDV2a, PI3v, BRSV 2- MLV BoHV-1, BVDV1a, BVDV2a, PI3v, BRSV	None recorded
6	1- MLV BoHV-1, BVDV1a, BVDV2a, PI3v, BRSV 2- MLV BRSV	1- ML *M. haemolytica, P. multocida*
7	1- Killed BoHV-1, BVDV1a, PI3V, BRSV 2- Killed BoHV-1, BVDV1a, PI3V, BRSV	None recorded
8	1- Chem altered ML BoHV-1, PI3v, killed BVD1a, BVD1 2- Chem altered ML BoHV-1, PI3v, killed BVD1a, BVD1	1- *M. haemolytica* bacterin-toxoid
9	1- MLV BoHV-1, BVDV1a, PI3v, BRSV 2- MLV BoHV-1, BVDV1a, PI3v, BRSV	1- *M. haemolytica* bacterin-toxoid
10	1- Chem altered ML BoHV-1, PI3v, killed BVD1a, BVD1 2- Killed BoHV-1, BVDV1a, PI3V, BRSV	2- *M. haemolytica* bacterin
11	1- Killed BoHV-1, BVDV1a, PI3V, BRSV 2- Killed BoHV-1, BVDV1a, PI3V, BRSV	1- *M. haemolytica* bacterin 2- *M. haemolytica* bacterin
12	1- Chem altered ML BoHV-1, PI3v, killed BVD1a, BVD1 2- MLV BoHV-1	None recorded
13	1- MLV BoHV-1, BVDV1a, BVDV2a, BRSV 2- MLV BoHV-1, BVDV1a, BVDV2a, BRSV	1- *P. multocida* bact extract and *M. haemolytic*a toxoid 2- *M. haemolytica* toxoid
14	1- MLV BoHV-1, BVDV1a, BVDV2a, PI3v, BRSV 2- MLV BoHV-1, BVDV1a, BVDV2a, PI3v, BRSV	1- ML *M. haemolytica, P. multocida* 2- ML *M. haemolytica, P. multocida*
15	1- MLV BoHV-1, BVDV1a, BVDV2a, PI3v, BRSV 2- MLV BoHV-1, BVDV1a, BVDV2a, PI3v, BRSV	1- Leukotoxoid and antigens from *M. haemolytica* and *P. multocida* 2- Leukotoxoid and antigens from *M. haemolytica* and *P. multocida*
16	1- MLV BoHV-1, BVDV1a, BVDV2a, PI3v, BRSV 2- MLV BoHV-1, BVDV1a, BVDV2a, PI3v, BRSV	1- ML *M. haemolytica, P. multocida* 2- ML *M. haemolytica, P. multocida*
17	1- MLV BoHV-1, BVDV1a, PI3v, BRSV 2- MLV BoHV-1, BVDV1a, PI3v, BRSV	None recorded
18	1- MLV BoHV-1, BVDV1a, PI3v, BRSV 2- MLV BoHV-1, BVDV1a, PI3v, BRSV	1- *M.haemolytica* bacterin-toxoid

Table III: Antibodies to viruses and bacteria at time of entry in study B

Herd	N	Mh WC	Mh LK	Pm MP	BVDV1a	BVDV2	BHV-1	PI-3V	BRSV
1	86	0.81777^{e}	0.55071^{de}	0.66687^{c}	454^{ef}	101^{g}	126^{d}	160^{g}	29^{ef}
2	5	0.16640^{abc}	0.29080^{abcd}	0.27780^{abc}	12^{a}	0^{a}	47^{c}	8^{cde}	11^{abcd}
3	18	0.85039^{e}	0.86711^{e}	1.5739^{de}	1448^{g}	45^{ef}	59^{c}	84^{f}	29^{def}
4	10	0.33680^{abc}	0.70060^{e}	0.51627^{abc}	104^{c}	3^{ab}	48^{c}	362^{h}	37^{efg}
5	19	0.20663^{abc}	0.25626^{abc}	0.09189^{a}	275^{def}	319^{h}	143^{d}	10^{de}	23^{cde}
6	8	0.10913^{ab}	0.13538^{a}	0.59113^{bc}	64^{bc}	117^{gh}	5^{a}	4^{abc}	117^{g}
7	5	0.05820^{ab}	0.07900^{a}	0.20920^{abc}	8^{a}	3^{ab}	35^{bc}	42^{f}	14^{abcde}
8	12	0.48883^{bcd}	0.67708^{de}	0.37267^{abc}	29^{ab}	3^{ab}	54^{c}	40^{f}	20^{bcde}
9	10	0.06230^{ab}	0.15470^{a}	0.19360^{ab}	194^{cde}	18^{de}	10^{a}	5^{bcd}	11^{abc}
10	27	0.02878^{a}	0.19633^{a}	0.30274^{abc}	102^{c}	3^{ab}	55^{c}	396^{h}	37^{efg}
11	20	0.68310^{de}	0.50750^{cde}	0.45170^{abc}	14^{a}	1^{a}	27^{bc}	375^{h}	5^{a}
12	10	0.13350^{ab}	0.25330^{abc}	1.44460^{ef}	23^{ab}	4^{bc}	89^{cd}	2^{ab}	10^{ab}
13	10	0.27110^{abc}	0.36820^{abcd}	0.50020^{abc}	64^{bc}	274^{h}	56^{c}	1^{a}	84^{g}
14	15	0.55453^{cd}	0.49280^{bcde}	1.35933^{ef}	354^{def}	223^{h}	138^{d}	40^{f}	51^{fg}
15	14	0.42864^{bcd}	0.36536^{abcd}	0.71893^{c}	1521^{g}	55^{fg}	6^{a}	3^{abc}	5^{a}
16	7	0.19314^{abc}	0.72443^{e}	0.78871^{cd}	345^{def}	13^{cd}	171^{d}	53^{f}	24^{cde}
17	10	0.12770^{ab}	0.20540^{ab}	1.76150^{f}	128^{cd}	11^{cd}	92^{cd}	42^{f}	9^{ab}
18	5	0.75380^{de}	1.55200^{f}	0.29420^{abc}	1024^{fg}	32^{def}	13^{ab}	28^{ef}	7^{efg}

Within each column, herds with the same superscript letter are not significantly different ($P < 0.05$)

The mean titers of antibody to the 5 viral and 3 bacterial antigens were compared among the 24 herds in the Study A, and the 18 herds in Study B. There were significicant differences for each antigen among the respective herds each year. For selected herds and antigens, lower titers occurred in herds with a high morbidity rate, whereas high titers occurred in several herds with lower morbidity rates. Although the data is not presented, there are some general observations regarding killed versus MLV viral vaccines, and also regarding bacterial vaccines such as *M. haemolytica.* In the Study A, the herds with the highest morbidity used killed viral vaccines, with only one dose or poorly timed with the second dose near delivery. In the Study B for example, the three herds with the lower treatment costs received vaccine containing *M. haemolytica.* Herds with the highest net value to the owner and had significant lower number of treatments received bacterial vaccine, *M. haemolytica* and/or *P. multocida*.

Regarding antibody responses and vaccine usage, in general, herds with the highest *M. haemolytica* WC titers received *M. haemolytica* immunogens with similar relationships with *M. haemolytica* LKT titers and vaccination. Antibody responses were higher to BVDV in herds using MLV vaccines compared to killed. Antibody levels in herds using MLV or killed BoHV-1, PI3V, and/or BRSV did not reveal consistent differences. The antibody titers for each antigen for an animal and the herd averages for the represented calves were used to correlate antibody levels to health status and feedlot performance (Table IV and Table V) indicating significant relationships between serology, health status, and feedlot performance. Numerous relationships based on lower or higher antibody levels were associated with either enhanced (positive) or detrimental (negative) impact on the feedlot performance.

Table IV: Parameters of performance and health status at delivery for calves and subsequent feedot period

Performance parameter	
Shipping weight	At delivery to feedlot
In-value	Price per 100 lb x weight
Total cost	Feed cost + trucking + yardage + options + processing + treatment costs + carcass data + identification
Sickness	Treated once of multipl times
Treatment	Number of treatments
Treatment costs	Addition of all treatment costs
Total value	Carcass value
Cost of gain	
Average daily gain (ADG)	
Net value to owner	Carcass value - total cost
Gross margin	Carcass value - total cost - in-value
Health status at entry	
Organisms for which antibodies were measured	BoHV-1, BVDV1a, BVDV2a, PI3V, BRSV, BoCV, *M. haemolytica* WC and *M. haemolytica* LKT, and *P. multocida* OMP
Organisms isolates from nasal swabs	Bacteria detected by culture *M. haemolytica, P. multocida,* and *H. somni*

Table V: Summary of significant relationships between health status and performance (P < 0.05) for two studies

For individual animals	
Sickness	Low *M. haemolytica* WC titer Low BVDV1a titer Low BoHV-1 titer Low PI3v titer
Decreased net value to owner	Low *P. multocida* OMP titer Low *M. haemolytica* LKT titer Increased number of treatments Increased treatment costs Low BVDV1a titer Low BVDV2a titer Low PI3V titer
Decreased gross margin	Low *P. multocida* OMP titer Low BVDV1a titer Low PI3v titer
Increased average daily gain	High *P. multocida* OMP titer High PI3V titer Sickness reduced gain
Increased number of treatments	Low *M. haemolytica* WC titer Low BVDV2a titer Low BVDV1a titer Low BoHV-1 titer Low PI3V titer Low BRSV titer Low BoCV titer Low *M. haemolytica* LKT titer
Increased total treatment costs	Low *M. haemolytica* WC titer Low BVDV1a titer Low BVDV2a titer Low PI3V titer Low BRSV titer
Higher carcass grade	High BVDV1 titer High BVDV2a titer High PI3V titer
Herd averages	
Sickness	Low BVDV1a titer Low BoHV-1 titer Low *P. multocida* OMP titer
Decreased net value to owner	Low *P. multocida* OMP titer Low BVDV1a titer Low BVDV2a titer
Increased number of treatments	Low *M. haemolytica* LKT titer Low *M. haemolytica* WC titer Low BVDV1a titer
Increased total treatment costs	Low BVDV1a titer Low BVDV2a titer Low *M. haemolytica* LKT titer Low *M. haemolytica* WC titer Increased number of treatments
Increased total costs	Low *P. multocida* OMP titer Low PI3V titer
Decreased gross margin	Low BVDV1a titer

In these studies, there were certain predictors such as antibody levels to six viruses and two bacteria regarding feedlot performance in animals prior to delivery to the feedlot. This is important as there are studies indicating that waiting until the feedlot to vaccinate may be too late. Calves vaccinated at entry to the feedlot with a *M. haemolytica* bacterin-toxoid and their performance measured by BRD mortality, morbidity, or average daily gain was not different than unvaccinated controls (MacGregor et al., 2003). Thus management of cattle before delivery offers a potential to enhance resistance to BRD. The management issues for preconditioning such as weaning and holding for 30-45 days are also factors to enhance performance. This is illustrated in another study where calves from a single source were retained on the ranch for 45 d after weaning and the calves exhibited less morbidity and less health costs during the receiving at the feedyard than when cattle were commingled or trucked to the feedyard immediately after weaning (Step et al., 2008).

The results of these studies reported here reaffirm that post-weaning calves with increased immunity as measured by antibody levels to BoHV-1, BVDV1a, BVDV2a, PI3V, BRSV, BoCV, *M. haemolytica*, and *P. multocida* after vaccination perform better in the feedlot and have less clinical disease. Thus management of the beef breeding herd should stress vaccination against these pathogens with sufficient time to develop immunity before shipping.

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Use of in-house multiplex real-time PCR for simultaneous diagnosis of six major respiratory pathogens in cattle: two years of experience in Burgundy

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Purpose

Respiratory diseases affecting young cattle are a dominant multifactorial pathology of livestock in Burgundy with serious economic consequences. In the purpose of improving our performance of diagniosis, we have developed and validated four years ago three multiplex real-time PCR for simultaneous detection of Bovine Respiratory Syncitial Virus, Parinfluenza Virus 3, *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni* and *Mycoplasma bovis*. After three years of use, we can point out the contribution of molecular tools for the diagnosis of multifactorial diseases compared with traditional techniques used so far.

Methods

For molecular tests, a single nucleic acid extraction protocol using RNeasy mini kit (Qiagen) has been established to recover both the genomes of bacteria and viruses. The three PCR are performed on the same run (QuantiFast Probe RT-PCR or QuantiFast Probe PCR kit (Qiagen)), on ABIPRISM 7500 (LifeTechnologies).

The results of three years with molecular tests are compared to five years using traditional bacteriological and virological techniques.

Results

We compared the apparent prevalence of six respiratory pathogens according to the techniques used. It can be seen that if the data are comparable for the two viruses, the prevalence of bacteria was considerably improved with real-time PCR especially for slow-growing bacteria such as *H. somni* and *M. bovis*.

Conclusions

The use of real-time PCR for the simultaneous diagnosis of respiratory pathogens provides several major advantages in terms of feasibility, cost and delay of analysis. With a single test, a single operator can detect at least one major pathogen in 80% of analyzed samples.

Influence of bovine viral diarrhoea virus (BVDV) infections on milk yield and fertility in dairy herds: a retrospective case-control study

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Bovine viral diarrhoea (BVD) not only leads to the death of cattle caused by mucosal disease but also to economic losses in infected herds through reduction in milk performance and fertility. In order to economically assess the progression of BVD eradication in Styria, Austria, the consequences of a bovine viral diarrhoea virus (BVDV) infection on the milk performance and fertility in small dairy herds were examined in a retrospective case-control study.

In Styria, a BVDV eradication program is implemented since 2001. Within this period, a BVDV infection was detected in 1.154 farms (maximum herd prevalence 2005 = 2.07%). 389 of these farms were selected as case farms for the present study. To every case farm a control farm, which proved BVDV uninfected since the beginning of the eradication program, was selected. Case and control farms were registered in the official milk recording scheme. Milk yield per test day, calving dates, insemination data, breeding values for milk and breed for all cattle were available for these herds. Breed, production area, number of calvings and milk production of the herd served as selection criteria for the control herds.

In a further scenario, the case farms themselves acted as control farms. The milk performance and the fertility during the period 15 months to 3 months prior to the birth of the first persistently BVDV-infected cattle (PI) and during the period of 12 months after the eradication of the BVDV infection in this herds was proven (control periods) were compared to the milk performance and the fertility during the period 3 months prior to 21 months after the birth of the first PI.

The influence of the BVDV infection on milk yield, first service conception rate, calving interval, premature birth rate and rearing results was estimated as a function of the BVDV infection pressure using general linear mixed models. The results of the study provide the basis for calculating the BVD-related economic losses in the Styrian cattle population and for the assessment of the cost-efficiency of the BVD surveillance after completion of the BVD eradication.

Effectiveness of vaccination and/or antibiotherapy to control *Coxiella burnetii* infection in dairy herds: impact on clinical signs, shedding and reproductive performances

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The aim was to assess the effectiveness of vaccination and/or antibiotherapy to control *Coxiella burnetii* (Cb) infection in 120 dairy herds experiencing abortions due to Cb, and followed over 18 months. Four strategies applied to cows (vaccination using phase 1 vaccine Coxevac®, vaccination and oxytetracycline at drying off or calving, oxytetracycline at drying off or calving, nothing) were randomly assigned to herds. All nulliparous should be vaccinated in each herd. The reduction of frequency or amount of Cb shedding was assessed using real time PCR (i) monthly on bulk tank milk (BTM) and (ii) at calving on a subsample of 22 herds where vaginal swabs were performed. The effectiveness to reduce Cb load in BTM was assessed through logistic hierarchical models. The impact on shedding at calving was investigated using a mixed logistic regression assessing the risk for a cow to be detected shedder or high shedder, depending on its medical strategy, after adjustment for serological status, age and herd (random effect). The occurrence of abortions was compared over time and fertility was assessed through the probability of return to service using mixed logistic regression.

The probability of the reduction of Cb load in BTM was significantly increased in herds vaccinating > 80% of cows in comparison to those vaccinating [0-20%] (OR]80-100%] = 5.9, [1.1-16.7]) confirming the interest of a whole vaccination strategy. At calving, 18.3% of cows were detected as shedder. Tetracycline used once at drying off was associated with a lower risk of being shedder (OR = 0.40, [0.21-0.75]). Vaccination did not significantly prevent shedding but was significantly (OR = 0.15, [0.03-0.85]) associated with lower Cb load shed at calving. For practical reasons, some nulliparous were not vaccinated: seronegative ones vaccinated before service had a significant lower risk of shedding at calving (OR = 0.37, P = 0.04) and a decreased risk of return to service (OR = 0.54 [0.30-0.96]). Vaccination of cows was associated with a reduction in abortion rate (OR = 0.69 [0.45-1.06], P = 0.09).

This study reports the benefit of vaccination for both heifers and cows in terms of prevention of Cb shedding and reproductive performances and provides original knowledge for rationale and evidence-based use of antibiotics.

Towards genomic characterization of *Anaplasma phagocytophilum* pathogenic and non pathogenic strains of ruminant origin using a Multiple-Locus Variable-number tandem repeat Analysis (MLVA)

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Anaplasma phagocytophilum is a zoonotic and obligate intracellular bacterium transmitted by ticks (mainly the *Ixodes* genus), which infects multiple mammalian species. In ruminants, it is the causative agent of bovine granulocytic anaplasmosis (BGA), characterized by anorexia, agalactia, "pasture fever", respiratory signs, and in some cases, reproductive disorders such as abortions. BGA is therefore responsible for significant economic losses in domestic ruminants.

It is crucial to better understand the transmission cycles of *A. phagocytophilum* - in particular the reservoir(s) of bovine strains is(are) still unidentified - in order to control more efficiently bovine infection. The animal source(s) of *A. phagocytophilum* for humans in Europe are also unknown. Severe cases of HGA are present in the USA but are rare in Europe, whereas BGA is common in Europe but not described in the USA. This situation suggests considerable strain variation.

In a previous study, Bown et al. (2007) developed a MLVA technique, a tool that is usually very helpful for epidemiological studies. However, this technique was presently too discriminatory, as it allowed distinguishing different variants within the same cattle herd.

Our objective was to develop a MLVA technique that would be more appropriate for *A. phagocytophilum* typing.

For this purpose the following criteria were preferentially selected: i) intragenic VNTR (vs intergenic VNTR) ii) minisatellites vs microsatellites (< 9 pb). To date, several VNTR candidates have been identified, which: i) are amplified in all samples of ruminants tested ii) are polymorphic between and within different ruminant species.

20 samples from cattle, 7 from sheep, 5 from roe deer, 8 from izards, 25 from ticks, 2 from horses and the Webster human strain have been tested. All the animals have been sampled in different French regions.

To date, we have selected 4 VNTRs. Among our VNTR, one is able to discriminate between strains from domestic vs wild ruminants whereas three VNTRs allow discriminating strains within a given species. With our combination of different VNTR, the strains from the same cattle herd gave the same MLVA profile, whereas a high diversity was obtained at the scale of all tested samples.

Thus, according to our results, our technique has the potential to allow studying the links between *A. phagocytophilum* strains of various origins, for epidemiological and clinical purposes.

Demonstration of efficacy against challenge of an inactivated Schmallenberg vaccine in sheep

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Introduction

In August 2011, outbreaks of an unknown disease of cattle were reported in both the Netherlands and Germany. From December 2011, abortion and foetal abnormalities, were reported in sheep and cattle in several European countries. A new virus was identified in November 2011 and was associated with both conditions. This agent was named Schmallenberg virus (SBV) after the German town where the virus was first identified.

Schmallenberg virus is in the Simbu serogroup of the Orthobunyavirus group. This group of viruses includes many viruses occurring in the Tropics. None had been previously identified in Europe. Although some uncertainty remains on the transmission of SBV, it seems primarily spread by biting insect vectors (midges/mosquitoes).

Here, we present the results of a vaccination / challenge study showing that a single administration of an inactivated SBV vaccine was able to prevent viraemia in sheep.

Material and Methods

Eleven weaned lambs were randomly allocated to one group of 5 vaccinates and one group of 6 control sheep. Vaccinates were subcutaneously treated once on day 0, with 1 mL of an inactivated SBV vaccine (Merial). The other group was left unvaccinated and served as control. Twenty one days after vaccination, all sheep were challenged with a virulent SBV strain. All sheep were then monitored for rectal temperature, clinical signs and viraemia (quantitative RT-PCR) from D22 to D31.

Results

Hyperthermia: no hyperthermia was observed in any of the groups.

Clinical signs: no significant clinical sign was observed in any of the groups.

Viraemia (qRT-PCR): all controls were found positive on 3 consecutive days. None of the vaccinated animals was ever detected positive.

Conclusion

In the present study, single vaccination of sheep with the product tested provided full and significant protection against viraemia following a SBV challenge.

Protozoan infections (cryptosporidiosis and giardiasis) of the gastrointestinal tract of the calf: an update

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General introduction

Cryptosporidium was first described in 1907 in the gastric mucosa and later in the small intestine of laboratory mice. The first detailed description of *Giardia* dates from 1859, although Antonie van Leeuwenhoek already described protozoan trophozoites looking like *Giardia* in 1681. Cryptosporidiosis is nowadays considered as a life threatening disease in AIDS patients and as a common cause of diarrhea in human patients, both in sporadic cases and in outbreaks, such as the large waterborne cryptosporidiosis outbreak in Milwaukee in 1993. *Giardia* is recognised as the most common parasitological cause of human diarrhea, with an estimated 280 million infections worldwide per year. Both giardiosis and cryptosporidiosis are a frequently diagnosed waterborne infection and are a major concern to water utilities. In veterinary medicine, *Cryptosporidium* and *Giardia* are at present considered as important pathogens in the aethiology of calf diarrhea. Surprisingly, the pathogenicity of both parasites was not recognised until the 1980's, but since then *Cryptosporidium* has been consistently identified in up to 70% of the faecal samples collected from diarrhetic calves younger than 1 month. Therefore, *Cryptosporidium* is considered as the most important pathogen in the aetiology of neonatal calf diarrhea (de Graaf *et al.*, 1999). Although the first case of bovine giardiosis was reported in 1921 and despite the high prevalence in farm animals, the clinical importance of giardiosis in cattle was not described until quite recently (St. Jean, 1987). Since then *Giardia* has increasingly been recognised as an important pathogen in young calves and is considered as an important differential diagnosis for coccidiosis in the aetiology of diarrhea in calves older than 1 month (Geurden et al., 2009).

Background and Life cycles

Cryptosporidium is an apicomplexan protozoan parasite and was until recently classified as a coccidian parasite, despite the many unique characteristics, such as the lack of host specificity, resistance to anti-coccidial treatment, ability for autoinfection and the particular location within the host cell. It is now believed that *Cryptosporidium* should be placed in a taxonomic group separate from the coccidians and close to the gregarine parasites. To date multiple *Cryptosporidium* species and genotypes have been described (Xiao et al., 2004; Ryan et al., 2004; Fayer et al., 2005).

Cryptosporidium has a complex life cycle with an asexual and a sexual developmental phase. The life cycle can be completed within 3 to 5 days in the host small intestine. The life cycle initiates with the ingestion of the infective oocyst and the excystation of sporozoites. After cell invasion, the sporozoite forms a circular stage known as the trophozoite. The first part of the cycle consists of an asexual followed by a sexual development into sexual stages known as micro- and macrogametes. The microgametes are released from the host cells and penetrate cells harbouring macrogametes. Their union results in a zygote, which further develops into an oocyst with resistant oocyst wall. After 2 asexual divisions (sporogony) the oocyst contains 4 sporozoites.

Giardia is a flagellated protozoan parasite that possesses paired organelles, including two equivalent nuclei, and a ventral adhesive disc. At present, 7 major assemblages (A to G) have been identified within *G. duodenalis*, some of which have distinct host preferences or a limited host range. Next to the assemblages A and B which are both prevalent in human patients, several host-specific assemblages have been identified in animals. Assemblages C and D primarily infect dogs, the hoofed livestock assemblage or assemblage E is identified in artiodactyl species, assemblage F is primarily identified in cats and assemblage G in rats (Thompson and Monis, 2004; Geurden et al., 2008a).

In contrast to *Cryptosporidium*, *Giardia* has a simple and direct life cycle consisting of two stages: an infectious cyst which is resistant to many environmental stressors, and a trophozoite stage, which colonizes the intestinal lumen of the host and is responsible for the clinical symptoms. After oral ingestion, cysts are exposed to the acidic environment of the proximal gastro-intestinal tract resulting in the release of the trophozoite in the upper part of the small intestine. For the colonisation of the duodenum and the jejunum, attachment to epithelial cells is essential, but unlike *Cryptosporidium*, the trophozoites of *Giardia* are not invasive. They multiply asexually by binary fission in the lumen of the small intestine. Finally, exposure to biliary salts leads to encystation of trophozoites in the jejunum. Cysts are passed in the faeces and are immediately infectious upon excretion allowing completion of the life cycle within 72h.

Epidemiology

Calves are infected by oral ingestion of infectious *Cryptosporidium* oocysts or *Giardia* cysts. As soon as 3 days after infection calves start to excrete (oo)cysts. *Cryptosporidium* excretion peaks in calves younger than one month, whereas *Giardia* excretion peaks in calves between 1-3 months of age. The oro-faecal transmission may be direct through contact with infected animals, or indirect through a contaminated environment or through the ingestion of contaminated feed and water. Especially young calves and to a lesser extent the dam should be considered as direct sources for calf infection. As indirect sources for infection the housing should be contemplated, such as the maternity pen and the calf facilities. In group housing direct contact between calves favours transmission, but also in individual pens transmission is frequent, either by a vector, such as the care-taker, or by effluent of faecal material. Furthermore, transmission to subsequent calves in one particular pen is possible. In general, calves housed in indoor calf pens are at greater risk of infection than calves housed outside in hutches. Several parasite characteristics contribute to the successful transmission of protozoan infections in calves (Table I). The high number of infective (oo)cysts excreted by infected calves is in contrast with the low number needed for infection. Furthermore, (oo)cysts are very resistant and are able to survive for several months in the environment, resulting in a gradual increase in environmental infection pressure.

Table I: Parasite characteristics of *Cryptosporidium* and *Giardia* and epidemiological consequence in calves and the appropriate preventive measures

Parasite characteristic	Epidemiological consequence	Preventive measure
high excretion of (oo)cysts in the faeces	infection pressure can increase in a short period of time	avoid crowding isolation of excreting calves hygiene*
(oo)cysts are extremely resistant to environmental conditions	excreted oocysts can survive for several weeks or months in the environment	hygiene*
(oo)cysts are extremely resistant to chemical disinfection	disinfection with common disinfectants (chlorination) is not effective enough	products based on ammonia, chlorine dioxide, hydrogen dioxide or ozone
(oo)cysts are only sensitive for heat and desiccation	infection is mostly seen in the humid environment of stables	disinfection using steam avoid crowding hygiene*

*hygiene: - frequent removal of faeces
- thorough cleaning, preferably high pressure water cleaning
- if possible, followed by vacancy during several weeks

Prevalence

Overall, the highest *Cryptopsoridium* prevalence is observed in animals under the age of 4 weeks, with farm prevalences between 50-100% in developed countries. Worldwide cross-sectional studies have reported a *Giardia* prevalence varying from 20% to 73% in calves younger than six months. In calves older than 6 months the prevalence is lower. Similar to *Cryptosporidium* the farm prevalence is close to 100%. On positive farms, the cumulative incidence is almost 100%, indicating that every calf on that farm will get infected.

Next to the ubiquitous *C. parvum* the ruminant specific species *C. bovis and C. andersoni,* and the widespread *C. ubiquitum*, previously known as the cervine genotype (Fayer et al., 2010), have been described on a regular basis in cattle. Recent data indicate that *C. parvum* is most prevalent in calves up to 3 months. In older calves, *C. bovis* predominates, and in adult cattle *C. andersoni* and *C. bovis* are most frequently reported. In cattle, the livestock specific *Giardia* assemblage E is most prevalent, although up to 20% zoonotic assemblage A isolates have been reported (Geurden et al., 2008a).

Pathogenesis and clinical symptoms

The invasion and colonisation of the epithelial surface by *Cryptosporidium* results in loss of epithelial cells and microvillus brush border. Furthermore, the epithelial tight junctions are disrupted leading to an increased epithelial permeability. Clinical symptoms are most frequently observed in calves between the age of 5 days and 1 month and include a malabsorptive and secretory diarrhea which is usually self-limiting within 2 weeks after onset. The diarrhea can be mild to severe with pale yellowish watery or mucoid faeces. Calves can be dehydrated, depressed and anorectic. The severity and duration of the clinical symptoms is highly variable, depending on concurrent viral, bacterial or parasitic infections. Mortality is variable and is most often observed in calves with multiple infections. Calves with severe cryptosporidiosis can take several weeks to fully recover, and there is certainly an initial negative impact on production due to weight loss or impaired weight gain, and due to treatment expenses. Infection with *C. andersoni* has been reported world-wide in post-weaned cattle (Enemark et al., 2002; Olson et al., 2004). *C. andersoni* invades the peptic and pyloric glands of the abomasum causing glandular dilatation and hypertrophy of the gastric mucosa and

thinning of the epithelial lining. Although occasionally reported (Daniel et al., 2005), *C. andersoni* does usually not result in a pronounced diarrhea, but mainly causes maldigestion by inhibition of protein digestion due to increased gastric pH and subsequent decreased gastric proteolytic activity. These results in a moderate to severe impairment of weight gain, decreased feed efficiency and reduced milk production (Esteban and Anderson, 1995; Ralston et al., 2003). The pathogenesis of giardiosis is a combination of parasite and host factors and the subsequent clinical symptoms may vary considerably from host to host. The diffuse microvillus shortening leads to a decrease in overall absorptive area in the small intestine and an impaired intake of water and nutritients. The combined effect of the decreased resorption and the brush border enzyme deficiencies results in malabsorptive diarrhea and lower weight gain. The reduced activity of lipase and the increased production of mucine by goblet cells may explain the steatorrhea and muceous diarrhea which has been described in *Giardia* infected hosts. Clinical symptoms include the excretion of pasty to fluid faeces, often with mucus. Sometimes steatorrhoea is observed. The diarrhea does not respond well to antibiotic treatment. Although acute diarrhea can occur, more often a chronic or intermittent diarrhea is observed. Next to diarrhea, an impaired weight gain despite good appetite seems to be a typical clinical symptom of giardiosis in calves. The primary pathogenic effect of natural *Giardia* infections in ruminants was prone to debate, but is at present well recognised (Geurden et al., 2006b and 2010a).

Diagnosis

The laboratory diagnosis of these protozoal infections in calves was traditionally performed by microscopical examination of a faecal sample, although immunological assays are increasingly applied. The clinical symptoms and the age range, as well as the high excretion of *Cryptopsoridium* oocysts by clinically affected calves, facilitates the diagnosis of cryptosporidiosis. This is in contrast to *Giardia,* with vague clinical symptoms and intermittent cyst excretion in the faeces. Given the intermittent excretion of cysts, especially in the chronic phase of infection, multiple samplings can be necessary, either from the same animal for 3 consecutive days or from several calves within the same housing. Since the peak cyst excretion is observed in animals around 4 weeks of age, preferentially young animals are sampled for diagnosis, even if they have not developed clinical symptoms.

Microscopical examination

The most widely used technique for the diagnosis of *Cryptosporidium* is the detection of oocysts in a faecal smear, either native or after staining. The most commonly used staining methods are the modified Ziehl-Neelsen staining and the carbolfuchsine stains. For the dignosis of giardiasis, both the trophozoites and the cysts can be detected by microscopy, either directly or after concentration with sucrose, zinc sulphate or formalin. Trophozoites can sometimes be detected in faecal samples from calves with diarrhea due to the increased peristalsis. Most frequently the detection of cysts in the faeces is preferred for diagnosis. Prior to examination cysts can be stained. Frequently used stains are iodine and trichrome. Overall, microscopy has a lower sensitivity compared to immunological techniques.

Immunological assays

Immunological assays commonly used in veterinary medicine comprise immunofluorescence assay (IFA), antigen enzym-linked immunosorbent assays (ELISA) and solid-phase qualitative immune-chromatographic assays (dip-sticks). Especially for *Cryptosporidium*, serological detection of specific antibodies is unsuitable for clinical diagnosis, as the increase in specific antibody titre cannot be detected until after the clinical phase of infection. Overall, dip-sticks were found to be less sensitive than IFA (Geurden et al., 2008a and 2010b), but can be used on site.

Treatment and control

Paromomycin or aminosidin is a broad-spectrum amino-glycoside antibiotic, with well-known efficacy after oral treatment against several protozoan parasites like *Cryptosporidium* and *Giardia* in calves (Chartier et al., 1996; Geurden et al., 2006a). Nevertheless, paromomycin is not registered for the treatment of *Cryptosporidium* or *Giardia* in calves.

Several chemotherapeutic agents have been tested for the treatment of bovine cryptosporidiosis, but none resulted in a complete prevention of clinical symptoms or reduction of oocyst excretion. In European countries, halofuginone lactate is registered for treatment in calves, at a dose rate of 100 µg/kg bodyweight (BW) per day during seven consecutive days. For preventive treatment, halofuginone should be administered within 48 hours after parturition, and for curative treatment, within 24 hours after the onset of the clinical symptoms. Treatment with halofuginone lactate reduces the occurrence of diarrhea and postpones the oocyst excretion. Overall, results were better in experimental settings than under natural conditions with a high environmental infection pressure. Since infection is not completely eliminated, treatment with halofuginone lactate allows the development of a specific immunity (de Graaf et al., 1999).

For the treatment of giardiasis in cattle, there are no registered products. The benzimidazole compounds (BZs) are well known anthelmintics, and have been used for the treatment of giardiasis as well. In calves, data on reduction in cyst

excretion are available for treatment with fenbendazole (Xiao et al., 1996; O'Handley et al., 1997: Geurden et al., 2006b and 2010a) and albendazole (Xiao et al., 1996). Both have been shown to significantly reduce the peak and the duration of cyst excretion and to result in a clinical benefit (O'Handley et al., 2000a; Geurden et al., 2010a), although the dosage of both BZs needed for *Giardia* treatment (5 to 20 mg per kg bodyweight per day during three consecutive days) is higher compared to helminth treatment. The cyst suppressing effect of BZ treatment is either not complete or short-lasting in field conditions, despite the high *in vitro* efficacy of both drugs. This might be either due to a high environmental infection pressure which counters the effect of treatment or to the lack of persistent efficacy of BZs against *Giardia* in calves, resulting in a rapid re-infection shortly after the end of the treatment.

Since the treatments only partially or temporarily reduce the (oo)cyst excretion under natural conditions, the control of these protozoal infections relies on a combination of animal treatment and appropriate hygienic measures and management. Due to the particular parasite characteristics (Table I), such as the high (oo)cyst excretion by infected calves, the environmental resistance of excreted (oo)cysts and the presence of asymptomatic carriers, these protozoal infections should be considered as an endemic problem on infected farms. Hygienic measures must therefore aim to minimize the environmental infection pressure in order to prevent the spread of infection to susceptible calves and to break the transmission cycle. Frequent removal of bedding and thorough cleaning combined with disinfection help to reduce the (oo)cyst load in the environment. Unfortunately, these protozoa are extremely resistant to commonly used disinfectants. The most effective, but also the most toxic disinfectants are ammonia, methyl bromide, ethylene oxide and ozone (Fayer, 2004; Geurden et al., 2006b). (Oo)cysts are susceptible to extreme temperatures and to desiccation. Cleaning with hot water followed by drying is therefore an effective way to reduce (oo)cyst infectivity.

Good management practices include warm and dry individual calf facilities, preferably outside. High stocking densities should be avoided. Furthermore, a quarantine unit should be present at the farm to isolate clinically affected calves, with separate tools, boots and coveralls to prevent spread of infection. It is also important to ensure adequate colostrum intake, since colostral antibodies protect calves from developing severe clinical symptoms by blocking parasite invasion and immobilisation of gut luminal parasitic forms.

Since most treatments do not have a persistent efficacy, vaccination could provide a valid and long term alternative, especially for the prevention of disease. A *Giardia* vaccine is commercially available for use in cats and dogs (Fel-O-Vax *Giardia* or *Giardia* Vax, Fort Dodge Animal Health), but the efficacy of a preventive or curative vaccination seems to be variable. In calves vaccination against *Giardia* did not result in a protective immune response (Uehlinger et al., 2007).

Conclusions

Many studies indicate that *Cryptosporidium* and *Giardia* occur in cattle worldwide, although the prevalence reports vary markedly. Both infections can lead to gastro-intestinal disease, but symptoms caused by *Giardia* infection are usually less acute and in calves older than 1 month. Halofuginone is registered for the preventive and curative treatment of *Cryptosporidium* in calves, and was shown to be efficacious both in experimental and in natural conditions. For the treatment of *Giardia* in calves, there is however no drug registration, although several benzimidazole compounds were shown to be efficacious in experimental and to a lesser extent in natural conditions. Moreover, the efficacy of animal treatment in combination with hygienic measures in natural conditions has never been evaluated in cattle.

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Molecular characterization of *Cryptosporidium* spp. in pre-weaned dairy and beef calves and in adult dairy cattle in Western France

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Cryptosporidiosis is a very common infection in cattle worldwide. The agent responsible for this intestinal disease is *Cryptosporidium*. This parasite is considered as a major cause of neonatal diarrhea in calves. *Cryptosporidium parvum* has been frequently recorded as the dominant species in diarrheic calves but others species could occur in adults, particularly in subclinical situation.

In this study, pre-weaned calves and cows, from dairy and beef cattle herds located in Western France, were sampled in order to characterize oocyst output, parasite species and clinical features associated with infection. Fecal samples were screened for the presence of oocysts using immunofluorescence analysis after concentration. DNA was extracted and PCR-RFLP (or sequencing) was performed on the SSU rRNA gene of *Cryptosporidium*. Finally, for the genotyping of *C. parvum,* a gp60 PCR was realized.

The prevalence of *Cryptosporidium* sp varied according to the age of animals with the highest prevalence observed in pre-weaned calves (92 to 100%). Prevalence in adults was very low (1.6%) compared with younger animals. The level of excretion ranged from 100 to 1.7 x 10^7oocysts/g of feces (opg) in pre-weaned beef and dairy calves while this level was very low in adults (< 20 opg). The species *C. parvum*, *C. bovis* and *C. ryanae* were identified and different profiles were observed according to the age and date of sampling. Pre-weaned calves excreted first the species *C. parvum* or *C. bovis* from 5 or 7 days of age and then the species *C. ryanae* from 17 days of age. Significant differences concerning the clinical signs were observed depending the age of animals and the species identified. *C. parvum* and *C. bovis* were found both in diarrheic and non diarrheic beef calves.

Species excreted by adults could not be identified because of the low level of excretion. Finally, four subtypes of *C. parvum* belonging to the IIa zoonotic family were identified in calves.

This study shows that different patterns in *Cryptosporidium* species as well as in prevalence and intensity of infection may be observed according to the age, breed (beef or dairy calves) and conditions of sampling. *C. parvum* is undoubtedly the main *Cryptosporidium* species involved in neonatal diarrhea of calf far ahead *C. bovis*.

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Bovine neonatal diarrhea: *in vitro* inhibitory activities development of new drugs against *Cryptosporidium parvum*

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Cryptosporidiosis is a zoonotic disease caused by a protozoan parasite, *Cryptosporidium parvum*. In animals, it is considered as an economically important disease with clinical signs and death in young ruminants. The usual clinical course is acute diarrheoa affecting animals from 1 to 3 weeks old. Today, no drugs are fully effective in the treatment of cryptosporidiosis in man and animals.

Therefore the research for new therapeutic agents is crucial.

We report here details of the adaptation of *in vitro* culture systems (HCT-8 and Caco-2 cell lines) for *C. parvum* to investigate the "anticryptosporidial" activity of drugs and the results obtained with two new molecules (Chitosan NAG and Chitosan Mix). Chitosan, a natural polysaccharide compound, has been found to be active against a variety of diseases including antimicrobial and antitumoral effects. We investigated the effects of Chitosan in our two *in vitro* models we recently established in the laboratory. Paromomycin, a classical drug used in veterinary medicine, was used as a positive control. Immunofluorescence technique was used for the quantification of the parasites.

Our results showed a very significant reduction of viability of *Cryptosporidium* oocyts (> 95%) after pre-incubation of 24h at 37°C with Paromomycin ($P < 0.001$), Chitosan Mix and Chitosan NAG ($P < 0.001$). On the other hand, Paromomycin, Chitosan Mix and Chitosan NAG inhibited significantly the development of *C. parvum* in HCT-8 and Caco-2 cell lines ($P < 0.005$).

In conclusion, these findings provide for the first time the evidence of *in vitro* inhibitory activities of natural polysaccharides against *C. parvum*. *In vivo* preliminary data showed clearly that these drugs are effective in decreasing diarrheoa in ruminants.

Toxoplasma gondii infection in bovines: is it true?

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Objectives

Toxoplamosis is a zoonosis caused by the protozoan parasite *Toxoplasma gondii*. Toxoplasmosis is generally transmitted by ingesting tissue cysts from undercooked or raw meat or consuming food or drink contaminated with oocysts from the environment. France is the most important consumer of bovine meat within the EU, with a traditional raw meat consumption of it. A nationwide study was conducted to evaluate the prevalence of *T.gondii* in fresh bovine meat and to identify the risk factors of *T.gondii* carriage of an animal (age, sex, geographical origin, type of production).

Methods

2349 hearts and diaphragms of bovine originating from 46 selected slaughther-houses from all over France has been analyzed in correlation with their age, sex and geographical region. 570 muscles samples (diaphragms) originating from imported bovine carcasses (from EU and Brasil) has been supplementary included in the analysis. Serology has been performed using the MAT (Modified Agglutination Test) on cardiac fluids. An exclusion test has been performed on the tissue fluids against *Besnoitia besnoiti* and *Neospora caninum*. Direct detection of parasites has been performed after trypsic digestion of the tissue-samples by mouse bio-assay and real time-PCR.

Results

The overall estimate of *Toxoplasma* seroprevalence was 3% (2-5%) for calves and 18% (16-20%) for adult bovines ($P < 0.0001$). No significant difference was observed between imported and French meat. The proportion of French carcasses carrying live parasites according to bioassay results was of 2/207 (2 genotype II) for bovine carcasses.

Conclusion

This study represents the first large-scale analysis of the *Toxoplasma* infection in bovine population slaughtered in France. The seroprevalence is as high as 18%, with a strong predilection for old female animals. Concerning the geographical distribution more delicate analysis need to be done in order to understand the origin of the contamination and its link to the type of farming. Two isolates were identified, which emphasize the risk of human *Toxoplasma* contamination following the bovine raw meat consumption.

Selective treatment against gastrointestinal nematodes in dairy cows: identification of a cow profile with improved milk production after treatment

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To optimize treatments against gastrointestinal nematode (GIN) in adult dairy cows by selective treatment, we need to identify cows with an improved milk production (MP) after treatment.

The objective of this study was to determine whether there exists a typical profile of cows showing a milk production response after anthelmintic treatment. A field trial involving 20 grazing dairy herds in Western France was conducted in autumn 2010 and autumn 2011. In each herd, lactating cows were randomly allocated to a treatment group (fenbendazole) (541 cows), or a control group (547 cows). Daily cow MP was recorded from 2 weeks before until 14 weeks after treatment. Individual blood and fecal samples were taken at the time of treatment. Moreover, in each herd, were collected: (i) bulk tank milk samples, (ii) information regarding heifers' grazing history to assess the time of effective contact in months (TEC) with GIN larvae before the first calving. This TEC was expected to reflect the development of immunity against GIN. Thus, each cow included in this study could be characterized by: 3 production based indicators (parity, days in milk at the time of treatment, and production level), 3 parasitological indicators (serum anti-*Ostertagia* antibody levels (expressed as ODR), pepsinogen levels, and fecal egg count), and 3 herd level indicators (TEC, bulk tank milk ODR and percentage of positive egg counts). Statistical analysis is being carried out. Milk production models constructed with control cows' MP as the reference will enable to predict the expected MP of treated cows if they had not been treated. Then by comparing this expected MP with the observed MP, cows with an increased MP following treatment will be identified. These cows will be qualified as a "responding cow". The three types of indicators will then be tested in logistic regression models to identify those which are associated with the risk of being a "responding cow". A potential typical profile based on these indicators will be evaluated, and would be a useful tool to implement selective treatment against GIN in adult dairy cows.

Comparative long term efficacy of diclazuril and toltrazuril against natural infection by *Eimeria bovis* and *Eimeria zuernii in calves*

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Objective of the study

To investigate the effects and the efficacy of metaphylactic treatment with diclazuril against natural infections with pathogenic *Eimeria* spp in dairy calves, compared with toltrazuril, on a specially long follow-up (78 days after treatment).

Protocol of the study

The study was conducted as a randomised, blinded clinical field study of 199 calves in 9 french dairy farms. The study started on the day of treatment (SD1) and finished 78 days there after (SD 78). On SD1, 23 calves remain untreated and 88 healthy calves each were allocated to two separated groups (mean age at SD1 = 37 to 41 days). Both groups - diclazuril and toltrazuril- were treated orally once (diclazuril, VECOXAN® at 1 mg/kg BW and toltrazuril, BAYCOX® at 15 mg/kg BW).

Clinical and parasitological parameters were evaluated.

From SD1 to SD 78 (22 examination days), individual faecal examination were carried out twice a week (faecal score, coccidial OPG by Mac-Master countings, *Eimeria* species determination). Body weights (BW) were determined on SD1, SD22 and SD78.

Results

Tested qualitatively with Fischer's exact test and quantitatively with Mann-Whitney test, effects will be considered significant at $P = 0.05$.

At SD1, all parameters were not statistically different in the two groups.

During the whole study period, the mean number of days with diarrhea was also similar (0.7).

The animals in the toltrazuril group had significantly ($P < 0.05$) lower average OPG than animals in the diclazuril group from SD11-SD13 to SD32-SD34. From SD36 to SD78, no significant difference was observed excepted for the SD54, SD57, SD64 and SD68 which diclazuril group had significantly ($P < 0.05$) lower average OPG than toltrazuril group.

Between SD1 and SD22, the average daily gain (ADG) was not different between diclazuril and toltrazuril groups, whereas the ADG of diclazuril group was higher (74 g/day) between SD22 and SD78 comparatively to toltrazuril group. During the whole study period, the animals in the diclazuril group had an ADG significantly ($P = 0.01$) higher of 57 g/day comparatively to those of the toltrazuril group.

Discussion

It is possible to differentiate two periods in this study; first one : between SD1 and SD22, despite a higher prevalence excretion of pathogenic *Eimeria* spp (P.E.sp) in diclazuril group, the ADG and the faecal score were similar between the two groups, second one : between SD22 and SD78, when the prevalence excretion of P.E.sp.in toltrazuril group increases, the ADG becomes higher for diclazuril group than toltrazuril group, inducing the total difference of ADG between the both groups.

Conclusion

This study confirms that the rational metaphylactic treatment with benzene acetonitrile drugs is efficient for the control of pathogenic coccidia, with here the best result for diclazuril at 1 mg/kg BW. The right period for this treatment must be carefully determined, usually 15 days after allotment and stress in farms, or 1 week before usual onset of clinical cases.

Emergence of bovine besnoitiosis in Europe: how to stop the spread?

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Bovine besnoitiosis (BB), which is caused by *Besnoitia besnoiti*, is an emerging disease in France, Spain, Italy, Germany, Belgium and Switzerland. The control of BB is not an easy task given that there are currently no efficient treatment and no registered vaccines in Europe. Therefore, the control of this parasitic disease entirely relies on vector control and management measures coupled with diagnosis.

Here, we report the first results of a pilot project implemented in southeastern France where BB is emerging. Two main issues have been addressed: i) implementing a serological control before entry of new animals in uninfected farms and ii) testing the efficacy of a selective culling program in some infected farms.

A serological control of introductions has been applied on a voluntary basis on commercial trades in the pilot area. During the first year, 464 animals have been tested with a prevalence of 0.43%. Therefore, two previously uninfected farms have been preserved of the introduction of infected animals. Cost/benefit ratios of this measure are discussed.

In parallel, eleven infected and adjoining beef-cattle farms were enrolled in an evaluation of a selective culling program. In March 2010, serological prevalences were evaluated by ELISA and Western Blot. In four farms, the prevalences were low (from 1.2 to 5%) and exhaustive culling of seropositive animals was applied. Higher prevalences (from 13.6 to 57.6%) were noted in the seven remaining farms where regular applications of insecticides were proposed. Annual incidences of *B. besnoiti* infections were evaluated by serology one, two and three years later. No seropositive animals were detected in 2011, 2012 and 2013 in the four farms where an exhaustive culling was done. On the contrary, serological incidences were high (from 10 to 80%) in the adjoining seven farms during the same period. In conclusion, the rapid elimination of seropositive animals in lowly infected farms seems to be an efficient option to stop the spread of BB even if active "intra" herd transmission occurs in neighboring farms. Here, the absence of "between" farms transmission could be due to the feeding behavior of vectors and to the mechanical nature of transmission.

Milk analyses as a tool for assessment of digestive and metabolic imbalances of energy nutrition

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Introduction

Meeting energy requirements of dairy cows is a major goal to sustain milk production and needs a high digestive efficiency, mainly at the ruminal level where a major part of dietary organic matter is fermented to volatile fatty acids, and also needs an accurate metabolic utilisation of absorbed nutrients. This is especially critical during early lactation, due to the discrepancy between a mammary demand that increases faster than voluntary intake. This results in a negative energy balance (NEB) that can lead to subclinical or clinical ketosis, impaired immunity and reproduction. To limit this NEB, high levels of energy concentrates, especially grain, are usually given to cows, but they can result in subacute ruminal acidosis (SARA). This later abnormality can also occur during mid-lactation, due to abrupt dietary changes, excess starch or lack of effective fibre. Management of these adverse conditions at herd level requires a precise monitoring, and some biochemical parameters can be helpful to assess abnormalities of ruminal digestion or energy balance. Among these biochemical parameters, milk analyses have the advantage of an easy sampling, including by farmers, and are easily available in farms.

Assessment of energy balance

NEB results in the mobilisation of fatty acids (FA) from adipose tissues. Part of these FA, which circulate as non-esterified fatty acids (NEFA), are taken up by the liver. A part is metabolised to acetate in the liver, but not all this acetate can be oxidised du to the competition of gluconeogenesis with tricarboxylic acids cycle for acetoacetate. Hence, the remaining part of acetate is converted to ketone bodies: beta-hydroxybutyrate (BHB) and aceto-acetate, which can be converted to acetone. Another part of mobilised FA is re-esterified in the liver, resulting in triacylglycerols that are either exported to the blood flow after assembly into VLDL, or stored as lipid droplets in liver cells. Because NEB is more frequent during the peripartum period, including the two last weeks of gestation and the two first months of lactation, ketosis and fatty liver are more usually observed during early lactation.

Due to this strong relationship between NEB and ketosis, these two conditions are often confused, and most literature regarding criteria for assessment of NEB are actually criteria for assessment of either adipose tissue mobilisation (plasma NEFA) or ketosis (ketone bodies accumulation). For ketosis assessment, the usual biochemical gold standard is blood BHB, with a threshold value 1200 to 1400 µmoles/l for subclinical ketosis. However, NEB and ketosis are not tightly linked; for example, Heuer et al. (2000) showed that blood BHB has only a 25% sensitivity to detect cows having the highest NEB in a herd. For NEB, changes of body condition score over time are often considered as the best assessment method, but these changes can only measured over a several weeks period of time, so that they cannot be easily compared to biochemical measurements, which reflect metabolic changes over a shorter period of time.

Test-day milk analyses are usually made at monthly intervals by dairy herd improvement organisations, and include milk fat, milk protein, and in many cases, milk urea. Analysis of some ketone bodies becomes more and more available. Moreover, especially in herds using an automatic milking system, additional kits can be available for milk analyses.

Milk fat and milk protein contents

FA mobilised from adipose tissue during NEB periods are partly available for mammary uptake, resulting in high milk fat content. At herd level, Nordlund and Cook (2004) proposed 10% of cows less than 60 days in milk having a milk fat content over 55 g/l as being indicative of ketosis or fatty liver.

Conversely, in cows with a low body condition score at calving, milk fat content is low during months 2 and 3 (Holter et al., 1990). More generally, at individual level, a strong drop of milk fat content between the two first monthly tests is usually considered as being due to a very high loss of fat between the two controls, and indicates a very poor body condition after the production peak. Finally, interpretation of milk fat content needs to consider the herd status regarding SARA, which can negatively affect milk fat (see later). However, some observations by Enemark et al. (2004) suggest that the milk fat content increase due to mobilisation surpasses the decrease due to SARA.

Low milk protein content is a usual effect of NEB in dairy cows: lowering intake by 1700 kcal of net energy (7.1 MJ) results in a 0.3 and 0.6 g/kg drop in milk protein content in early and mid-lactation cows, respectively, whatever initial milk production and milk protein content (Coulon and Remond, 1991). These changes are in a narrow range, so that

values in cows with NEB can remain in a physiological range. As a result, using a unique threshold for all herds can lead to strong misinterpretations, and the threshold should take into account the mean values at cow or herd level, which roughly reflect genetic merit. Martinot (2006), in a survey relative to the relationship between NEB and fertility, proposed a 28 g/l threshold for milk protein content at individual level in early lactation, and proposed to increase this threshold to 29 or 30 g/l in herds selected for high milk protein content.

Brun-Lafleur et al. (2010) showed an interaction between NEB and dietary protein level on milk protein content: the negative effect of NEB on milk protein content is high when protein requirements are met or exceeded, but is very low in protein deficient diets. Milk urea content can help interpretation of milk protein content, with low urea concentrations observed with low protein diets as opposed to high values with low energy diets. This underlines that interpretation of milk protein content must be cautious: in most herds, abnormally low milk protein contents are due to NEB, but insufficient protein supply, insufficient methionine supply or high fat diets can also result in low milk protein, which probably explains the moderate specificity (65%) observed by Duffield et al. (1997) when using a 29 g/l milk protein content for ketosis detection.

Because milk fat and milk protein contents follow opposite patterns when energy balance is negative, the fat / protein ratio can be expected to be more heavily modified than either one of these contents. Grieve et al. (1986) showed that this ratio more strongly correlates with energy balance in early lactation cows than either milk fat or milk protein contents. This ratio has been proposed by many authors as a better tool than either milk fat or milk protein for evaluation of NEB or subclinical ketosis. For example, at individual level, Heuer et al. (1999) found that a fat/protein ratio over 1.5 at first test-day is associated with a high body condition loss. Heuer et al. (2000) showed that a fat/protein ratio over 1.5 is a better predictor of NEB between weeks 2 and 12 of lactation than either milk fat or milk protein contents (Table I). Van Knegsel et al. (2010) and Krogh et al. (2011) proposed to declare ketotic cows having a fat/protein ratio over 1.5, measured weekly during 9 weeks or measured once during weeks 2 or 3, respectively. At herd level, Duffield (2011) proposed to declare at high risk of ketosis herds with more than 40% of cows having a fat/protein ratio over 1.33 at first test-day.

The relationship between fat/protein ratio and energy balance is weaker in mid-lactation than early-lactation cows (Buttchereit et al., 2010). Genetic merit of cows can affect the interpretation of values, and Stoop et al. (2009) proposed to use the difference between fat to protein ratio observed in a sample and the average value of the same cow over an entire lactation, to avoid cows with a high genetic merit for fat percentage being always defined as energy deficient.

Table I: Sensitivity and specificity of milk test data to detect negative energy balance (from Heuer et al., 2000)

Criterion / threshold	Sensitivity / specificity to detect the 10% cows with the highest NEB	Sensitivity / specificity to detect the 25% cows with the highest NEB
Milk fat > 48 g/l	39 / 97	28 / 89
Milk protein < 29 g/l	17 / 85	18 /86
Fat / protein > 1.5	51/ 87	41/ 91
Milk BHB strips > 100 µmoles/l	29 / 91	24 / 93

Interpretation of both milk fat and milk protein content could be improved by taking into account days in milk because typically, these two values strongly decrease during the first month of lactation and reach a nadir during the second month (Reist et al., 2003), so that the fat/protein ratio at first control (between 7 and 35 days) poorly correlates with the fat/protein ratio seven days after calving, which best predicts health or milk production (Toni et al., 2011). However, at herd level, days in milk at first test-day differ among cows and cannot easily be taken into account.

Milk ketone bodies

Ketone bodies (BHB, acetoacetate and acetone) produced by the incomplete oxidation of FA are in part taken up by the mammary gland, and their milk concentration can reflect the metabolic status of the animal. Milk acetone concentrations are similar to blood acetone concentrations, whereas milk BHB and milk acetoacetate concentrations are far under blood concentrations (Enjalbert et al., 2001) due to BHB utilisation for short-chain FA synthesis by the mammary gland and acetoacetate - BHB interconversions.

Milk BHB and acetone can be analysed:

- quantitatively by laboratory methods, using chromatography or enzymatic kits,
- quantitatively as a cow-side test, using Abbot Precision Xtra Meter™ (Iwersen et al., 2009),
- quantitatively by Fourier-transformation mid-infrared spectroscopy (van der Drift 2012), whose values correlate around 0.80 with values obtained with chemical methods (de Roos et al., 2007); this analysis can be included in test-day milk analyses laboratories, which routinely use mid-infrared spectroscopy for

determination of fat and protein contents.

- semi-quantitatively using test-strips for BHB (Ketolac™, also distributed as Ketotest™), which can be used as cow-side tests. Farm analysers associated with automatic milking systems can also use strips for BHB analysis.

Milk ketone concentrations have been extensively studied for detection of subclinical ketosis (Table II), compared to blood BHB as the gold standard. Cut-off values differ among studies: some authors published sensitivities and specificities for several thresholds, others proposed a threshold maximizing the sum of sensitivity and specificity, and another gave priority to sensitivity considering that the cost of a false positive is under that of a false negative. This results in cut-off values ranging from 23 to 200 µmoles of BHB/l, with a low specificity for thresholds under 100 µmoles/l and a low sensitivity for high thresholds.

At individual level, detection of subclinical ketosis should be done in very early lactation cows, since Mc Art et al. (2012) showed that peak prevalence of subclinical ketosis occurs during the first lactation week, and that milk production and fertility are more affected in cows that are ketotic during the first lactation week than in cows that become ketotic later. Such a time effect is a limit to test-day milk analyses, which exclude the first week of lactation.

The relationship of milk ketones with energy balance is far less known than the relationship with subclinical ketosis. Heuer et al. (2000) showed that milk BHB with a 100 µmoles/l threshold, measured by strips, has a 29% sensitivity for detection of a high NEB in cows between 2 and 12 weeks of lactation. This sensitivity was far lower than the sensitivity to detect subclinical ketosis (Table II).

Finally, the relationship between milk ketone and NEB can depend on lactation stage: an induced nutrient restriction results in increased milk BHB in early lactation cows, but not in mid- and late-lactation cows (Bjerre-Harpoth, 2012).

Regarding milk acetone, Clark et al. (2006) underlined a strong day-to day variation, making it necessary to average samples taken at intervals < 3 days, which is possible in robotic dairies. They proposed a threshold value of 140 µmoles/l and outlined that the correlation coefficient between milk acetone and energy balance is -0.64, but they did not determinate sensitivity and specificity of milk acetone for detection of subclinical ketosis or NEB.

Table II: Sensitivity and specificity of milk BHB and acetone to detect subclinical ketosis

Criterion /threshold (µmoles/l)	Reference	Sensitivity	Specificity
BHB ≥ 70	Enjalbert et al., 2001	92	64
BHB ≥ 200	Iwersen et al., 2009	60	89
BHB ≥ 300	Iwersen et al., 2009	40	97
BHB ≥ 23	Van Knegsel et al., 2010	80	71
BHB ≥ 76	Van der Drift et al., 2012	83	76
BHB strip ≥ 100	Enjalbert et al., 2001	96	63
BHB strip ≥ 50	Carrier et al., 2004	88	90
BHB strip ≥ 100	Carrier et al., 2004	73	96
BHB strip ≥ 200	Carrier et al., 2004	27	99
BHB strip ≥ 50	Oetzel, 2004	89	80
BHB strip ≥ 100	Oetzel, 2004	87	83
BHB strip ≥ 200	Oetzel, 2004	45	97
BHB strip ≥ 100	Iwersen et al., 2009	90	94
BHB strip ≥ 200	Iwersen et al., 2009	30	98
BHB strip ≥ 200	Krogh et al., 2011	58	99
Acetone ≥ 160	Enjalbert et al., 2001	92	57
Acetone ≥ 70	van Knegsel et al., 2010	80	71
Acetone ≥ 131	Van der Drift et al., 2012	71	89

Milk fatty acids composition

During periods of NEB, FA originating from adipose tissue mobilization are in part available for mammary uptake. Briefly, milk FA originate either in a mammary synthesis (FA with 4 to 16 carbons) or in an arterial uptake (FA with 16 carbons or more), so that palmitic acid (C16:0) has both origins. The FA composition of adipose tissue differs from that of milk fat, which has much more FA with less than 16 carbons, and less FA with 18 carbons, especially *cis*-9 C18:1 (around 20% in milk fat *vs* 30 to 40% in adipose tissue, Hostens et al., 2012). As a result, when the mammary gland takes up FA from adipose tissue in the arterial blood, the proportions of C18:0 and *cis*-9 C18:1 can be expected to increase, and the proportions of FA with less than 16 carbons can be expected to decrease. Experimental data support these trends at various lactation stages (Table III), and indicate that changes of C16:0, branched-chain FA and *trans* FA including conjugated linoleic acid (CLA) are much less reliable.

Variations of FA profile suring NEB are relative to major FA, so that it is possible to use results of mid-infrared analysis, which allows an accurate separation of major milk FA, including individual and total saturated FA, and oleic acid (Rutten et al., 2009).

However, milk FA composition also greatly depends on diet, especially fat addition and dietary FA profile, so that individual milk FA profiles should preferably be compared with herd average FA profiles than to standard average values. At this time, no cut-off point or NEB prediction model has been proposed for milk FA proportions or deviations.

Table III: Changes in milk FA proportions due to negative energy balance

	Van Haelst et al., 2008	**Stoop et al., 2009**	**Gross et al., 2011**	**Knapp et al., 2012**
Lactation stage	< 9 weeks	day 63 to 282	< 26 weeks	week 1 to fertilization
Method for energy balance estimation	blood BHB > 1200 µmoles/l	deviation of fat / protein ratio relative to entire lactation	individual measurement of intake and milk yield or NEB induced by feed restriction	NEFA classes
C6 to C14	decrease	decrease	decrease	decrease
C5 to C15		decrease		
C15			decrease	
C16:0		increase	decrease	decrease
Branched-chain FA		no change	increase	
Total C18	increase	increase		
C18:0		increase	increase	increase
Total unsat. C18		decrease		
cis-9 C18:1	increase		increase	increase
trans C18:1			no change	
Polyunsat. C18			no change	no change
CLA		decrease	increase	

Other milk components

In a recent study Bjerre-Harpoth (2012) observed a strong increase of milk citrate during induced feed restriction, whatever lactation stage, and suggested this metabolite to be a promising biomarker. This increase of citrate concentration could be due to a decreased cytosolic conversion of isocitrate to α -ketoglutarate for production of NADPH, which is necessary for mammary FA synthesis, knowing that FA synthesis decreases during feed restriction du to increased availability of plasma FA. However, more studies, especially regarding other possible origins of milk citrate variations such as mastitis or lactation stage (Garnsworthy et al., 2006), are necessary.

Multiple criteria models

Some authors have proposed prediction models for assessment of NEB based on several criteria. A selection of models mainly based on milk criteria is presented in table 4. Reist et al. (2002) and Friggens et al. (2007) underlined the lack of precision of their models at individual level. Moreover, Reist et al. (2002) and Heuer et al. (2000) outlined the importance of herd size for an accurate prediction at herd level, so that Reist et al. (2002) proposed to use his models in herds with more than 100 to 400 cows according to seasonal grouping of calvings.

A promising alternative approach has recently been developed by McParland et al. (2011, 2012). Test-day milk analyses are routinely performed using mid-infrared spectrometry, which allows determination of most milk fat components, including protein and fat contents, or FA composition, with calibration equations. Instead of using milk composition derived from mid-infrared spectra, these authors developed a calibration equation to directly predict energy balance of cows from spectrometric spectra, which has the advantage to avoid restricting the use of the spectra to what is necessary to predict milk composition.

Table IV: Some multicriteria models for evaluation of negative energy balance

	Lactation stage Estimation of NEB	Milk components	Other criteria	r^2
Heuer et al., 2000, 2001	< 21 weeks calculated from energy intake and milk yield	protein fat / protein ratio	lactation week parity milk yield	0.25
Reist et al., 2002	< 20 weeks calculated from energy intake and milk yield	fat / lactose ratio acetone	% concentrate lactation week milk yield	0.69
Reist et al., 2002	< 20 weeks calculated from energy intake and milk yield	protein urea fat / lactose ratio acetone		0.33
Friggens et al., 2007	all calculated from body weight and body condition score changes	fat fat / protein ratio changes of fat / protein ratio, milk yield and protein yield from the day before	days in milk	0.39
McParland et al., 2011, 2012	all calculated from energy intake and milk yield	mid-infrared spectrometry spectra	milk yield	0.56

Assessment of ruminal carbohydrate digestion

Carbohydrates provide the largest part of dietary energy available for ruminants. They are fermented in the rumen, resulting in the production of volatile fatty acids (mainly acetic, propionic and butyric acids), gases (mainly CO2 and methane), and energy used by the microbiota for its growth. Most volatile fatty acids are absorbed by the rumen mucosa. They are used by animal metabolism, including for gluconeogenesis from propionate and milk fat synthesis from acetate and butyrate, so that their proportions are of importance for meeting energy requirements and providing precursors for the synthesis of milk constituents.

When the production rate of volatile fatty acids exceeds absorption rate, rumen pH drops, which leads to changes of carbohydrate fermentation, including an increase of butyrate or propionate productions at the expense of acetate, and a net lactate production. These rumen changes affect the microbiota. They can also affect rumen lipids metabolism: biohydrogenation of FA can shift to unusual intermediates, which are absorbed in the small intestine and partly exported into milk, and can result in strong changes of lipid mammary metabolism, including milk fat synthesis.

Milk fat content

The general trend for milk fat content is to decrease during SARA; a field study showed an average 3 g/kg improvement of milk fat content at herd level when solving a SARA problem (Stone, 1999). This change has long been considered to result from a lowered proportion of rumen acetate, which is a precursor of mammary FA synthesis. Milk fat depression during SARA is now mainly considered to result from ruminal synthesis of *trans*-10 FA during biohydrogenation of linoleic acid (see later). Consistent with this relationship, Nordlund et al. (2004) proposed to suspect SARA in herds where more than 10% of cows have a milk fat content under 25 g/l.

Milk fat content is however a controversial tool for SARA detection, because the determination coefficient with rumen pH is very variable among studies, varying from 0.18 (Enemark et al., 2004) to 0.39 (Allen et al., 1997). Moreover, Enemark et al. (2004) only observed this relationship when grouping cows by lactation stage and estimated that, due to fat mobilisation in early lactation, milk fat percentage is of no value for detecting SARA during the first month of lactation, which represents a strong limit since this period is at high risk of SARA due to dietary changes.

Other dietary factors can affect the relationship between milk fat content and SARA, especially the interaction of unsaturated fat addition, which strongly increases the effect of a low rumen pH on milk fat content (Griinari et al., 1998). Moreover, dietary supplementation with *trans*-10,*cis*-12 CLA, FA formed in the rumen during SARA (see later) can be used in farms to manage milk fat production, resulting in low milk fat content (Chouinard et al., 1998) without any relationship with SARA.

Because milk fat changes due to SARA can be low (Stone, 1999), taking into account the "normal" values at individual or herd level can be useful. Moreover, because milk protein content is poorly affected by SARA, using fat / protein ratio or fat – protein difference is not expected to increase the accuracy of milk fat content for SARA detection (Nordlund et al., 2004).

Milk fatty acids composition

Changes of ruminal fermentation can affect milk FA profile by different ways:

- different bacteria or bacterial groups have different FA profiles, especially for odd- and branched-chain FA,
- changes in rumen fermentation can result in changes of ruminal biohydrogenation of dietary unsaturated FA, especially regarding *trans* FA isomers,
- mammary uptake of some specific FA, including some isomers of CLA, can affect mammary metabolism (*de novo* synthesis of FA, Δ9 desaturation of stearic acid to oleic acid).

A first objective of assessment of rumen fermentation is the prediction of volatile fatty acids proportion and methane production, with mainly a nutritional and environmental interest. Milk odd-and branched-chain FA as a tool to evaluate rumen fermentation have been extensively studied at Ghent University, with the initial study of Vlaemink et al. (2006) and the recent update of Fievez et al. (2012). This team proposed models allowing prediction of 50 to 75% of variations of rumen volatile fatty acids and methane proportions. FA with a high predictive value are:

- *iso* C14:0, which is abundant in fibrolytic bacteria and correlates positively with acetate and methane, and negatively with propionate and butyrate,
- *iso* C15:0 which strongly and positively relates to acetate and methane,
- C15:0 and C17:0 which are abundant in amylolytic bacteria and negatively relate with acetate and methane, and positively correlate with propionate and butyrate,
- *anteiso* C15:0 which negatively correlates with butyrate (Vlaeminck et al., 2006).

The same team proposed another model emphasizing the predictive interest of *iso* C14:0, C15:0 and C17:0 (Bhagwat et al., 2012). However, other recent data (Patel et al., 2013) showed that C15:0 and C17:0 increase when grass silage proportion in the diet increases at the expense of concentrates, which is not consistent with their abundance in amylolytic bacteria, and suggests that models must be validated over a great variety of diets.

Other models, mainly dedicated to prediction of methane production, have been proposed. The model of Chilliard et al. (2009) was based on a limited number of experimental observations with linseed addition. Dijkstra et al. (2011), using a larger dataset of experimental data, also found a positive relationship between *iso* C14:0 and *iso* C15:0 and methane production, and outlined that various *trans* C18:1 isomers negatively relate with methane production.

A second objective of assessment of rumen function is the detection of SARA. During SARA, changes of rumen bacteria occur, especially a drop of fibrolytic bacteria and an increased abundance of amylolytic bacteria, which result in decreased acetate and increased propionate proportions, and can be reflected by odd- and branched-chain FA as stated above. Moreover, during SARA, rumen biohydrogenation of unsaturated FA is modified, especially that of C18:2 (linoleic acid) with a shift from the usual *trans*-11 pathways, whose intermediates are *cis*-9,*trans*-11 C18:2 (rumenic acid) and *trans*-11 C18:1 (vaccenic acid) to the *trans*-10 pathway with *trans*-10,*cis*-12 C18:2 and *trans*-10 C18:1 as intermediates (Griinari et al., 1998). This shift is more likely to occur when high starch diets are associated with supplementation with a high C18:2 fat source, like soybeans and sunflower (Zened et al., 2013). These later authors showed that this shift occurs when the acetate / propionate ratio is close to 1, far under the "normal" 2.5 to 3.0 value. This *trans*-11 to *trans*-10 shift can also occur without C18:2 dietary addition, and Enjalbert et al. (2008) showed, using experimental acidosis induced by abrupt wheat addition to the diet, that the *trans*-10 / *trans*-11 FA ratio strongly increases when rumen pH was under 6.0, but outlined that the relationship between rumen pH and this ratio could depend on fat content of the diet and the nature of forage, making it difficult to use this ratio as a tool for ruminal acidosis detection. They also outlined high negative correlation coefficients between rumen pH and several milk FA proportions, including C10:0, C12:0, C13:0, C15:0 and total odd-chain FA. Similarly, Colman et al. (2012) showed that during induced acidosis, milk proportions of odd-chain FA (C15:0 and C17:0) increased whereas those of *iso* FA (from C13:0 to C17:0) decreased. They also observed that milk proportions of *trans*-10 isomers increase when rumen pH exhibits high daily variations, but can remain low when rumen pH is low but stable. This is consistent with the results of Weiss et al. (2013), showing that abrupt changes of unsaturated fat content in the diet result in the highest milk proportions of *trans*-10 FA, reflecting alterations of rumen microbiota.

Most FA that can reflect rumen digestion are minor milk FA, and cannot be quantified by rapid determination using infra-red techniques (Rutten et al., 2009), which makes investigations costly, especially at individual level.

Conclusion

Milk analyses can be a useful tool for assessment of energy digestion or metabolism in dairy cows. Some values are available in test-day milk analyses reports, some others can be determined as cow-side tests, or by analysers associated with automatic milkers. On the contrary, some analyses, like quantitatively minor milk FA, need a more expensive laboratory assay. Because analytical data are usually stored in the farms, they offer the possibility of a retrospective investigation of nutritional status at individual or herd level.

Parameters can be indicative of NEB and/or ketosis, or of rumen function or dysfunction. Except milk ketone bodies that directly relate to ketosis, the other criteria are not sufficient to establish a diagnostic due to possible confusion with other sources of variation. Hence, they must be associated with investigations regarding dietary origin and clinical effects of suspected imbalances.

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Investigation into the prevalence of ketosis in peri-parturient dairy cows in EU dairy herds in 2011-2012

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The aim of this study was to determine the prevalence of ketosis in West European dairy cows and its association with fresh cow diseases.

A total of 4709 transition cows from 130 dairy farms were enrolled in Germany, France, Italy, the Netherlands and the UK during 2011-2012. A milk-based test for ketones (Keto-Test, Elanco) was used for screening fresh cows and ketosis was defined as Keto-Test ≥ 100 µmol/l between 7-21 days after calving. Study cows were observed for clinical disease up to 35 days post calving. Multivariate analysis (GEE logistic regression) was performed to determine country, farm, management, feed and cow factors associated with ketosis and the models controlled for random effects of cows within farms within country. The associations between ketosis and fresh cow diseases or conditions within 35 days of calving were assessed through a series of multivariate GEE logistic regression models for each individual diagnosis.

Thirty-nine percent of the cows were classified as having ketosis. The herd average of ketosis was 43% in Germany, 53% in France, 31% in Italy, 46% in the Netherlands and 31% in the UK. While clinical ketosis was not reported in most farms, 111 out of 130 (85%) had 25% or more of their fresh cows screened positive for ketosis. The risks of ketosis were significantly lower in Italy and the UK compared to France, the Netherlands and Germany. Larger herd size was associated with a decreased risk of ketosis.

The odds of ketosis in parity2 and parity3-7 was significantly higher (1.5 and 2.8 times higher) than the odds of ketosis in parity1. The odds of ketosis was significantly smaller in parity2 compared to parity3-7. Ketosis was associated with significantly higher odds (OR) of all common fresh cow conditions; milk fever (OR:2.1), retained placenta (OR:1.6), metritis (OR:1.5), mastitis (OR:1.9), displaced abomasums (OR:3.4), clinical ketosis (OR:14.7), lameness (OR:1.8) and gastro-intestinal disorders (OR:3.8).

This study shows that ketosis is very common in European dairy cows and that ketosis may be a gateway disease for peri-parturient disorders.

Longitudinal analysis of milk somatic cell counts: a case study

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The Somatic Cell Count (SCC) is a main indicator of milk quality. French dairy farmers are financially penalized for having averaged SCC > 250 000 cells/ml via quality penalties subtracted to each milk payment. Hence, identifying specific udder health risk factors associated with herd management is a major issue to improve milk quality. In the present study, data stored on an individual animal basis by the national milk recording system from a herd suffering endemic high SCC were analysed using a linear mixed model. Test-day milk yields (kilograms of milk and fat protein ratio), parity rank, length of lactation, time factor in days were included as fixed effects and SCC as the response variable. The herd with a milk production (at the end of the study) of 9.800 kg (n = 115) milk per lactation was enrolled from October 2007 to February 2013.

A total of 5333 Test-day observations from 418 Holstein cows were included in the study. Ranges and averages of investigated variables were: SCC [3 000 cells/ml to 8 777 000 cells/ml] with an average of 263 7500 cells/ml; parity rank [1 to 7] with an average of 1,9; milk production [5 kg to 57 kg] with an average of 27,48 kg; length of lactation [1 control to 33 controls] with an average of 5,20 controls; fat protein ratio [0,29 to 2,99] with an average of 1,25. As expected, the results showed that parity rank and length of lactation affected negatively on the SCC level (high parity rank and high length of lactation were associated with high SCC). Milk production was negatively associated with SCC (low milk productions were associated with high SCC). The interaction between time factor and fat protein ratio was found significant. This significant interaction revealed that during the first part of the study high fat protein ratio (cows suffering from sub-ketosis) were associated with high SCC. On the other hand, during the last period no statistical relationship was observed between fat protein ratio and SCC. This latter period was associated with a lower starch diet since grass silage and sugar beet pulp were added to the feeding of the cows.

Conclusively these results suggest a link between cow feeding and SCC. Moreover, high fat protein ratio was found to be a higher risk factors even though a possible sub-acidosis diet was given to the cows.

One case of suspected bovine Marfan syndrome: pathological investigations

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Marfan syndrome (MFS) is a human autosomal dominant disorder caused by a mutation in the FBN1 gene which encodes fibrillin-1 a major component of extracellular microfibrils. MFS is characterized by cardiovascular, ophthalmic and muscoloskeletal symptoms.

Cattle can exhibit the major clinical and pathological signs of human MFS and are recognized as potential models of the human pathology

The authors report the gross pathology and light microscopic lesions in n 3 young adults which died of sudden death while they were grazing.Gross lesions observed were laceration of the aorta and cardiac tamponade.

Aorta, heart, liver, lung and kidney were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned and stained with hematoxylin and eosin and Elastic Van Gieson stain (EVG).

Histological examination of EVG stained sections of aorta revealed severe elastic fibers degeneration in the tunica media. Elastic fibers were frequently fragmented, lacking parallel orientation and vertical fibers were numerous. Smooth muscle cells in the media were large and abundant, organized in big clusters surrounded by degenerated elastic fibers. Aortic rupture showed multifocal hemorrhages in the media and adventitia tunica; granulation tissue, characterized by numerous small blood vessels, near rupture sites indicated an acute lesion. Tissue from lung, heart, kidney and liver contained no disorganization of elastic fibers.Ultrastructural investigations are in progress.

Antibiotic use and trends in resistance in cattle

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Antibiotics were introduced as therapeutic molecules in the 1940's and considerably improved the treatment of diseases in all animal species, particularly food-producing animals. Their use was also closely associated with an increase in food production capacity all over the world. On the other side, antibiotic use is the primary driving force for the selection and dissemination of resistant bacteria, including pathogenic and commensal ones. The vast diversity of the molecular mechanisms allowing bacteria to resist to antibiotics is also a main component of the dynamics of resistance. As an example, mobile genetic elements carrying resistance genes -such as plasmids- are able to propagate resistance very efficiently among bacteria, including of different species. Those plasmids are specifically abundant in Gram negative bacteria and certainly contribute to the spread of some major resistance traits, such as genes encoding Extended-Spectrum beta-Lactamases (ESBL) conferring resistance to last-generation cephalosporins.

This lecture will consider various aspects of the use of antibiotics in cattle and discuss possible options to tackle this problem, in line with an overview of the trends in resistance in this animal species. This latter issue will in particular include recent data on the most important resistance traits in cattle, such as ESBLs and Methicillin-Resistant *Staphylococcus aureus* (MRSA), but will also highlight to what extent the molecular mechanisms of resistance may contribute in different ways to the dynamics of this burden. The importance of cross- and co-resistance and the impact of the antimicrobial use on pathogenic but also commensal bacteria will be also discussed.

The use of antibiotics: lessons from the past and future options

Even though the emergence and spread of resistant bacteria has been growing over the last decades, the continuous introduction of new molecules has been considered the main option to raise this issue for a long time. This strategy was at first developed in human medicine and subsequently in animals. However, the development of new antibiotics has become limited over the years, and it is now obvious that medical and veterinary practitioners will have to deal principally with the existing stock in the future. Consequently, one major sound strategy against the wide spread of resistance must include a prudent use of these molecules by ensuring that antibiotics are not used if not needed. Considerable efforts are being made in several countries, particularly in Europe, to produce guidelines on the appropriate use of antibiotics in the different diseases (Garcia-Alvarez, al., 2012). In particular, the major selective pressure exerted on the whole bacterial ecosystem by prophylactic treatments is a main reason for stopping those practices. Considering that bacteria can spread widely among animals and to the environment, these guidelines should be set up in all animal sectors as well. However, besides limiting the overall use of antibiotics, other strategies are needed which should include measures to prevent infections at farm, development and use of alternative options such as vaccines, and improved efficiency of diagnostic methods.

Antimicrobial sales, antimicrobial use and selection pressure in cattle: how to provide an appropriate assessment?

The selection pressure exerted by the use of antibiotics is a very important step for an accurate control of the emergence and spread of resistance in animal and human populations. In cattle, as in any other food-producing animal species, the antibiotic consumption was historically expressed in tons of molecules that were sold for use. This was systematically contributing to a confused situation when comparing data in veterinary and human medicine, leading to disproportionate figures when expressed in absolute numbers. To this respect, tetracyclines and sulphonamides are the top two most important families sold in food animals worldwide, and one could even consider that those antibiotic molecules sold for food animals such as cattle were not overlapping that much with those prescribed to humans.

This has changed recently in several countries where appropriate parameters of exposure were considered by experts, which include numerous items such as the number of treatments per animals, an estimate of the body weight at the time of treatment or the duration of treatment (Callens et al., 2012; Pardon et al., 2012; Persoons et al., 2012; Timmerman et al., 2006). Hence, the use of treatment incidences based on animal daily doses has now become a commonly accepted way to assess the selection pressure by antibiotics in animals. This was proposed a few years ago by comparison with standardized measurement units introduced internationally in human medicine and based on daily defined doses.

Difficulties for an efficient strategy: multidrug resistant bacteria and impact on the commensal flora

Both pathogenic and commensal bacteria are being exposed to several classes of antibiotics and the selection of so-called multidrug resistant bacteria has now become a growing and important issue in livestock populations. As an example, most ESBL-producing *Enterobacteriaceae* found in cattle also harbor resistances to non beta-lactam antibiotics, such as phenicols, sulphonamides, quinolones or tetracyclines (Valat, 2012). Consequently, the use of any of those antibiotics not only favors their own resistance but also co-select for all other resistance genes present in the same bacteria. This highlights the importance of a global strategy aimed at reducing/avoiding any unnecessary antibiotic use in food animals without strictly focusing on so-called critically important molecules (last-generation cephalosporins and fluoroquinolones).

This strategy should be extended to other nutriments supplemented in the cattle diet, such as copper. A recent study highlighted the consequences of including copper in diets at elevated doses in U.S. cattle for growth-promotional effects in feedlot animals (Amachawadi, 2013). This study demonstrated that the prevalence of fecal enterococci carrying the *tcrB* gene -conferring resistance to copper- was higher among cattle fed diets supplemented with copper (6.9%) compared to controls (0.7%). Those isolates harbored also resistance to macrolides and tetracyclines, conferred by the *ermB* and *tet*(M) genes, respectively, which were co-localized with the *tcrB* gene on the same plasmid. Again, these data demonstrate the complexity of the selection pathways of resistance, and the major role of co-resistance (and subsequently, co-selection).

Another major issue to consider is the general impact of any antibiotic prescription on the level of resistance of the enteric flora. This can be for instance highlighted through the example of the use of florfenicol for the treatment of respiratory tract infections in cattle. Whereas the resistance of florfenicol in target pathogens such as *Pasteurellaceae* is more than limited in cattle, the dissemination of the *floR* gene in enteric *E. coli* isolates has been increasing overtime. To this respect, recent data from the French Surveillance Scheme in animal pathogens (Resapath, www.resapath.anses.fr) indicate that ca 25% of the *E. coli* isolates from diarrheic calves do possess the *floR* gene, including on plasmids carrying resistance to last-generation cephalosporins (Meunier et al., 2010). Moreover, this prevalence has gradually increased since the introduction of the florfenicol on the veterinary market. Considering that florfenicol has no indication for the treatment of diarrheic calves, this strongly suggests indirect pathways for the selection of florfenicol resistance at farm.

Resistance in cattle: where are the hotspots?

In cattle as in other food-producing animals, the selection and spread of antimicrobial-resistant bacteria refer to a considerable number of genes and bacteria species in line with the diversity of the antimicrobials used. However, ESBL and MRSA are two main concerns for the veterinary medicine, not only with regard the growing prevalence of these traits in livestock but also because of their potential transfer to the human population (Garcia-Alvarez et al., 2012; Pitout and Laupland, 2008).

MRSA are major pathogens in humans that emerged rapidly after the introduction of methicillin in the early 60's. Despite a variable incidence depending on countries and continents, MRSA have become widespread in the last decades in humans both in healthcare settings and the community. In animals, MRSA were first identified in cattle mastitis in 1972 in Belgium, but a major focus resulted from the report of a specific pig-associated MRSA strain (ST398) in 2005, next to infections of humans in close contact with pigs in the Netherlands (Huijsdens et al., 2006). Of note, the prevalence of MRSA in veal calves is high in certain European countries as well (Graveland et al., 2010). MRSA ST398 isolates harboring the Immune Evasion Cluster (IEC) complex, suggesting a possible evolution of this clone towards an adaptation to humans, were also identified in veal calves in France (Haenni et al., 2011). *S. aureus* is also a commonly isolated mastitis-pathogen but was considered till now as mostly spared from resistance to methicillin in cattle. In particular, MRSA from cattle were not considered at significant risk for humans, and MRSA infections in dairy cows were even found occasionally to be caused by human MRSA clones (Haenni et al., 2011). Nevertheless, this picture has changed in 2011 with the report of a new bovine-associated MRSA strain carrying a novel *mecA* gene (namely LGA251, and later on *mecC*) also disseminating in humans in the UK and Denmark (Garcia-Alvarez et al., 2011). This novel MRSA was not detectable through conventional molecular methods and the current knowledge suggests that cattle (particularly dairy cows) may act as a potential reservoir for human infections, possibly also through occupational exposure.

On the other hand, ESBLs are of much more concern than MRSA in cattle. The corresponding genes are located on mobile elements such as plasmids and disseminate very efficiently in *Enterobacteriaceae*, mostly through the enteric flora (Liebana et al., 2006). The ESBL spread is therefore highly difficult to control and the precise pathways for their transfer among animals -and from animals to humans- are poorly understood. It is however clear that the growing use of third generation cephalosporins, such as ceftiofur, successfully promotes the selection of ESBL producers. One should note that ESBL-producing bacteria are mostly prevalent in veal calves compared to other cattle sectors, as demonstrated by many studies (Dahmen et al., 2013; Hordijk et al., 2013; Madec et al., 2008; Watson et al., 2012). In particular,

even though many antimicrobials are used on dairy farms for the treatment (or prevention) of cattle mastitis, those molecules are usually locally delivered and highly dosed. Also, the mammary gland does surely not constitute a favorable ecological niche for genetic exchanges compared to the digestive tract.

To what extent humans are concerned with resistance in cattle?

The contribution of resistance in food animals to the situation in humans is still highly debated. Food contamination through resistant pathogens such as *Salmonella* is a confirmed route for the transfer of resistance but *Salmonella* carriage and infections in cattle have considerably decreased these last years (Madec et al., 2011). ESBL-producing *Salmonella* isolates from cattle are also rare. Also, contrary to the situation in pigs, the occupational exposure risk of *mecC*-carrying MRSA isolates transferring from cattle to humans still needs to be further clarified at this time.

On the other hand, several studies highlighted the major role of plasmids (rather than clones) in the spread of ESBL genes. For instance, identical ESBL plasmids were found in veal calves and hospitalized humans, suggesting a common molecular reservoir among cattle and humans (Madec et al., 2012). These results however do not indicate the extent to which cattle may share common resistance genes with humans. They do not argue for any direction of transmission either, if any. However, the growing prevalence of ESBL genes spreading in multidrug resistant enteric pathogens in both reservoirs is a major possibility for genetic exchanges at the ecosystem level. This uncontrolled trend is becoming highly scaring.

Which attention to pay to cattle environment in the spread of resistance?

This question was raised at numerous occasions (Allen et al., 2010; Call et al., 2013; Subbiah et al., 2012). It is now clear that many resistance genes are present in the environment, not only as a natural source but also subsequently to antimicrobial practices. This was for instance highlighted in a recent study on the occurrence of CTX-M-producing *E. coli* in cattle and soils, possibly through manure application or irrigation with waste water (Hartmann et al., 2012). These bacteria constitute a reservoir of multidrug resistance whose potential risk for animal and public health needs to be considered. In the U.S., recent data also showed an increased prevalence of ceftiofur-resistant enteric bacteria in cattle in association with a local administration of this molecule (prevention of intra-mammary infections), and this would suggest an intermediate role of the cattle environment in the dissemination of those resistant bacteria.
Many factors are of course needed for antibiotics present in the environment possibly having an impact on the selection of resistant bacteria. To this respect, appropriate methods are obviously still lacking to deeply investigate these issues and to provide a ranking list of situations which would be more at risk than others of selection and spread of resistance. This would also include a better understanding of the impact of excreted residues or metabolites from antibiotic-treated cattle with regard to various parameters, such as types of infections, types and doses of molecules, fitness cost of resistance or herd management practices. Similar difficulties are encountered to deciphering the main driving forces of the dissemination and persistence of resistant bacteria in soils and water.

Conclusion

The interplay between the different sources of resistant bacteria and pathways for transfer of resistance among animals, humans and the environment is more than complex. Among them, the use of antimicrobials in cattle indisputably contributes to the global burden of antimicrobial resistance. The most important concern probably relies to the spread of multidrug resistant Gram negative bacteria producing ESBL enzymes, which constitutes a non visible dissemination through genetic exchanges rather than expansion of bacteria clones. The ESBL prevalence is particularly high in veal calves and this should be a focus of urgent interest in the future. The role of direct contamination of cattle environment through manure spread on lands or water flow is also an issue. Many groups and committees were set up over time in Europe to consider a revision of veterinary/farmer practices and to propose control options at a country level. Until now, the surveillance of resistance was not mandatory from the EU in cattle, but a very recent legal basis will change this from 2015 in healthy animals. In France, a very long-term surveillance network -so-called Resapath- publishes the trends of resistance in pathogens from all animal species (including cattle) on an annual basis. In all, one should consider that, even though cattle was probably not equally highlighted compared to pigs (ST398 MRSA) and poultry (ESBLs and ceftiofur-resistant *E. coli*) as a main contributor to the burden of resistance, an intersection of the three main food animals sectors (pig, poultry, cattle) relies on similar difficulties with regard to intensively reared animals. The intensification of food animal production is frequently associated with mass medication and prophylactic and/or metaphylactic treatments. Precise dosing is also more difficult in this context. To this respect, veal calves are obviously a major hotspot to consider, both in terms of antimicrobial use and resistance.

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Pulmonary lesions and clinical disease response to *Mannheimia haemolytica* challenge 10 days following administration of tildipirosin, tulathromycin or saline

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Bovine respiratory disease (BRD) causes significant economic loss to the beef cattle industry. *Mannheimia haemolytica* (MH) is considered the predominant bacterial pathogen associated with BRD. This randomized, blinded, controlled clinical trial evaluated the impact of metaphylactic antimicrobial administration 10 days prior to experimental inoculation with MH to mitigate pulmonary lesions.

Thirty-three crossbreed heifers were randomly allocated to 1 of 3 replicates and to treatment (tildipirosin, tulathromycin, or saline) within replicate. Calves within each replicate received an endoscopic guided MH challenge 10 days following treatment administration and were housed in individual indoor stalls for 3 days post challenge. Lungs were weighed following necropsy and lesion scores were calculated based on a standardized formula. Rectal temperature and individual animal clinical illness, respiration quality, and appetite scores were recorded daily following challenge.

Pulmonary lesions ranged from 3.3 to 39.8% with 92% (11 out of 12) of tildipirosin treated calves having less than 10% lesions. Differences (P less than 0.05) in lung lesion scores were found among treatment groups. Lung weight as a percentage of body weight was lower (P less than 0.05) in tildipirosin calves compared to tulathromycin and saline treated calves. The probability of calves receiving abnormal clinical illness scores and appetite scores was lower (P less than 0.05) in tildipirosin calves compared to tulathromycin and saline treated calves.

This research showed that calves treated with tildipirosin 10 days prior to MH challenge have less pulmonary damage, lower lung weight as a percent of body weight, and fewer clinical signs of illness compared to tulathromycin and saline treated calves.

Monitoring the use of antibiotics in dairy cattle in Austria

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The use of antibiotics in animal production is under growing criticism. Which antibiotics are going to be approved for veterinary use in the future essentially depends on the prudent use of these substances. Critically important antimicrobials may only be used for specific veterinary needs and their use must be justified by objective diagnostic measures.

The Austrian Agency for Health and Food Safety was assigned by the Austrian Ministry of Health to develop methods with which the quantity of antimicrobials used in the Austrian cattle, pig and poultry production can be determined and monitored. Within this project an attempt was made to assess and statistically evaluate the consumption of antimicrobial substances in dairy cattle farms under performance recording. The data were derived from treatment records and prescriptions acquired from veterinary practices active in dairy cattle farms between 2008 and 2010. As units of antimicrobial consumption, the amount of active substances used per livestock unit (LU) per year and the number of prescribed daily doses used per LU per year were considered. These parameters were estimated by applying Monte Carlo simulation techniques, where uncertainties in the annual working hours in the veterinary practices, in the number of produced animals and in the proportion of the non-treated population were taken into account.

Three quarters of the consumed doses belonged to antiinfectives for systemic use. Antibiotics ranked as critically important antimicrobials (Cephalosporines, Macrolides and Quinolones) were used by the participating veterinarians. Cephalosporins were most frequently applied to dairy cattle in the therapy of udder diseases and diseases of claws and legs. Compared to pig and poultry production, the total amount of antibiotics used in dairy cattle production is of minor importance.

From an end-consumer´s perspective the health risks caused by drug resistant bacteria are of particular interest. There is growing demand for consumer confidence in animal health and drug use. The documentation of treatment data along with diagnostic data and their association gives valuable information on prudent use of veterinary drugs in cattle production and serves as a basis for research on the impact of drug use on antimicrobial resistance.

Colonization rate by extended-spectrum producing *Enterobacteriaceae* in dairy cattle in France

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Background

The occurrence of extended-spectrum ß-lactamase (ESBL) producing *Enterobacteriaceae* in animals is increasingly reported, and several studies showed that food-producing animals might be reservoirs of ESBL producers. Our study aimed to evaluate the colonization rate by ESBL-positive or carbapenem-resistant *Enterobacteriaceae* of cattle in France.

Materials

Rectal swab samples (n = 260) were recovered, (i) from cows during a one-year period in 2011 in different farms located in the suburb of Paris, France and (ii) from cattle hospitalised in the clinic for ruminants based at Alfort National Vet School. Samples were pre-cultured in buffered peptone water and incubated 18 h at 37°C. Cultures were inoculated by streaking 100 µl of the suspensions onto Drigaslki agar plates containing either ceftazidime (2 µg/ ml) or imipenem (2 µg/ml). Multilocus sequence typing (MLST) was undertaken to identify the genetic background of the strains. PCR experiments and DNA sequencing were used to identify the ß-lactamase genes. Plasmid typing was performed by PCR-based Replicon Typing (PBRT).

Results

Only 14 isolates in total exhibiting an ESBL phenotype were recovered from this screening, being all *Escherichia coli* isolates. Among them, two isolates expressed TEM-52, had been recovered from the same farm, and were clonally-related, belonging to sequence type ST359. One isolate was an ST1421 and produced CTX-M-27, whereas the fourth isolate was an ST244 and produced CTX-M-32. The plasmids carrying the ESBL encoding genes were not typeable by PBRT, except the $bla_{\text{CTX-M-32}}$-positive plasmid that was typed as an IncF plasmid. No imipenem-non susceptible enterobacterial isolate was recovered during this screening, however carbapenem-resistant *Acinetobacter* genomospecies 15TU expressing the carbapenem-hydrolyzing class D beta-lactamase OXA-23 were obtained (published study). Ten other isolates obtained from hospitalized cattle were ESBL positive *E. coli* producing either CTX-M-1 or CTX-M-9.

Conclusion

Our study revealed the rate of ESBL-producing enterobacterial isolates in healthy dairy cattle and in clinically ill hospitalized cattle. Only *E. coli* isolates were identified as ESBL producers. In healthy dairy cattle, a relatively low rate was evidenced, and all isolates belonged to three distinct ST types rarely identified in humans. Interestingly, different types of ESBLs were identified, all of them being quite frequently identified in human isolates. By contrast, the rate of ESBL-producing enterobacterial isolates in clinically ill cattle was higher, with different types of ESBLs.

The role of communication supporting prevention strategies in dairy farms

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Prevention has a key role in dairy farms because is an important part of an efficient health management program. Its importance is furthermore augmented by the increasing concern on antimicrobial resistance in human and veterinary medicine.

The positive influences of an effective communication in dairy farms have been demonstrated, particularly in mastitis control. Moreover, implementation of prevention strategies needs a communication approach.

In this study, in cooperation with Hipra Italia, we investigated farmers and veterinarian attitude towards prevention. Furthermore, we investigated how communication supports prevention strategies and vaccination plans implementation. Several aspects of prevention were considered, particularly mastitis.

A questionnaire specifically developed was administered to farmers, and about 100 of them were collected up to now. Analogously, a specific questionnaire was developed for practitioners, and an equal number was collected. All questionnaires were recorded into a database and analyzed by FREQ procedure of SAS 9.2 (SAS Institute, Cary NC).

The preliminary results showed as one-third of farmers thinks that prevention plans are difficult to apply, 50% that prevention is not always an advantage and just 30% of them that it is useful. Half of the farmers thinks that management is more important than vaccines and drugs. Considering farmer-practitioner communication aspects, data show as both farmers and practitioners agree on that prevention planes are usually explained by practitioners, even if participants included into the two data sets aren't professionally related. However, nearly 40% of practitioners feel to have insufficient communication capability, and this is reflected by the 60% of them having difficulties in applying prevention practice by the farmers.

Half of farmers thinks that the most important consultant for prevention is the veterinarian. However, farmers consider themselves the most important subject for the mastitis management in 40% of the cases and practitioners just in 22% of the cases. Conversely, 40% of practitioners think to be the most important consultant for mastitis control, and farmers only in 22% of the cases.

These preliminary data will be useful to develop new strategies to improve practitioners' skills in communication and to identify the most efficient means to increase farmers' acceptance of preventive control practices.

Evaluating and reducing pain due to lameness and other health problems in dairy cattle

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Introduction

The 18th century philosopher Jeremy Bentham said of animals "…the question is not, Can they reason? nor, Can they talk? but, Can they suffer?". This widely used quote from Bentham describes the view that it is not necessary to judge animals' abilities by our own standards, i.e. whether they have speech or sophisticated decision making capacities, but that we should be most concerned about how they feel and whether they themselves are alright. The International Association for the Study of Pain (IASP) definition outlines that; [pain is] "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (International Association for the Study of Pain, 1983). It is important to note that this definition recognizes that pain has an emotional as well as physical component; this implies that some level of consciousness is required to fully experience pain in the way that humans do. Interestingly, despite the amount of value put on whether animals can have experiences akin to humans, it is only relatively recently that medical science has recognized that all adult humans experience pain to a similar degree regardless of race, gender and wealth. Even now the debate continues as to the levels of pain experienced by neonates. This uncertainty about whether neonates can experience pain illustrates the problem that we have to overcome when trying to understand whether non-human animals feel pain. It means that a) despite the obvious merit of exercising the precautionary principle it is still not standard practice in all neonatal care units to provide analgesia when dealing with poorly babies, and b) when examining the reason for this uncertainty about human neonates ability to suffer pain much of the problem seems to be that because young children cannot communicate through language there is room for doubt as to their actual pain experiences.

In both humans and animals the apparent pain experience is not always consistent between individuals or with what might be expected. Severe fractures or wounds might be apparently pain free while what looks like the merest scratch may be reported or elicit behaviours akin to agony. In 1965 Melzack and Wall described seven inconsistencies in the behaviour of pain:

- the relationship between injury and pain is highly variable,
- innocuous stimuli may produce pain,
- the location of pain may be different from the location of damage,
- pain may persist in the absence of injury or after healing,
- the nature and location of pain changes with time,
- pain is not a single sensation but has many dimensions,
- there is no adequate treatment for certain types of pain.

While pain science gives us explanations, or at least partial explanations, for these inconsistencies dealing with the reality of this in a clinical situation, especially with non-verbal animals remains extremely challenging.

Evidence that cattle feel pain

The question of whether animals, in this case cattle, experience pain is clearly not straight forward to answer and a considerable weight of evidence has to be examined and considered before reaching any conclusion. Firstly, for cattle to experience pain the underlying physiological mechanisms of pain, the receptors, nerves and neurochemicals that are activated by noxious stimuli, should be similar to those of humans; which indeed they are. Further to this, the behavioural responses of the cattle to noxious stimuli should closely mirror those of humans; which they do. However, some people have then questioned whether animals [cattle] might experience the sensations of pain without actually suffering (Iggo, 1984). This might suggest that cattle have insufficient cognitive ability to allow them to experience pain or to put it another way "they might be too stupid to feel pain". Science continues to increase our knowledge about animal cognition and most who work in the field, while acknowledging that no definitive answers exist, point out that we have no proof that animals do not have subjective experiences; therefore the benefit of the doubt should be afforded to them (Nicol, 1996). To convince ourselves that cattle experience pain we might expect them to respond to the administration of analgesics, for example a lame cow should, as indeed it does, bear weight on the affected limb once it has received effective local anesthesia. However, it should also show a change in what might be termed "quality of life": This might take the form of either resting comfortably or alternatively becoming active and performing tasks, such as eating, which it was reluctant to do prior to receiving pain relief. The evidence for this is largely empirical but does

exist. It appears when examining the available information that the balance tips towards the likelihood that cattle do suffer pain and so we are ethically obliged to take steps to both prevent and properly manage their pain whenever possible.

Effects of pain and benefits of pain management

The term 'pain' is extremely generic and does not in itself convey the range of qualities of pain that may be experienced; stabbing, throbbing, burning, aching, grinding, piercing, radiating and tearing to name but a few. Cattle infected with Bovine digital dermatitis (BDD) show behaviours, repeated lifting and shaking of the affected limb, that seem to indicate that the lesion 'stings' under some circumstances. It is also notable that the IASP have also extended their descriptors of pain to include itching. In addition to the different qualities of pain, there is also a severity component which can range between unpleasant to down right intolerable for the sufferer. Pain also has ancillary effects that cause problems for both cattle and their carers.

Ancillary effects of pain include:

- slowing down healing,
- causing a negative energy balance (at the very least through inappetance),
- decreases in productivity,
- impairment of cardiovascular and respiratory function,
- aggressive behaviours,
- further associated problems (e.g. postural changes leading to muscle wastage or joint damage).

It is clear that pain in cattle is not only a serious animal welfare concern but that it should also be a cause of considerable management concern. The effective management of pain in cattle, using lameness as an example, can be divided into four phases (Whay, 2002):

1) Recognition of pain: unless a painful clinical problem, for example lameness is detected no management action will follow. The earlier lameness is detected the more effective pain management will be. A study described by Whay and colleagues in 2002 (Whay et al. 2002) found that three out of four cases of lameness in UK dairy cattle were going unreported.

2) Treatment: rapid and effective treatment will often immediately reduce suffering and will decrease the chances of chronic pain developing.

3) Sympathetic care: the chances of a full and quick recovery will be greatly increased by providing the cow with an environment in which she can rest comfortably, eat easily without having to compete for food and where she does not have to walk long distances (especially over rough or difficult walking surfaces). Again the quicker and more complete the recovery the greater the likelihood of avoiding long-term complications and chronic pain.

4) Analgesia: using drugs to interrupt or modulate the pain experienced by cattle will promote recovery, reduce the risk of prolonged suffering and limit production losses.

Effective pain management requires an integration of these approaches and should not rely on one single element; for example administration of analgesics without effective treatment. There is research evidence that lame cattle benefit significantly from the receiving the aspirin-like Non-Steroidal Anti-Inflammatory Drug (NSAID) ketoprofen when it is given in association with effective lesion treatment (Whay et al., 2005) and that these combined approaches can also promote recovery of milk yield (O'Callaghan-Lowe, 2004). However, as Flower and co-workers (Flower, 2008) demonstrated in Canada, when a NSAID is given without associated treatment of the cause of lameness an improvement in gait is detected but to a very minor degree, reinforcing the message that a multilateral approach to pain management is required.

In the example of mastitis in dairy cattle, there seems to be a consensus of opinion that severe cases of mastitis cause 'significant' pain and distress to the affected animals (Hewson et al., 2007). However moderate to mild cases of mastitis still present challenges in terms of early recognition of the disease and recognition that there is associated pain. Signs of pain associated with mastitis in cattle such as altered stance, and higher heart rates, respiratory rates and rectal temperatures (Fitzpatrick et al., 2000) may also be indicators of the disease process itself, making clear cut recognition of pain difficult. In addition, the use of NSAID's for the treatment of severe endotoxic mastitis is normal practice, as the anti-endotoxic effects of NSAID's are well documented. However, this practice has perhaps deflected attention from the value of NSAID's in providing analgesia for cows with mastitis. Furthermore, veterinary practitioners are not always directly involved in the treatment of moderate and mild cases of clinical mastitis. There is however increasing evidence that NSAID's do provide pain relieving benefits in cases of moderate severity mastitis (for example see Milne

et al., 2004).

The influence of human attitudes towards cattle pain

How individuals, veterinary surgeons, farmers and herdspeople respond to pain in the cattle under their care is likely to be influenced by a number of factors. These include their beliefs about whether or not cattle feel pain, their own personal attitudes to and experiences of pain and what they believe they or others around them can do to manage it. In a survey of UK veterinary surgeons, Huxley and Whay (2006) found that cattle practitioners varied considerably in their estimates of the levels of pain associated with a range of conditions and procedures. As has been previously reported, in most cases women rated pain higher than men. However, most importantly and regardless of gender, a practitioner's perception of pain severity influenced their likelihood of giving analgesics; those that perceived pain to be more severe were more likely to give pain relief in more cases. In addition, 65% of practitioners surveyed reported a belief that farmers would not be willing to pay for analgesics as a barrier to their use. Interestingly, in a corresponding survey of farmers 53% agreed with the statement "Veterinary surgeons do not discuss controlling pain in cattle with farmers enough" (Huxley and Whay, 2007). While this is clearly not an open mandate for veterinary surgeons to prescribe analgesics for cattle it does suggest that they should not assume that all farmers will be unwilling to pay for them.

Concluding remarks

The challenge of pain is that for all individuals it is a private experience. Humans overcome this by using language as well as behaviour to convey how they feel and the extent of their suffering. Animals do not have the facility of describing their pain to us which means that, although they cannot be accused of exaggerating, we sometimes take this as leave to assume that they are not hurting. As yet no definitive answer can be given as to whether animals feel pain in a manner and intensity comparable to humans, however, the weight of evidence suggests that they do suffer and that they also benefit greatly from receiving the best treatment that we can offer them.

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Ultrasound-guided femoral nerve block as a diagnostic aid in demonstrating quadriceps involvement in bovine spastic paresis

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The aim of this study was to evaluate the clinical effects of diagnostic anaesthesia of the femoral nerve performing a dorsal paralumbar injection technique in healthy calves and calves suffering from bovine spastic paresis. Based on bony landmarks and with ultrasound guidance, the femoral nerves of eight healthy calves were blocked with a 4% procaine solution containing blue dye. After euthanasia of the calves successful location of the injection was confirmed during dissection work. In 69% of the cases a paralysis effect of the quadriceps muscle was obtained after performance of the injection technique. A total paralysis of the quadriceps muscle was obtained in 50% of the cases. In 75% of the cases, the blue dye was less than 2 mm perineurally. Clinical use of the technique is demonstrated in two clinical cases suffering from atypical presentations of bovine spastic paresis. In calves suffering from these presentations of bovine spastic paresis an objective diagnostic tool is needed to declare an animal suitable for surgery to avoid unwanted aggravation of symptoms following partial tibial neurectomy. Femoral nerve blocking has the potential to be a valid diagnostic method to establish involvement of the quadriceps femoris muscles in young calves suffering from the quadriceps form (BSP-Q) or mixed presentation (BSP-M) of bovine spastic paresis.

Use of static intramedullary interlocking nailing for the fixation of diaphyseal tibial fracture in bovines: 10 cases

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Static intramedullary interlocking nailing (SIILN) has been used successfully for the treatment of diaphyseal long bone fractures in small animals. However, its use in large animals is still in its infancy. The objective of the present study was to assess the clinical findings, surgical repair, post-operative complications and outcome of 5 mature bovines and 5 calves with diaphyseal tibial fracture that were treated with open reduction and internal fixation using SIILN.

The feasibility of repairing the fracture was assessed on the ability of the animal to bear weight on unaffected limbs, body weight, type of fracture (open or closed), first aid and duration prior to presentation, and pre-treatment radiographs. The different methods of anesthesia followed in the study were general anesthesia (n = 7) and spinal anesthesia (n = 3). Custom-built intramedullary interlocking nails of different diameters (9, 10, 12 and 14 mm) and variable lengths (22-32 cm) were used for fracture fixation and ancillary support was provided with lag screws, hemicerclage and full cerclage wires. These stainless steel nails were solid except at the holes.

Fracture reduction was satisfactory in all the cases on immediate postoperative radiographs. Weight bearing on the operated limb was observed on 2nd post-operative day in seven of ten animals due to the excellent stability provided by the implant at the fracture site. The surgical wound and open wounds at the time of presentation were managed by regular dressing. In 7 animals, operative wound healed by 15-20 days post operatively without any complication and sutures were removed. Periosteal bridging callus was observed in 7 animals presented for follow up by 1 month post operatively. Exuberant callus formation was observed in the fractures with complete union of fracture and no evidence of fracture line. The complication observed were incisional infection (n = 1), implant dislodgement following post-operative fall (n = 1) and inability to stand (n- = 1). These animals were euthanized as they were unresponsive to treatment.

Complete recovery and optimal weight bearing was observed in 6 animals and were declared fit for intended activity. Weight bearing with occasional lameness was observed in 1 animal. Rigid fixation using nails and screws can be successful in treatment of complete diaphyseal tibial fractures in mature bovines and calves.

Pain management in the disbudding of calves with Flunixin

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The objective of this randomized and blinded experiment was to determine the effect of a dose of 2.2 mg/kg Flunixin before dehorning (Dx) of 5 to 9-week-old calves on cortisol concentrations in serum, behaviour and heart rate variability.

Material and methods

64 calves were randomly allocated to 4 groups. In 3 treatment groups calves received a local anaesthetic (LA) and a first treatment with Flunixin or a placebo 20 min before hot-iron dehorning, and a second treatment with Flunixin or a placebo 3 h after Dx. Groups received for both treatments a placebo (PP), 2.2 mg/kg Flunixin followed by a placebo (FP), or for both treatments 2.2 mg/kg Flunixin (FF). Calves in the control group CON were restrained only without dehorning or injection; most of them were dehorned one week later. Blood samples were collected from all groups several times starting 20 min before restraint and ending 8 h after Dx. Samples were analyzed for concentration of cortisol by Enzyme-Immunoassay (EIA). Heart rate variability (HRV) during resting was measured in the period 9-11 h after dehorning and compared with the day before (baseline). Additionally, behavioural pain responses were observed for the first 9 hours after Dx.

Results

Concentrations of cortisol, analysed as area under the curve, was greater in PP compared with FF and tended to be greater compared with FP. Flunixin-treated groups and CON were at the same level throughout the observation period, indicating a certain level of stress for the calves fixed in a headlock but not a higher level in Flunixin-treated groups after Dx. PP-calves showed more head shaking, scratching and ear flicking than Flunixin treated calves in the first 30 min after DX and ruminated less than Flunixin-treated calves 4 hours after DX. . HRV decrease from baseline was less in controls than in PP calves. HRV-parameter “trend” of FF calves differed from PP and FP, but not from control.

Conclusion

All parameters clearly showed that the pain response after dehorning can be reduced by the administration of Flunixin before dehorning. HRV suggests that some beneficial effects of a second dose of Flunixin might exist.

New and simple tools for easier obstetrics in cows - a tribute to female vets

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Obstetrics in dairy and beef cows are always a challenge for vets and farmers in daily life. Due to dystocia and other calving difficulties a significant percentage of calves are not able to survive. Around 10% of calves are stillborn, and another 5-10% are dying due to stressfull delivery. More than that, calves delivered from dystocias are of a higher risk for subsequent diseases like scours, bovine respiratory diseases and a higher culling risk later on in their life. Costs for dystocias are estimated to contribute for losses between 480 to 530 Euros for each single case.

With regard to obstetric tools-other than calf pullers- in order to ease calvings for cows and people, no remarkable new and revolutionary devices have been able to come to market for the last 30-40 years. As a practitioner in daily practice I am facing personal challenges with dystocias. Also as an instructor to teach new assistants for a convenient way of obstetrics to handle even difficult cases of calvings by themselves with special regard to their personal health and quality of work. Therefore I invented 4 new devices that are aimed to a special attidute: To "make live easier" – for people, for cows and last (but not least for the calf itself) for a good start: GYNstick® - A new multipurpose tool for bovine obstetrics: A modern and safe torsion fork with additional function as an obstetrical crutch.

Uterine torsion is a common cause of bovine dystocia. The classical and primary choice of treatment is the manual rotation of the fetus per vaginam, which has its physical limitations. When the calf is in an anterior position the head or shoulders need to be within good reach, in a posterior position it is difficult to find a suitable location for applying sufficient rotational force. In addition, the manual method is physically demanding and can put exceptional pressure onto the shoulders and back of the bovine practitioner over the years. Sometimes, the Caemmerer's torsion fork is used to instrumentally support the manual/vaginal method. However, this method is quite controversial due to the risk of fracturing the calf's leg.

The GYNstick is a new, safe and simple instrument which minimises the risk of complications. Using the GYNstick as a torsion fork, it has a closed aperture at the anterior end for fixating the calf`s legs. After applying calving ropes to the calf's legs, the ropes are threaded crosswise through the anterior aperture. Then, the ropes are tightly tied to the aperture at the posterior end of the stick, which is also used for the torque rod. Now the assistant can apply force to the torque rod and generate a torsional moment.

Several significant improvements are apparent compared tovthe Caemmer's fork. The complicated cuff system is replaced by two calving ropes. The risk of a cuff slipping off is eliminated and it is easy to keep an overview during the process of rotation. The optimal length (> 1m! instead of < 70 cm) of the GYNstick also affords the veterinary practitioner enough space for manual assistance during rotation of the calf. Even under severe structural strain, the plastic GYNstick is stable but also displays good flexibility. These features allow accurate control of the rotational force applied, preventing fracturing of the calf`s legs. To provide a versatile tool, the posterior end of the GYNstick is modified based on the principles of the Kühn`s obstetrical crutch. It is formed into two rounded arms with eyelets for the string, which are wider than the ones of the original Kühn`s obstetrical crutch. The danger of injuring the cow is therefore substantially reduced.

Additional new application possibilities include correction of bilateral hip flexion and the bilateral shoulder flexion posture. As well as a delivery of gascalves (in order to avoid fetotomy) or malformated calves due to Schmallenberg Virus infections. Also for a proper preparation of a C-section, the calf can be placed in the desired position for a good surgery.

Slinge - the existing flat slinge (part of the Gynstick set and also crucial for fetotomy) was significantly improved. Whereas the traditional 2 sloop end piece is flat, the loop ends are here twisted toward each other in a 90° angle that facilitates working around body shape of the calf in a more comfortable way.

HEADhunter® - premium calf a snare. Head malpositionings can lead to very exhaustive calvings. A quick and safe method to bring the head of the calf into the birth-channel might be life-saving. This model is more tighter than the existing ones on the market. The loop is also covered with a soft plastic shelter that allows a very smooth, but effective ceiling around the calf`s extremities. Another application might also be the fixation and leverage of uterus prior to a C-section.

EYEhook® - a new onehand fixation system for easier calvings. Eyehooks are used in piglets and calfs for fixation of the head to keep in the birth canal. Whereas the traditional models are hard to keep them in place, the new approach

mimikes a google-like system that allows the hooks to snap safely into the angles of the eye-pair. Once attached they cannot fall off and might damage the tissues.

Conclusion

Taken these 4 new innovations into account, calvings and dystocias can be done much easier. With the help of these tools it should be much easier to improve calving assistance in any case of dystocia. And, more than that - in a changing buiatrics environment, the future trend is female. We should make life easier, wherever it is possible.

Further informations and videos on www.gynstick.com

Indicators associated with the duration of lameness in dairy cows

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Lameness is a crucial issue in farms. To decrease efficiently the prevalence of lame cows within herd, appropriate control measures should be implemented. Indeed, preventive actions may be not sufficient when the prevalence of lameness is mainly due to a lack of detection and treatment. Therefore, it could be helpful to estimate the duration of lameness observed at a given time in order to provide relevant advice. We can assume that the longer cows lame, the higher the health status is impaired.

The objective of the study was to assess whether some health indicators could be used to differentiate between recent and chronic lame cows during a single farm visit. To reach that goal, two successive steps were carried out. First, a cross-sectional survey was performed in a random sample of 130 herds using the Welfare Quality® assessment protocol in order to select a limited number of health indicators associated with the presence of lameness. Three indicators were found using the Chi-squared test: low body condition score ($P < 0.001$), integument alterations ($P < 0.001$) and dirty patches on body ($P = 0.02$). However at this stage, it was not possible to determine the causative link between these indicators and the presence of lameness. Therefore in a second step a longitudinal survey was performed to investigate the relationship between their evolution over time and the duration of lameness at cow level. Thus, 871 Holstein cows located in 10 herds with cubicles facing high prevalence of lame cows (> 20%) were followed during seven visits for 3 weeks apart.

A total of 555 (63.7%) cows were lame at least once during the study period, with a high variability between herds (41.1 - 84.6%). Among the lame cows, 299 (53.9%) remained lame at least on two consecutive visits (25.0 - 67.1%). These results confirm the existence of both transient and persistent lameness. The last visits have just been performed. Statistical analyses are currently performed in order to generate profiles of lame cows (recent/chronic lame). The results should help to assess the duration of cows lameness from a cross-sectional intervention and in turn support farmers to take appropriate remedial preventive and/or curative solutions against lameness.


EBF 2013
European Buiatrics Forum
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Posters

Electrochemiluminescence immunoassay of the testosterone by using a heterologous system in plasma ovine: preliminary study

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Testosterone in males is a prerequisite for normal spermatogenesis (Goeritz et al., 2003), normal function of the reproductive tract and sexual behaviour (Luke and Coffey, 1994). The aim of the present study is the use of electrochemiluminescence immunoassay (ECL) method with the specific kit human testosterone (Elecsys 2010, Roche diagnostics) for measuring plasma testosterone in ovine. This study was carried out on 8 males of the local breed with mixed age. The animals were divided into two groups namely: Group male-1 (n = 4, aged 2-3 months) and Group male-2 (n = 4, aged 9-36 months). The experiment was conducted during the period of April 2012 in Bass Kabylie, Algeria (36°43'N, 5°04'W). Blood samples from the jugular vein were collected between 09:30 and 11:00 a.m. Blood samples were collected in tubes EDTA-containing and centrifuged at 1500 rpm for 20 min. Plasma was rapidly separated and stored at -20 °C until assayed. The estimated dose of testosterone measurement extends from 0.025 to 15 ng/ml and the minimal detection limit was 0.025 ng/ml. The test of reproducibility inter and intra-assay of the system electrochemiluminescence assay is satisfactory (5 and 7.3%, respectively). The accuracy (96-102%) and the test of parallelism were largely acceptable. As for specificity test, no cross-reaction was observed with different hormones (PMSG, hCG, progesterone, oxytocin and $PGF_{2\alpha}$) following concentrations 10 UI/ml and 10^{-3} UI/ml. Peripheral testosterone concentrations determined by ECL system in all male extends from 0.025 to 4.5 ng/ml. In conclusion, the preliminary results show clearly that human testosterone electrochemiluminescence kit can be used to measure testosterone in plasma ovine for the characterization of reproduction seasons, the evaluation of reproduction performance and possibly the diagnosis of some pathology in testicle.

Assessment of the prevalence of bacterial bovine respiratory disease (BRD) pathogens in feedlots in Spain and Italy

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Backgound
Mannheimia haemolytica, Pasteurella multocida, Histophilus somni and *Mycoplasma bovis* are considered to be major primary and secondary pathogens in bovine respiratory disease (BRD).

Objective
To determine the prevalence of bacterial respiratory pathogens in cattle during an outbreak of BRD on four commercial feedlots in Spain (n = 1206) and two in Italy (n = 1023).

Methods
An outbreak of BRD was confirmed during the first two days following arrival at the farms and deep nasopharyngeal swabs were collected for bacteriology from at least 5% of each batch of cattle followed by administration of injectable antimicrobials to all animals. Additional swabs were collected from all cattle requiring re-treatment during the following 60 days, prior to treatment administration.
Swabs were transported to the laboratory in transport media containing activated charcoal. Upon arrival, the swabs were suspended and seeded onto a range of plates containing suitable culture media. The bacterial colonies were identified according to morphology, Gram staining, and biochemical and growth characteristics. Identification of *M. bovis* was confirmed by polymerase chain reaction (PCR) test.

Results

Bacterial pathogen	Spain n (%)		Italy n (%)	
	On arrival	BRD re-treatment	On arrival	BRD re-treatment
Histophilus somni	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Mannheimia haemolytica	9 (14%)	7 (5%)	4 (8%)	11 (23%)
Pasteurella multocida	13 (20%)	41 (29%)	10 (20%)	8 (17%)
Mycoplasma bovis	21 (32%)	99 (70%)	6 (12%)	25 (52%)
n total	66	141	49	48

Conclusion
Mycoplasma bovis and *Pasteurella multocida* were the BRD bacterial pathogens isolated most frequently. The prevalence of *Mycoplasma bovis* was particularly high in animals which required retreatment during the course of the study which is probably due to the highly infectious nature of this pathogen. The results underscore the importance of selection for use in feedlots of a broad spectrum antimicrobial which is effective against *Mycoplasma bovis*.

Application of good feeding principle of Welfare Quality® assessment Protocol in dairy cattle farms of the province of Bejaia, Algeria

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The animal welfare is a very difficult concept to define that the assessment should cover several aspects, such as physical comfort, no hunger, no disease, the ability to express normal behavior. Today, there is a great disparity in the consideration of the concept of animal welfare worldwide. Indeed, the developed countries are now more looking for an increase in production. In these countries, people's expectations are that they are obliged to integrate welfare in livestock systems and maximize it. Thus they are equipped not only guidelines and laws to legally protect the welfare of animals, but several tools have been developed to evaluate properly. Among these tools, the assessment protocol welfare of dairy cows from the project Welfare Quality® (2009), provides a comprehensive assessment of the well-being of livestock through these four principles (good food, good housing, good health and appropriate behavior). However, in Algeria the issue of animal welfare is limited only to the legislative aspect and reliable assessment tools are far from being raised. In order to contribute to a better perception of the level of animal welfare on farms, an attempt to apply the principle of good nutrition Welfare Quality® protocol (2009) farms in the Wilaya of Bejaia.

The principle good nutrition of Welfare Quality® protocol including two sub-criteria : absence of thirst and hunger extended from 25 dairy cattle farms spread over nine communes of the province of Bejaia revealed that the sub-criterion absence of prolonged thirst enjoyed from the water supply capacity (number of drinkers, their rates and cleanliness), which aims to assess the availability of water, got a score of 3/100 of the theoretical maximum. The sub-criteria no longer hungry meter that allows focus on chronic hunger and thus the detection of lean animals by calculating the body condition score, got low scores: in fact 40% of farms respondents obtained scores ranging from 20 to 47.45% of the theoretical maximum. Thus, aggregation of scores as criteria: absence of hunger and thirst extended by the use of the Choquet integral recorded low scores of 4.53 to 11.73% for all farms surveyed. The analysis of the results reveals that 40% of surveyed farms are out classification and levels of well-being are considered unacceptable. This is a major impediment to the development and therefore to animal welfare.

Impact of sub-clinical mastitis on production performance of dairy cow: quantity and physico-chemical quality of milk in the demonstration farm of Baba Ali, Algiers

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In recent decades, the intensification of producing has led to ask issues about the health and welfare of animals. Indeed, poor husbandry practices have contributed to the emergence and recrudescence of many diseases (lameness, infertility, mastitis problems...). Thus, in Algeria, bovine mastitis constitutes the most dominant and the most devastating disease in dairy cattle. It is responsible for economic losses such as reducing the production and quality of milk, the high costs of treatment, prevention and reform. It remains more with the development of livestock above ground and use almost full mechanization of trafficking. Indeed, clinical mastitis is a serious problem in dairy farms Their goal detection is easy, while That of sub-clinical mastitis Requires the use of means clustering of detection (pH, CMT and somatic cell count, etc...) All which May Reflect the presence of Such infections. Objective of the study is to assess the health of livestock farm demonstration Baba-Ali (ITELV) through the use of the CMT test and study its impact on the quality and quantity of milk produced to reduce losses and ensure the welfare of farm animals.

The study was conducted over a period of one year (April 2010 in the month of April 2011) and a range of 41 cows (28 lactating and 13 dry). The 28 cows in production of different races (17 Holstein, 6 Montbéliard, 4 Brune des Alpes and 01 Fleckvieh) and intensive type conducted in loose housing. Samples of milk were made, one for the early detection of subclinical mastitis (Schalm test), the second for physico-chemical analysis of the milk to the individual scale (cow) and the herd level to assess the overall health status. The results of application of the test Schalm have revealed that out of 112 districts tested, 38 have shown a positive CMT (> score or = 2) with a frequency of 33.93% and 73 districts were negative (CMT score between 0 and 1), a rate of 65.18%.

The distribution of infected according to the rank of lactation neighborhoods has shown that the females of the first and 5th lactation have shown a high infection rate of about 35.49% and cows in mid-lactation are most exposed to sub-clinical mastitis with a number of areas reached 44.73 is equal to 17% of the total detected and degree less cows in late lactation with a number of districts affected equal to 8 or 21.05% of the total infected. While the distribution of infected based on milk production produced districts revealed that of 38 cows screened, 12 infected cows (42.85%) with a score of 2 were recorded milk production from 250 to 350 liters against eight cows (28.85 %) which produces a quantity of 350 to 500 liters. 14.28% were marked only a quantity greater than 500 liters. Also, the results showed that the Holstein breeds are more susceptible to subclinical mastitis with a frequency of 25% against 14.26% for the Montbéliard and Fleckvieh breed. While Brown Alpine breed has little exposure to mastitis (3.75%). The physico-chemical analysis of the collected milk from all four quarters of 28 cows revealed a slight decrease in milk components (protein, fat, and density, percentage of water and grease and total dry extract). So that milk tank has made no alteration in the milk constituents.

Evaluation of a commercial in-clinic diagnostic test for combined germ identification and antibiotic sensitivity testing in bovine mastitis

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Introduction

Clinical mastitis is a common disease in dairy herds. Rapid and efficient management is necessary to limit its clinical and economic consequences; an early and appropriate administration of an antibiotic treatment is one of the keys to success. The purpose of this study was to assess the performance of an in-clinic combined test (ICCT) for both identification of the main bacteria involved in bovine mastitis and evaluation of their susceptibility to a selection of veterinary antibiotics.

Material and methods

98 bovine milk fresh samples, analysed at the Laboratoire Départemental d'Analyses de La Manche, were included in the study. The samples were considered as positive if seeding onto blood agar, without previous enrichment, gave a positive bacterial culture. Identification, counting and a standard susceptibility profile (disc diffusion on agar NF U47-107) of the bacteria involved were performed on positive samples. The other samples were considered sterile.

Each fresh sample was also analyzed with the ICCT (Speed Mam Color, BVT, France), according to the manufacturer's instructions, to determine the susceptibility profile to 14 antibiotics and associations of antibiotics (within 24 hours), and to identify 7 bacterial species groups (within 48 hours). The antibiotic susceptibility profile was interpreted only for monomicrobial samples.

Results

Of the 98 samples analyzed, 39 samples were sterile and 59 samples were positive on blood agar plate culture. The analysis of the bacterial counts showed a positivity threshold of the ICCT of 5.10^3 CFU/ml, with a sensitivity and specificity of 92.5% and 94.9%, respectively. All samples containing at least 10^4 CFU/ml were positive with the in-clinic test. The test correctly identified the bacteria present in 94.6% of samples and the antibiotic susceptibility profile was consistent with the reference method in 95% of cases.

Discussion and conclusion

The ICCT test is easy to use and can be considered a quick and reliable tool for detection and identification of mastitis-causing bacteria when their concentration exceeded 5.10^3 CFU/ml, as frequently observed in clinical mastitis. ICCT also provided a reliable antibiotic susceptibility profile after only 24 hours, thus allowing an optimal treatment of mastitis.

Seroneutralization against Border Disease strains following vaccination with a BVD vaccine in sheep

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Border Disease (BD) is one of the most important infectious diseases of sheep. Whereas BD is a common condition in several areas in France, not any vaccine is registered for the prevention of the disease. The main objective of this study was to address preventive efficacy of a bovine viral diarrhoea (BVD) vaccine in sheep against various viral strains of BD.

Material & Methods
Eight Pestivirus free ewes (seronegative) were vaccinated with a commercial inactivated vaccine against BVD, 2 mL subcutaneously, twice, 21 days apart on days 0 and 21 (half the labelled dose for cattle). Two animals remained unvaccinated and served as control. All animals were sampled on d0, d21 and d42 (just before vaccination on vaccinated animals). Blood sera were tested for seroneutralization against five BD viral strains and NADL BVDV control strain.

Results
All animals were negative for the seroneutralization assay against all tested strains on d0. On d21 sera from a few animals were able to seroneutralize some BD viral strains. On d42, sera from 7 out 8 vaccinated ewes were able to seroneutralize all BD viral strains, and serum from the remaining vaccinated animal was yet able to seroneutralize all BD strains but an only one. No adverse effect, neither local nor systemic, was observed in animals after vaccination.

Conclusion
Vaccination in sheep with this vaccine induces seroconversion with seroneutralizing antibodies against almost all BD strains tested. This vaccination sounds safe in sheep. However further investigations are needed to properly assess efficacy of this protocol against Border Disease.

Efficacy of two cephalosporins dry cow therapies in UK dairy herds

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This study was performed to compare efficacy of two intramammary treatments at drying off (either 150 mg of cefquinome or 250 mg of cephalonium). A total of 739 cows from five farms in the UK were randomly allocated to one of the two treatment groups between December 2010 and February 2012. Quarter milk samples were taken on all cows before drying off and within the 10 days after calving for bacteriological analysis according to standard laboratory techniques. Cows were monitored during the first 100 days post-calving for the presence of clinical mastitis, bacteriological analysis being performed from milk samples of the mastitic quarters. The likelihood of infection by different types of pathogens post-calving was compared between groups at the quarter level. The rate of clinical mastitis due to different types of pathogens during the first 100 days in milk was also compared between groups at the cow level. Multivariable statistical analysis was used to take into account potentially confounding factors. Statistical analysis was performed either on all cows included or on a sub-population corresponding to cows with a low somatic cell count (SCC) before drying off (SCC < 200,000 cells/mL during the last three controls before drying off and no occurrence of clinical mastitis within this period).

No significant differences between groups were found on the whole population recruited according to the multivariable analysis. However the likelihood of being infected by an *Enterobacterial* pathogen was significantly lower in the cefquinome treated cows compared to the cephalonium treated ones on the sub-population with low SCC before drying off (Odds Ratio = 0.41; 95% confidence interval : 0.18-0.96). This finding may be linked to the enhanced activity of a 4^{th} generation cephalosporin (cefquinome) against *Enterobacteriaceae* compared to a 2^{nd} generation cephalosporin (cephalonium). Interestingly low SCC cows have been considered as more at risk of coliform mastitis in early lactation.

Field study about prevalence of wet areas trematodosis (*Fasciola hepatica, Calicophoron daubneyi)* in North West of France

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A field study regarding the prevalence of wet areas trematodosis (fasciolosis and paramphistomosis) was performed at the beginning of 2012 among 10 veterinary practices located in Normandy (North West of France). It aimed to assess the prevalence but also to increase farmers and vets' awareness about their potential clinical and zootechnical impact.

Materials and methods
An equivalent number of dairy and beef herds were investigated during the survey.
For fasciolosis, 10 less than 2 years old heifers and 10 adult cows were sampled in each herd for serological diagnosis using the INRA Elisa method performed in Nantes Vet School. Overall, 85 herds were investigated. When bulk milk tank or blended serology results (Iddex Elisa) were avalaible, only herds with negative results were investigated.
For paramphistomosis, 77 herds were investigated. Fecal samples concerned 5 adult cows and 5 less than 2 years old heifers. Fecal examinations were performed according to the Mc Master method in the veterinary laboratory located in Rennes (France).

Results
In average, 56% of the sampled herds turned out infested by *Fasciola hepatica.* That prevalence varied from 20 to 80% according to the practices, whatever the anterior known status; that means that, in some cases, 80% of the herds considered as healthy were infested!
In average, 56% of the sampled herds exhibited a *Calicophoron daubneyi* infestation with an individual prevalence of 27%. That one was higher in beef than dairy herds. According to the practices, the individual prevalence varied from 2 to 90%, the herd prevalence from 24 to 100%. The two prevalences were related.

Discussion
In that survey, the number of sampled animals permitted to assess correctly individual prevalences higher than 15% for fasciolosis and 30% for paramphistomosis. Herds in which the individual prevalence was less may have been considered by mistake as sound. Nevertheless, prevalences of that kind of level can be considered as inducing few zootechnical and pathological consequences.

That field study exhibits that wet areas trematodosis are plainly underdiagnosed in current vet practiceand that it is basic to implement a systematic screening toward them in each herd.

Farmer-practitioner communication in Italian dairy farms

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An effective communication approach showed to be important in improving dairy health management.

The aim for the study was to investigate veterinarian-farmer communication in Italy, including also an evidence-based approach in evaluating informational process. The study was designed to gather information both from farmers and practitioners, in order to identify farmers needs and to develop strategies helping practitioners.

Research involved three steps. The first step involved a questionnaire for farmers distributed by a farmer magazine (Informatore Agrario) and interviewing consultants during an interactive session at Mastitis Council Italia Congress. Then, an improved questionnaire was administered to other farmers. Finally, a questionnaire related to farmers' ones was administered to veterinarians.

Overall 400 questionnaires were collected both from farmers and practitioners.

Questionnaires collected in each phase were recorded into a database and analyzed by SAS 9.2 (SAS Institute, Cary NC).

Responding herd parameters (milking cows, SCC, production) match with current Italian dairy farm situation.
The study shows a general farmers dissatisfaction and a lack of trust in practitioners, even less in other consultants, and in salesmen from pharmaceutical and feeding companies. Data showed as all these professionals were not meeting farmers needs, even if with large difference among them.

Several factors affects problem perception: among them herd production and SCC. In example, the perceived utility of practitioners decreased as SCC increased, while, paradoxically, an increase of perceived utility of salesmen was recorded.

The ideal consultant is useful, practical and clear. Only veterinarian showed to be close to meet these needs, but with an overall low satisfaction level. Farmers reported as consultants are not used to communicate each other, and as they are too little proactive in supplying information. Furthermore, information are often conflicting.

Practitioner is rated around 50% as an information source, trusted consultant and reference for prevention. About 40% of practitioners think that this result is due to a lack of specific communication training, and just 16% of farmers is of the same opinion. In order to improve farmer-practitioner communication, practitioners ask for a specific communication training, while farmers ask equally for practitioners with higher communication skills and technical preparation.

Introduction of dairy cattle breeding in the Saharan region of Algeria

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This study aims to analyze the introduction of cattle breeding in Saharan regions and specially the dairy cows. The analysis of behavior of breeding in the area of Oued Souf (Saharan region of Algeria) shows that cows possesses a sizeable potentiality in matter of dairy production, however this intensive breeding is facing multiple constraints in all areas that slow down its development, such as: unavailability of forage and the few areas for forage production, non mastery of rationing; non respect of hygienic measurement, difficulties of adaptation of animals to the harsh environmental conditions of saharan climate; and failure to control the breeding behavior due to the lack of qualification and specialization of labor. Furthermore, we must adopt effective strategy based on the development of availability of forage and formation of qualified labor for dairy breeding succeed in these difficult regions.

Detection of *Streptococcus agalactiae*, *Staphylococcus aureus* et *Mycoplasma bovis* in bulk milk tank from various European countries using filter cards and real time PCR: preliminary results

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Epidemiology of sub-clinical intramammary infections in Europe is not well known. Shipment of liquid milk samples for bacteriological examination is a limitation to large scale epidemiological studies. Authors present a reliable method for collecting and shipping bulk milk tank (BMT) samples, and thus testing them for *Streptococcus agalactiae*, *Staphylococcus aureus* and *Mycoplasma bovis*.

Material and Methods
Sampling BMT with Whatman™ FTA™MiniCard (GE Healthcare UK Ltd) was identified as an interesting procedure to ease sample shipment. However, due to low concentration of bacteria into BMT, DNA extraction from cards for real time PCR assay (PathoProof™Mastitis-Major-3 PCR Assay, ThermoFisher Scientific) was expected to be more difficult than with liquid milk samples, implying impairment of the analytical sensitivity. Serial dilutions of pathogens, and comparative PCR on FTA cards and liquid milk samples from commercial dairies, were used to assess test characteristics. Cycle thresholds (Ct) were recorded. Finally, corresponding veterinarians from 14 European countries were provided with FTA™MiniCard for a pilot study; they were asked to collect BMT samples on cards in dairy operations with average BTM SCC > 300.10^3 cell.ml^{-1} and to send them to a reference laboratory in France for PCR analysis.

Results
The association of FTA™MiniCard and PathoProof™Mastitis-Major-3 allows detection of *S. aureus*, *Str. agalactiae* and *M. bovis* in BMT samples at concentrations $\geq 10^3$ CFU.ml^{-1}, concentrations usually observed in bulk milk. Assuming results where positive when Ct $\neq$ 0, PCR testing carried out on FTA™ cards or liquid milk yielded similar results in 86.6% of cases tested (30 tests). Compared with direct RT-PCR on liquid milk, sensitivity and specificity of the card/PCR test were 90.9 and 94.7% respectively. At the time of abstract submission, veterinarians from 8 countries collected 57 BMT samples. From this limited data set, current herd-prevalence of *Str. agalactiae*, *S. aureus* and *M. bovis* in Europe is estimated to be 8.8, 40.4 and 0% respectively, *Str. agalactiae* being identified in Belgium and Denmark only.

Conclusion
Storage and shipment of BMT with FTA™MiniCard does not impair results of RT-PCR testing. This technique allows cheaper and easier BMT bacteriology testing and would be recommended for large scale epidemiological surveys as well as veterinary diagnostic.

Comparison of the assessment of sanitary quality of colostrums by two methods

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A study was conducted during autumn 2012 on 8 farms near Lyon, France. The objective was to determine the bacterial quality of colostrum and the potential sources of contaminations. Sixty-six cows were sampled for their two first milking after calving, one sample corresponding to the raw product of the milking and the second being sterile sampled. Bacterial cultures were performed with a laboratory standard analysis, and with a bacteriological assay consisting in a culture plate divided in three sectors, each sector containing a selective growth media. Raw milk samples were too heavily contaminated to be analyzed. However the results for the sterile samples were congruent for both culture methods: the main species isolated were *Staphylococcus* (*aureus* and coagulase-negative), *Escherichia coli*, *Enterococcus faecalis* and *Klebsiella pneumoniae*. This study demonstrated that bacterial quality of colostrums was not only the reflect of cow's infections but also of the contaminated environment.

Ovarian activity and sex ratio in cattle

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In cattle, studies have shown that there are more ovulations in the right ovary than in the left. Few data, however, are available regarding how ovulations alternate between ovaries. Limited information is available regarding sex ratio of calves in relation to the uterine horn of gestation. The objectives of the study were to record which ovary had ovulation during a number of subsequent heats and to evaluate the sex ratio of calves in the right and left uterine horn.

A number of 107 animals (Norwegian Red and Hereford) were palpated per rectum, once a week. The ovulations were recorded in 323 heats and 214 interovulatory intervals. A total number of 365 animals (Norwegian Red and Hereford) were pregnancy diagnosed six weeks after mating. The side of *corpus luteum graviditatis* and the pregnant horn were recorded. No transuterine migration was found. Calving data, including calf sex, were collected immediately after birth and later compiled with palpation findings collected earlier in gestation. Eight pregnancies were excluded; seven of them had twins and one had a *corpus luteum* in each ovary, bearing a female foetus.

Among the 323 heats, 182 (56.3%) ovulations were in the right ovary (RO) and 141 (43.7%) in the left ovary (LO). There were 25% ovulations in RO and 16% in LO for two subsequent heats. Ovulations alternated from RO to LO in 26% of the heats and from LO to RO in 33%. Thus, 59% of the ovulations changed side. One animal had ovulation in the left ovary for four subsequent heats.

Sixty four per cent of the pregnancies were in the right uterine horn and 36.0% in the left. In both uterine horns the proportion of males was 49% and females 51%. The difference in the sex ratio of the calves between the two uterine horns was not significant. These results contrasted with the reported significantly greater proportion of males gestated in the right uterine horn and a greater proportion of females in the left horn (Hylan 2009, Giraldo 2010).

Efficacy and safety of a single injection of marbofloxacin in the treatment of bovine acute mastitis in a European field study

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The efficacy of marbofloxacin administered as a single-dose treatment to dairy breed cattle with acute mastitis was evaluated in a masked, randomised, European (France, Germany), multicentre field study conducted on commercial farms.

A total of 354 lactating dairy cows were enrolled with local and general clinical signs of untreated acute mastitis on one quarter and treated on day 0 with either marbofloxacin (10 mg/kg, single intramuscular administration of a 16% marbofloxacin solution for injection; n = 178) or danofloxacin (6 mg/kg, single subcutaneous administration; n = 176). Milk samples were taken systematically for mastitis pathogen isolation prior to any treatment and all cows received an oxacillin intramammary treatment. The selected cows were examined on days 1, 2, 3 and 7 following treatment. Among the 354 enrolled animals, *E. coli* was isolated from the milk of 194 animals (54.8%). The second most prevalent pathogen was *S. uberis* with 57 strains (16.1%). Forty one (11.6%) milk samples were sterile. The high prevalence of *E. coli* cases can be explained by the fact that the investigators had to focus on this pathogen.

Success rates (cure + clear improvement) were compared statistically at each examination time. Animals presenting with an adverse event not related to the disease or the products, or presenting with a new affected quarter were excluded from the analyses. The differences between groups were never significant ($P > 0.05$). Similar trends were observed between the success rates concerning only *E. coli*-positive animals and the overall success rates, with non significant higher success rates in the marbofloxacin groups.

Success rates on Day1, Day2, Day3 and Day7 according to the treatment groups were respectively:

for all animals:
23.2%, 52.4%, 69.5% and 80.1% in the marbofloxacin group
26.9%, 50.6%, 65.4% and 78.1% in the danofloxacin group

for *E. coli* positive animals:
14.8%, 37.5%, 59.1%, and 76.1% in the marbofloxacin group
15.4%, 37.4%, 55.6% and 70.0% in the danofloxacin group

In conclusion, a single injection of marbofloxacin associated with a Gram-positive targeted intramammary antibiotic, is efficacious for the treatment of acute mastitis induced by the main Gram- positive and Gram-negative pathogens met in daily practice.

Demonstration of efficacy against challenge of an inactivated Schmallenberg vaccine in sheep

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Introduction
In August 2011, outbreaks of an unknown disease of cattle were reported in both the Netherlands and Germany. From December 2011, abortion and foetal abnormalities, were reported in sheep and cattle in several European countries. A new virus was identified in November 2011 and was associated with both conditions. This agent was named 'Schmallenberg virus' (SBV) after the German town where the virus was first identified.

Schmallenberg virus is in the Simbu serogroup of the Orthobunyavirus group. This group of viruses includes many viruses occurring in the Tropics. None had been previously identified in Europe. Although some uncertainty remains on the transmission of SBV, it seems primarily spread by biting insect vectors (midges/mosquitoes).

Here, we present the results of a vaccination / challenge study showing that a single administration of an inactivated SBV vaccine was able to prevent viraemia in sheep.

Material and Methods
Eleven weaned lambs were randomly allocated to one group of 5 vaccinates and one group of 6 control sheep. Vaccinates were subcutaneously treated once on day 0, with 1 ml of an inactivated SBV vaccine. The other group was left unvaccinated and served as control. Twenty one days after vaccination, all sheep were challenged with a virulent SBV strain. All sheep were then monitored for rectal temperature, clinical signs and viraemia (quantitative RT-PCR) from D22 to D31.

Results
Hyperthermia: no hyperthermia was observed in any of the groups.
Clinical signs : no significant clinical sign was observed in any of the groups.
Viraemia (qRT-PCR): all controls were found positive on 3 consecutive days. None of the vaccinated animals was ever detected positive.

Conclusion
In the present study, single vaccination of sheep with the product tested provided full and significant protection against viraemia following a SBV challenge.

Emaciation in cattle in small holder farms in Edfu (Upper Egypt)

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Cattle have an economic importance in Aswan governorate and considered the main source of income for the most farmers. Emaciation in cattle is considered a wide complain affect cattle productivity leading to highly economic losses, so this study aimed to evaluate the relation between emaciation, and clinical and some biochemical parameters in blood serum of cattle in this area.

A total number of 200 heads selected from nineteen villages belong to Edfu, Aswan Governorate over a period of 9 months (Nov. 2010 - July 2011). 160 females and 15 males emaciated cattle the rest 5 male and 20 female cattle were apparently healthy and kept as control groups.

Clinical examination of cattle revealed poor body condition with variable signs of debility, alopecia without itching signs, rough coat and pale mucous membranes.

There was a decrease in serum calcium, inorganic phosphorus and magnesium levels but there was a significant decrease in serum calcium of some animals. There was a significant increase in serum Aspartate-amino-transferase activities in some female cattle infected with helminthes while there was a non-significant increase in serum Alanine-amino-transferase activities in all animal groups. A highly significant decrease in serum total proteins and albumin levels recorded in emaciated cattle while a non-significant increase in serum creatinine level in emaciated animals.

The parasitological examination revealed that 65.54% of emaciated examined cattle were infected with helminthes.

The study concluded that emaciation and ill-thrift in Edfu, Aswan governorate constitute a serious problem, affecting cattle health and productivity. It could be attributed to many factors such as inadequate feeding of protein and mineral deficiency, infection with helminthes these factors together with the environmental stressors of the arid and semi-arid zones and possibly other factors constitute the main impact on health and productivity of cattle in Aswan governorate.

Bactericidal speed of 10 field strains of *Streptococcus uberis* by the main antibiotics used for treatment of mastitis in France

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Introduction
An antibiotic or a combination of antibiotics is considered as bactericidal for a bacteria species if it has the ability to reduce by at least 3 log initial bacterial population in less than 24 hours. The time-kill curve of a bactericidal antibiotic is an important key to improve the time of clinical recovery. The present study has been performed by the ISAE laboratory and consists to investigate the bactericidal speed of different antibiotics commonly used among veterinary products in the treatment of mastitis due to *Streptococcus uberis*.

Material and methods
10 strains of *S. uberis* from field cases of mastitis were selected as representative of the distribution of MICs of various antibiotics tested (ISAE). Comparisons of time-kill curves for different bactericidal antibiotics and associations have been performed on the basis of a concentration equivalent to 2 x MIC. A Kruskal-Wallis test was used for statistical comparison of all data and, in case of significant difference, pairwise comparisons were performed with the Mann-Whitney-Wilcoxon test.

Results
The results show that different tested antibiotics were bactericidal with different time-kill curves ($P < 0.001$). The time kill curve data for the association bacitracin- neomycin, with a median value of 3 hours, were better (and significantly different) from those obtained with all other tested antibiotics ($P < 0.05$). Penicillin G (median value = 15 hours) and cephalexin (median value = 17 hours) showed the longer time-kill curves, corresponding to significant differences in comparison with cloxacillin, cephapirin and cefquinome (which have a median value of 6 hours).

Mycoplasma bovis: first detection in Finland

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Mycoplasma bovis is recognized as a worldwide pathogen of farmed cattle. Finland has belonged to the few countries notably free from *M. bovis* infection. There have been efforts to prevent the spread of *M. bovis* to Finland through importing only cattle that are negative for *M. bovis* antibodies (no testing for cattle imported from Sweden), and early detection of the disease. We conducted a survey on respiratory pathogens from calf rearing units presenting signs of respiratory disease during 2002-2004, but could not find *M. bovis* by culture and PCR. Since 2004, we have analysed all samples from bovine respiratory tract and eye infections as well as from abortions for *M. bovis*. Altogether 532 deep nasopharyngeal swabs (NS) and 268 pathological samples *were examined during years 2006-2012 with negative results.* Since February 2012, most milk samples from *mastitis* have been analysed in laboratories using a PCR test detecting *M. bovis*.

In November 2012 we detected *M. bovis* for the first time in NS of 4 to 6-week-old fattening calves showing signs of pneumonia and poor response to antibiotics. Later *M. bovis* was found from five farms that had bought calves from the first case farm. Mortality to pneumonia was 15-50% in the diseased lots. *M. bovis* was also found in NS of pneumonic calves from three calf rearing farms with no contact to the infected farms. In December 2012 *M. bovis* caused an episode of respiratory disease in a dairy farm and four cows out of 24 were culled because of *M. bovis* mastitis. This previously closed farm had bought a heifer 3 weeks before the outbreak.

To find the source and time of import, we analysed *M. bovis* antibodies with commercial ELISA in blood samples from possible sources. Few ELISA-positive animals were found. Ten suspected dairy farms were analysed with RT-PCR for *M. bovis* with negative results. We conclude that *M. bovis* has silently spread to the major cattle-producing areas of Finland. Clinical disease mostly occurs in calf rearing units buying calves from multiple farms creating thus a high infection pressure. The infection source remains obscure.

Comparative efficacy of tulathromycin and tildipirosin injection for the treatment of experimentally-induced *Mycoplasma bovis* infection in calves

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Background
Mycoplasma bovis is a major primary and secondary pathogen in bovine respiratory disease (BRD).

Objective
To compare the efficacy of tulathromycin and tildipirosin in an experimental *M. bovis* infection model in calves (intra-tracheal, 3 x 10^9 CFU, three consecutive days, tulathromycin and tildipirosin MIC of challenge strain > 64 µg/ml).

Methods
Over a four-day post-challenge period, 126 animals with clinical signs of *M. bovis* infection were randomly allocated to one of three treatment groups: tulathromycin (2.5 mg/kg), n = 53; tildipirosin (4.0 mg/kg), n = 48; and placebo (0.9% saline injection), n = 25. Observations for clinical signs of respiratory disease (respiration, depression, nasal discharge, coughing), rectal temperature and injection site examinations were performed daily. At necropsy on Day 14, the animals were weighed, followed by examination of lung lesions and broncho-alveolar lavage (BAL) fluid collection to determine *M. bovis* concentrations. Injection site samples were collected for histopathology.

Results
Tulathromycin was superior to placebo treatment for all variables examined, with the exception of injection site reactions. One tildipirosin-treated calf died spontaneously and six others (three tildipirosin- and three placebo-treated animals) were euthanised on welfare grounds with severe signs of BRD. There were no BRD-related deaths or withdrawals in the tulathromycin group. Treatment with tildipirosin was significantly more effective than placebo for several variables examined but never superior to tulathromycin, expect for the diameter of injection site reactions from Day 3 onwards, although post-mortem histopathology changes were not significantly different between these two treatment groups. Tulathromycin was superior to tildipirosin regarding BRD-related mortality and withdrawals (0.0 vs 8.3%, P = 0.0477), Day 14 body weight (67.61 vs 65.65 kg, P = 0.0112), percentage of days with depression (0.9 vs 4%, P = 0.0486), and reducing Day 14 percentage of lung lesions (7 vs 12%, P = 0.0079). The mean concentration of *M. bovis* in BAL fluid was significantly lower in the tulathromycin group than in the placebo group (0.159 vs 1.678 x 10^6 CFU/ml, P = 0.0066). By contrast, the difference between the tildipirosin-treated group (0.812 x 10^6 CFU/ml) and the placebo group was not significant (P = 0.4054).

Conclusion
Tulathromcyin offers superior efficacy than tildipirosin for the treatment of *M. bovis* respiratory infections in calves.

Blood levels of immunoglobulins G and total proteins on fifteen dairy calves during the first month after birth

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Introduction
Veal calves coming from different farms are entered in stables at different ages, mostly older than eight days.
No information is known about the protection level of the calf obtained by colostrum transfer, usually measured between two and six days after birth.
The objective of this study is to investigate the potential correlation of immunoglobulin G and total protein levels in calves aged two to six days after birth and calves older than eight days.

Protocol
Fifteen dairy calves are sampled during the first month of life. Blood samples are taken once a day during the first ten days, and then every two days till day thirty.
All samples are analyzed and checked for immunoglobulin G level by radial immunodiffusion, and for total protein level.

Results
Before first colostrum intake, all fifteen calves show immunoglobulins G blood levels less than five grams per liter.
When two to six days old, fifty-three percent of the calves have an immunoglobulin G level over ten grams per liter of blood.
During this time, all calves with a blood level above twenty grams of immunoglobulins G per liter, still have blood levels over ten grams per liter at day thirty.
Immunoglobulin G blood levels slowly decrease during the first month after birth. In this study, the evolution of total protein blood levels is more variable and does not show any such trend.

Discussion
No transfer of immunoglobulins happens transplacental in ruminants; therefore, early colostrum intake is key to the calf's protection.
Failure of passive transfer is defined in literature as being less than ten grams of immunoglobulin G per liter of blood measured between two and six days of age. The total protein parameter, which is an indirect way of measuring passive transfer, should be above fifty-five grams per liter of blood at the same time.
The evolution of the immunoglobulin G levels is more repeatable than the evolution of the total protein level during the first month.
These results indicate that high immunoglobulin G level in older calves reflect sufficient protection levels due to passive transfer.

Validation of an in-house real-time quantitative PCR for quantification of *Coxiella burnetii* and using in a French national abortion control program

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Purpose
Following the recent cases of animal and human Q fever in the Netherlands, the EU calls for special consideration to the risks for humans and animals. In this context, the French government launched for a period of three years a national program for monitoring abortions in livestock related to Q fever in ten pilots french departments. The study focuses on the use of quantitative PCR to determine the amount of *Coxiella burnetii* present in vaginal secretions during an abortion and therefore establish a threshold beyond which we can consider that holding is clinically affected with Q fever and at risk for humans.

To participate in this program, the LDA71 has validated its own quantitative PCR according to the AFNOR XP-U47-600-2.

Methods
Nucleic acid extraction is performed on vaginal swabs using QIAamp DNA mini-kit (Qiagen). Quantitative PCR target sequence IS1111 (Qiagen PCR mix QuantiFastProbe on ABIPRISM 7500 Fast (LifeTechnologies).

Results
According to the standard AFNOR XP-U47-600-2 the features of quantitative PCR were defined for the PCR itself but also for the complete method. Analytical specificity was confirmed. The PCR LOD and LOQ were determined as one copy of genome. The linearity range was tested between 1 and 500,000 genome copies. The PCR efficiency is between 85 and 115%. Finally, LOD and LOQ of the complete method were determined as 200 bacteria per vaginal swab while LOQmax is 1.10^7 bacteria.

Conclusion
The quantitative PCR developed by LDA71 has been recognized by the French National Laboratory for *Coxiella* (ANSES Sophia-Antipolis) and COFRAC.

Dose confirmation and efficacy of intramuscular and intravenous single injections of marbofloxacin in the treatment of bovine acute *E. coli* mastitis compared with an untreated control group

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In order to confirm the efficacy of a 10 mg/kg dose of a 16% marbofloxacin solution as a single-dose treatment of severe *E. coli* mastitis in lactating dairy cows, an experimental *E. coli* bovine mastitis study was conducted evaluating the treatment effect of a single intravenous or intramuscular injection of marbofloxacin compared with an untreated control group.

E. coli strain was inoculated in one udder quarter of 24 healthy lactating Prim'Holstein cows after milking (day0 pm). Treatment started at onset of clinical signs. Marbofloxacin was injected once at 6.25 ml/100 kg either intramuscularly or intravenously in two randomised groups of 8 cows. Animals in the third group (n = 7) were left untreated. The 23 animals were examined from day0 to day12.

Both treatments with marbofloxacin were associated with an early return of rectal temperature to normal. From the first examination time after treatment, mean values of total clinical scores quickly decreased in both marbofloxacin treated groups. They were significantly lower in the intravenous treated group compared to those in the untreated group from day3 to day6 (P = 0.0109). Moreover, intravenous group values were lower than in the intramuscular treated group and significantly on days 4 and 5 (P = 0.0357) then showing a faster decrease of clinical scores values in the intravenous group. Individual general clinical scores (general behaviour, appetite, and hydration scores) followed the same trend as that of the total general score. Milk bacteriology showed a highly significant reduction of the milk *E. coli* isolation rate in the marbofloxacin treated animals on day4 (P = 0.0006). Milk yields plummeted less severely in the intravenous treated group than in the intramuscular group or in the untreated group. Increase of milk yields throughout the post-treatment period was stronger in both marbofloxacin treated animals compared to the untreated animals from day2 and significantly at days5-6 (P = 0.0436).

In conclusion, the 16% marbofloxacin solution administered intravenously or intramuscularly as a single-dose injection is efficacious in the treatment of acute *E. coli* mastitis and proved to have a significant clinical and zootechnical interest compared with no treatment. However, the intravenous route tended to give faster results than the intramuscular one.

A study toward a better understanding of the clinical expression of mastitis due to *S. uberis*: is severity and chronicity of disease influenced by cow factors, herd factors or bacterial factors?

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Mastitis, due to *S. uberis,* is a frequent disease on dairy farms associated with important economic losses. This disease has a large variety of clinical expression: in the severest cases, local symptoms at the udder level are associated with general symptoms. In the least severe expression form no clinical symptoms are detectable and infection is only associated with high somatic cell counts. There are also differences in chronicity of the disease: some cows heal in a few days and other will be infected for the rest of their lives. It would be interesting to be able to associate cow, herd or bacterial factors to the clinical expression form of the disease.

In this study, the influence of cow, herd and bacterial factors on the chronicity of *S. uberis* infections of the udder were investigated. This abstract describes the influence of cow and farm factors on. Milk samples of cows (n = 322) suffering from mastitis were analyses for the presence of *S. uberis*. The *S. uberis* strains were collected and stored at -20° C for later analyses. The *S. uberis* mastitis cases (n = 120) were classified according to chronicity of disease (acute disease: duration of infection less than 60 days (n = 59) and chronic disease: duration of infection more than 60 days (n = 61). For each group the influence of cow factors (DIM, rang of lactation, quantity of milk, milk fat and milk protein), housing factors (straw yard or cubicles) and factors associated to milking (presence of pre and post dip) was assessed. Statistical analyses were performed using the χ^2 test (Statistix®). Chronicity of *S. uberis* infection was influenced by DIM (P = 0,002), milk fat (P = 0,03) and number of lactations (P = 0,03). Factors such as milk protein, housing and milk hygiene did not influence the presence of chronic infections. These preliminary data indicate that chronicity of *S. uberis* disease is influenced by cow factors. In further research, the influence of cow and herd factors on severeness of clinical symptoms will be assessed, as well as the influence of bacterial factors on the clinical expression of *S. uberis* disease in dairy cows.

Composition and gene expression of peripheral blood leukocytes of cows with subclinical endometritis

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Subclinical endometritis (SCE) in dairy cows is usually diagnosed by invasive methods (cytobrush, endometrial biopsy and histology), which may interfere with fertility. The aim of this study was to analyze SCE-associated alterations changes in peripheral blood leukocytes (PBL) as potential diagnostic parameters. PBL subpopulations from cows with or without histologically proven SCE (45 to 55 days post partum) were flow cytometrically quantified and gene expression of whole blood PBL was assessed by PAXgene analysis. Blood mononuclear cells (MNC) and neutrophils of SCE cows were significantly increased with higher numbers of B-cells, NK-cells and monocytes among MNC. PBL of SCE cows expressed higher copy numbers of CXCL8, TNF and IL12 compared to non-SCE animals.

Blood plasma of SCE and non-SCE cows did not differ in their content of non-essential fatty acids, progesterone, calcium or beta-hydroxybutyrate. However, SCE blood plasma, when incubated with several isolated leukocyte subpopulations, selectively increased the expression of inflammatory mediators (CXCL8, CXCL1, IL1B) in intermediate monocytes. This observation suggests that yet unknown circulating factors in plasma of SCE cows alter the gene expression and probably the function of circulating precursor cells.

Whether the observed changes in the periphery of SCE cows are the consequence of the uterine inflammatory process, or whether they affect the course of the disease is currently unknown.

All experiments involving animals were carried out in compliance with national legislation and subject to local ethical review.

Wound healing from caesarian section in Israeli Holstein dairy cows using two different anesthesia approaches

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There are different surgical approaches to Caesarean operation in cattle. The most common approach is a standing position which is suitable for both left and right paralumbar fossa. For a flank incision, paravertebral anesthesia of the nerves associated with the transverse processes of T13, L1, L2 and L3 is commonly recommended. Signs of successful anesthesia are a warm and flaccid flank with no response to pain when tested with a hypodermic needle. A local anesthetic line block or inverted-L block of the flank is an alternative to paravertebral anesthesia.

The operations were approved by the "AEEC" 1994 of Volcani center. Nine Israeli-Holstein dairy cows, 6 primiparous and 3 multiparous, were operated electively for caesarian section and conducted at the Volcani Center experimental farm in Bet Dagan, Israel. All cows were examined before the operation for evaluation of the stage of pregnancy by examining the position of the fetus and state of the udder (273-289 d). Cows were shaved and scrubbed on the left flank. Anesthesia was done locally in a standing position, by using two different approaches:

- Lidocaine HCL 2% and epinephrine (injection USP) was injected (16G, 1.75" needle) paravertebral of the nerves of T13, L1, L2 and L3 inserted midway between the transverse process and the dorsal spinous process.

- Line blocks (LB) - 100 ml were given in 5 separated points.

The cesarean section was made with 25 cm incision line trough all the abdominal layers; Retracting the hind limb into the incision line and creating anther 15 cm uterus incision. The uterus was closed in 2 layers method (continues and modified Cusing). The abdominal wall was closed with 4 continues layers. Wound healing was monitored until complete healing. The wound was examined for inflammation and complete recovery. We compared the wound healing between Primiparous and multiparous and between both anesthesia approaches.

Two out of 6 from the primiparous comparing to 0 out 3 of the multiparous were detected with inflammatory process, whereas 0 out of 5 of the paravertebral anesthesia and 2 out of 4 of with the LB anesthesia were detected with inflammatory process.

Farmer-oriented mastitis research providing a basis for an evidence-based, prospective udder health program in Switzerland

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Milk produced in Switzerland is generally of high quality. Average national somatic cell counts (SCC) levels are around 115,000 cells/ml. However, large differences are seen between herds. Also, the use of intramammary antimicrobials in Swiss dairy herds is high compared to other European countries. Swiss dairy production is characterized by small dairy farms with an abundance of animal movements, including the sharing of communal alpine pastures by cows from different herds of origin. This is assumed to facilitate the spread of contagious mastitis pathogens between dairy herds.

Several research projects were started with the overall aim to provide a scientific basis for a new voluntary mastitis control program. Approximately 1,300 randomly selected farmers filled in an online questionnaire to determine the status quo on prophylactic mastitis practices in Switzerland and to assess farmers' knowledge and attitude towards mastitis. It was concluded that room for improvement existed concerning the implementation of several mastitis management practices. Moreover, half of the farmers indicated that they needed assistance in improving their udder health status, implying that a new mastitis control program would be appreciated by them.

To better understand farmers' perceived constraints and motivations towards udder health, semi-structured interviews with 2 groups of farmers were conducted. Eight herds had a low bulk milk SCC level (< 75,000 cells/ml), while another 12 herds had a high bulk milk SCC level (> 200,000 cells/ml). The study identified that mastitis was not a top priority for dairy farmers. The high workload and the lack of understanding of the cost-effectiveness of early mastitis prevention were identified as barriers for farmers to implement adequate mastitis management practices.

Currently, a mathematical model that simulates the dynamics of intramammary infections within dairy herds is being adapted. The goal is to study the dynamics of intramammary infections between dairy herds and to evaluate intervention strategies to limit the spread of contagious pathogens between dairy herds.

This information, together with results obtained from other studies, is assumed to provide the scientific basis for a new udder health program in Switzerland.

Economic value of optimal timing of anthelminthic treatment in dairy cows: a modeling approach

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Gastrointestinal nematodes (GINs) are an important cause of milk production losses in dairy cattle. An anti-*O. ostertagi* antibody ELISA on bulk-tank milk (BTM) samples can measure GIN exposure in dairy herds and estimate induced production losses: milk yield response to anthelmintic treatment is expected at optical density ratios (ODRs) above 0.5, with increasing response at higher ODRs. Timing of treatment is critical for optimizing treatment response. Current knowledge indicates that financial benefit is maximized when treating around calving: daily milk yield response is higher, and duration of this response is longer because of its potential to occur across the entire lactation period.

We developed a user-friendly model in MS Excel to compare the impact of different treatment timings on milk yield response, at varying BTM-ODR. Magnitude and duration of anthelmintic treatment effect in function of BTM-ODR and treatment timing were derived from peer reviewed publications; first author of these publications validated our model concept.

With this model, treatment at end of dry period (Txt-Dry) was compared to treatment during lactation (Txt-Lactation) on day 150, at BTM-ODR = 0.8 and for lactation period of 305 days. Daily milk yield response per cow with Txt-Dry was 0.96 kg/day versus 0.49 kg/day with Txt-Lactation, resulting in a 99.75 kg higher milk yield response across lactation (Txt-Dry=175.68kg; Txt-Lactation=76.11kg). In scenario analyses, ODR was varied from 0.5 to 1.1 and treatment timing in Txt-Lactation from 75 to 225 days in milk. As expected, treatment response is zero at ODR = 0.5. At ODR = 1.1, Txt-Dry results in a 199.14 kg higher increase in milk yield across lactation (Txt-Dry = 351.36 kg; Txt-Lactation = 152.22 kg). Varying timing of treatment in Txt-Lactation results in varying milk yield response per day and across lactation; at ODR = 0.8 and deworming in Txt-Lactation at day 75 and day 225, Txt-Dry results in a 43.20 kg and 155.28kg higher milk yield response across lactation, respectively.

In conclusion, BTM-ODR and timing of treatment are essential for predicting potential milk yield response to anthelmintics. At BTM-ODR = 0.8, treatment at end of dry period can result in 100 kg/cow higher milk yield response compared to treatment in mid lactation, corresponding to € 30 higher net income at current milk price to farmers (€ 0.30/kg).

Uterine infection exacerbates liver dysfunction during the early postpartum period in dairy cows

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Uterine disease develops in the early postpartum period, concurrent with the onset of lactation and associated metabolic changes that often result in negative energy balance (NEB) and uncoupling of the liver GH:IGF-1 axis. Indeed, it has recently been suggested that uterine disease may be associated with liver dysfunction. Thus, the aim of the present study was to test this assumption and to determine potential mechanisms mediating this relationship.

Fifty-three Holstein-Friesian cows were monitored for 12 weeks after calving. Energy status was calculated using standard equations and concentrations of NEFA's, BHB, urea and glucose were measured in weekly serum samples. Uterine health was assessed by vaginal mucus character score (range 0-3) on days 14 and 21 postpartum. Animals with a vaginal mucus score of 2 or 3 on day 14 and/or 21 postpartum were classed as 'infected' (n = 23) and animals with a vaginal mucus character score of 0 or 1 on both day 14 and 21 postpartum were classed as 'clean' (n = 30). Liver tissue was biopsied from all cows on day 35 ± 5 postpartum. In a separate experiment, Holstein heifers received an intrauterine infusion of lipopolysaccharide (LPS: n = 4) or saline (n = 4) and liver tissue was collected during necropsy 6h post-infusion. Validated real-time PCR assays were used to detect RNA transcripts of the key hepatic genes IGF-I and GH Receptor 1a (GHR1a).

There was no difference in daily energy balance (P = 0.57), cumulative energy balance (P= 0.59) serum NEFA (P = 0.10), BHB (P = 0.61), urea (P = 0.30) or glucose (P = 0.12) concentrations between clean and infected postpartum cows. However, infected cows took approximately 1 week longer to return to positive energy balance and had reduced liver GHR1a expression on Day 35 post partum compared to clean cows (P = 0.04). Furthermore, liver expression of IGF-1 and GHR1a was reduced in heifers following intra-uterine infusion of LPS (P = 0.04).

These data suggest that uterine infection may exacerbate uncoupling of the liver GH:IGF-1 axis in postpartum dairy cows thereby delaying the return to normal physiological function.

Efficacy of two dry cow therapies in Italian dairy herds

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This study was performed to compare efficacy of two intramammary treatments at drying off (either 150 mg of cefquinome or a combination of penethamate hydriodide (100 mg) + benethamine penicillin (280 mg) + framycetin sulphate (100 mg).

A total of 393 cows from two farms in Italy were randomly allocated to one of the two treatment groups between May and December 2011. Quarter milk samples were taken on all cows twice before drying off and twice after calving for bacteriological analysis and somatic cell counts according to standard laboratory techniques. Cows were monitored during the first 100 days post-calving for the presence of clinical mastitis. Bacteriological cure rate and new infection rate during the dry period were compared between groups at quarter level whereas incidence of clinical mastitis was compared at cow level. Comparisons were made by using either the chi-square or Fisher's exact test.

Among the included cows, 63 cases had to be excluded mainly for loss of monitoring (transfer between herds of the same organization) or for disease condition mostly unrelated to udder health. Around 46% of quarters were infected at drying off, major pathogens, coagulase negative *Staphylococci* and *Corynebacterium bovis* representing respectively 9%, 53% and 11% of positive samples. Overall bacteriological cure rate and new infection rate were not significantly different between groups. However, significantly higher cure rates were observed in the cefquinome treated group for quarters with subclinical mastitis at drying off and for those from cows entering in their second lactation. Moreover, in spite of small numbers, the cure rate against *Staphylococcus aureus* was significantly higher in the cefquinome treated group than in the combination treated group (100% vs 44.4%). A decrease of quarter somatic cell counts between drying off and post-calving was recorded in both groups from a mean value of 100,000 cells/ml to 10,000 cells/ml. The incidence of clinical mastitis during the first 100 days in milk was low and not significantly different between groups (3.5% in cefquinome treated group vs 5.0% in combination treated group).

Measurement of the preventive efficacy of enterotoxemia vaccine COGLAVAX in veal calves

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Aim of the study

Enterotoxemia is triggered by the sudden multiplication of Clostridial bacteria from the normal gut flora, especially *Clostridium perfringens,* leading to toxemia, peracute disease and sudden death in cattle. Treatment is generally unsuccessful as toxemia rapidly damages health status and creates irreversible lesions. Aetiology of the sudden multiplication of the gut flora may be multifactorial (modifications in the environment or feed, handling of the animals…). Preventive measures are possible, relying on adequate farm management and vaccination.

The aim of the study was to evaluate the efficacy of vaccination with Coglavax (Ceva Animal Health) in preventing mortality in cattle due to enterotoxemia.

Material and Methods

A field trial was carried out in 19 veal calves farms, involving a high number of study animals (n = 3975). The vaccinal status of mother cows was unknown.

In each farm, every pen was divided in 2 groups (even or odd number on ear tag). Each group was injected the vaccine or a placebo (saline), as a blind administration. Injection protocol of calves was as follows:

- 1st injection, 2 ml SC, within10 to 15 days after arrival of calves on the farm
- 2nd injection, 4 ml SC, 4 weeks apart (5 to 6 weeks after start of the fattening period)

Cases of suddenly dead calves that had spent more than 60 days in the fattening unit were clinically investigated for diagnosis including necropsy. Animals that met clinical criteria (acute mortality possibly associated with acute abdominal syndrome) and corresponding necropsy (acute necrotic and haemorrhagic enteritis with satellite lymph node inflammation without any other lesion that could have been lethal) were classified as dead from enterotoxemia. Number of calves dead from enterotoxemia was compared between the 2 groups.

Results

Mortality results on calves having spent more than 60 days in the fattening unit are presented in table below.

Vaccine group	Animal Numbers	Sudden death Numbers	Sudden death from enterotoxemia confirmed by necropsy
COGLAVAX	1960	7	2
PLACEBO	2015	19	6

Sudden death mortality resulted 0.94% in the Placebo group versus 0.36% in the Coglavax group.

It has to be noted that only 1/3 of suddenly dead calves showed enterotoxemia at necropsy.

Considering either sudden death or enterotoxemia as confirmed by necropsy, mortality in calves vaccinated with Coglavax was 3 times lower than in control calves.

Discussion

Considering the high animal numbers required in such a study and practical difficulties in performing systematic necropsy, few trials have been carried out to evaluate the clinical efficacy of enterotoxemia vaccines in cattle.

Mortality as measured in this study is compatible with published data: Institut de l'élevage (French cattle rearing institute) reported in a field study (2004-2007) a mean mortality rate due to enterotoxemia of 0.5%.

30% sudden deaths were identified as enterotoxemia in this study. It has to be noted that the strict assessment criteria as determined in the study to confirm diagnosis may have led to underestimation of the real incidence of the disease.

In this trial, vaccination with Coglavax was associated with a 3 times reduction of overall mortality.

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Performance evaluation of the ELISA Kit Prionics PrioCHECK® Besnoitia Ab 2.0 for serological diagnosis of bovine Besnoitiosis

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Introduction

This publication describes all the performance evaluation tests of PrioCHECK® Besnoitia Ab 2.0 realized by the French Departmental Veterinary Laboratory of Ariège (LVD 09) using samples from the national serum bank established by the National Steering Committee for bovine Besnoitiosis.

The LVD 09 is accredited by Cofrac for analysis in animal immune serology (program 109, date of publication of the report) with a scope of accreditation available on www.cofrac.fr. Until now, Besnoitiosis is not part of its scope of accreditation, but LVD 09 applies the same procedures and the same validation criteria if analyzes are covered by the accreditation or not.

Due to their respective expertise in the field and their geographic proximity, the LVD 09 and the National Veterinary School of Toulouse are combined to form a laboratory expert group under the umbrella of the National Federation of Sanitary Defense Groups (FNGDSB). This committee has set up a reference serum bank necessary for performance verification of Besnoitiosis diagnostic ELISA kits.

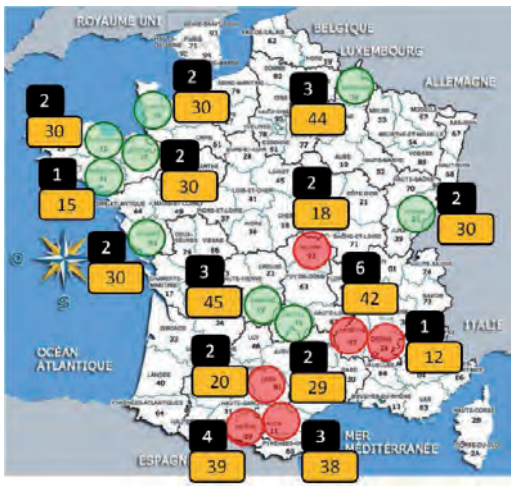
By using this serum bank, the following characteristics were evaluated:

- diagnostic sensitivity,
- diagnostic specificity,
- positive and negative predictive values,
- repeatability,
- reproducibility,
- analytical sensitivity.

The National Reference Serum Bank for Besnoitiosis

The Departmental Veterinary Laboratory Ariege (LVD 09), in collaboration with the National Veterinary School of Toulouse (ENVT) and the National Federation of Groups of Bovine Sanitary Defense (FNGDSB) formed during the first half of 2013 a serum bank from 452 cattle. The corresponding samples were taken in several French departments. The negative samples were collected from departments known as free of Besnoitiosis and positive samples from known as infected one's.

The map below shows the distribution of samples taken for the establishment of the serum bank (black background shows the number of herds chosen in the department, yellow background indicates the number of animals).



National serum bank is thus composed of:

283 cattle with a disease-free status (negative Western blot result),
169 animals with an infected status (positive Western blot result).

The LVD 09 provides on his location 452 serum samples centrifuged according to standard NF U47-020, corresponding to the respective cattle and stored at -80 °C in a freezer.

Material & methods

Following the test kit protocol, the following calculation and result interpretation was used:

Calculation of results

$$\text{Sample \%P (PP)} = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} * 100$$

OD_{PC} = mean value of Positive Control
OD_{NC} = mean value of Negative Control
The percent positivity (%P, PP) of PC is considered as 100%

Interpretation of results

PP ≥ 23 (positive)
17 ≤ PP < 23 (doubtful)
PP < 17 (negative)

Additionally to the package insert, LVD 09 conducted these analyzes respecting the NF U47-019 (2nd printing June 2010), accreditation standards EN ISO IEC 17025 (2005 version) and LAB REF 02, and the internal procedure.
The testing was performed by the manual method (no use of automatic ELISA).

Results and Discussion

Diagnostic Sensitivity (Se)

The sensitivity was calculated from known infected animals whose Western Blot analyzes showed a positive result. For the calculation of sensitivity, 169 cattle were selected.

Out of the 169 infected cattle, the results of the Prionics ELISA kit, Lot U130601L, are:

154 animals interpreted as positive,
9 animals interpreted as doubtful,
6 animals negative interpreted.

To calculate the diagnostic sensitivity, doubtful results were considered as positive results.

Table I: Results of 169 WB-positive tested cattle with PrioCHECK® Besnoitia Ab 2.0, lot U130601L

U130601L	WB +
Prionics +	163
Prionics -	6
Total	**169**

Sensitivity is equal to: 163/169 x 100 = 96.4%

With a provided repeatability and reproducibility given by CV = 5%, the sensitivity was recalculated by taking into account the uncertainties as required by the standard NF U47-019. If the estimated uncertainty (I) is equal to 2 times the CV%, then I = 10%.

The area of uncertainty calculated on the suspect area is 15.3% - 25.3%. All results included in that zone thus become uncertain.

After taking into account the uncertainty, the result of 1 sample becomes uncertain.

Table II: Results of 169 WB positive tested cattle with PrioCHECK® Besnoitia Ab 2.0, lot U130601L after taking into account the uncertainty

U130601L	WB +
Prionics +	164
Prionics -	5
Total	**169**

The sensitivity becomes 164/169 x 100 = 97.0%

Diagnostic specificity

Specificity was calculated from animals recognized as free and whose Western Blot analyzes showed a negative result. For the calculation of specificity, 283 bovine sera from the serum bank were selected.

The ELISA results (PrioCHECK® Besnoitia Ab 2.0, Lot U130601L) of the 283 negative tested cattle (WB) are:

269 animals negative interpreted,
14 animals interpreted as doubtful,
No animal interpreted as positive.

To calculate the diagnostic specificity, doubtful results were considered as negative.

Table III: Results of the 283 WB negative tested cattle in Prionics-ELISA with lot U130601L

U130601L	WB -
Prionics +	0
Prionics -	283
Total	**283**

The specificity is therefore equal to: 283/283 x 100 = 100%

Negative Predictive Value (NPV) and Positive Predictive Value (PPV)

The NPV is the probability that a negative ELISA defines an animal as non-infected with Besnoitiosis. The PPV is the probability that a positive ELISA defines an animal as infected with Besnoitiosis.

The calculation of NPV and PPV is as follows:

NPV = TN / (FN + TN) x 100
PPV = TP / (FP + TP) x 100
TN/TP = “true negative/positive; FN/FP = “false negative/positive”

Table IV: Result comparison of the of 452 bovine sera of the national serum bank tested by Western Blot and ELISA PrioCHECK® Besnoitia Ab 2.0, U130601L lot

U130601L	WB +	WB -	Total
Prionics +	163	0	163
Prionics -	6	283	289
Total	**169**	**283**	**452**

NPV = TN / (FN + TN) x 100 = 283 / 289 x 100 = 97.9 %
PPV = TP / (FP + TP) x 100 = 163 / 163 x 100 = 100 %

Repeatability

The repeatability of the ELISA kit was performed by analyzing 92 samples of internal reference material, where the PP is close to the doubtful area of the ELISA kit.

Negative controls were placed with A1 and G12 and positive controls A2 and H12.

The repeatability test was also used to evaluate the effect of edges.

The calculated CV% of repeatability appears acceptable as in all cases lower than 10%.

The small variation observed in the PP obtained at the center and the edge wells of the plate can be concluded as the effect of edges, although this can be considered insignificant and acceptable.

Reproducibility

The reproducibility of the ELISA kit was performed by analyzing 3 samples on different microplates at different positions and different days (same staff, same equipment). Samples with PP around the doubtful area of the ELISA kit were selected.

The obtained CV% values of reproducibility (4.6%, 5.1% and 5.5%) are all neighboring 5% and allow the assumption of a reproducible test kit.

Detectability (analytical sensitivity and specificity)

There is no national or international standard serum for Besnoitiosis serology up to now.

Analytical specificity

Analytical specificity was not evaluated for this ELISA kit, lot U130601L. Tests already done with the previous batch (U120601L) of PrioCHECK® Besnoitia Ab 2.0 during the constitution of the serum bank had ensured that there was no interference possible between *Besnoitia besnoiti* on one hand and *Toxoplasma gondii* and *Neospora caninum* on the other (main biological agents near *Besnoitia besnoiti*).

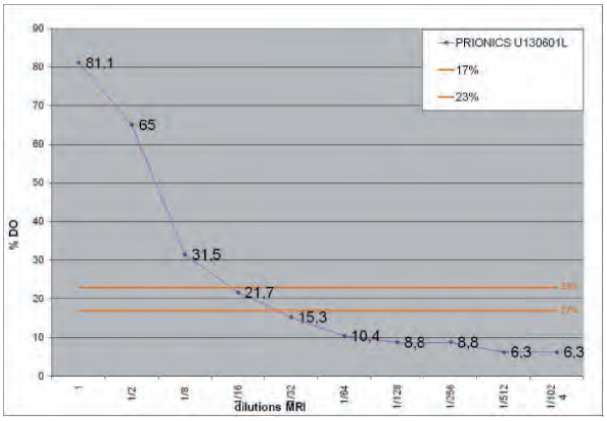
Analytical Sensitivity

In absence of a national or international standard reference, a serum from a region known as highly positive (serology ELISA and Western Blot) has been used for the analytical sensitivity. Analytical dilutions were made until 1/1024.

Positivity disappears down from the 1/32 dilution (doubtful at 1/16 dilution).

Figure 1 shows the correlation between successive dilutions of the serum and results in corresponding PP (% OD) obtained in the analysis (low dose-effect). The correlation of the low dose-effect is coherent as the development of positivity towards negativity passing the questionable results is observed.

Figure 1: Low dose-affect of a sample from a strongly positive territory



Summary of main results

In this study, PrioCHECK® Besnoitia Ab 2.0 (lot U130601L), showed a slight lack of sensitivity (6 false negatives) and a negative predictive value (NPV) of 97.9%.

The kit is very specific because of the lack of false positive results.

It is important to note that this kit, in addition to its good sensitivity and specificity, is repeatable and reproducible.