Paratuberculosis – current concepts and future of the diagnosis

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ABSTRACT

Intra-vitam diagnosis of paratuberculosis is performed either directly by detection of the causative agent, or indirectly by measurement of immune reactions. The detection rate of diagnostic methods is largely dependent on the state of the disease in infected animals. Direct identification of Mycobacterium avium subsp. paratuberculosis in faecal samples is limited by biological factors (sheding of low amounts of bacteria, intermittent shedding, clumping) and methodological influences. The presence of ubiquitous non-pathogenic mycobacteria in the environment of farm animals can impair the specificity of indirect tests. Up to now, none of the diagnostic procedures available enables a reliable diagnosis of paratuberculosis on the individual animal level. Estimation of prevalence is also complicated.

Enormous research efforts are taking place to improve existing and to develop novel diagnostic procedures for paratuberculosis. However, control of the disease is possible even now by combining regular diagnosis with strict sanitary measures and removal of infected animals.

KEYWORDS

Cultivation, polymerase chain reaction, ELISA, γ-interferon test, skin test

INTRODUCTION

Diagnosis of paratuberculosis aims at two main purposes, herd screening and monitoring on the one hand and identification of individual positive animals on the other hand. At present, intra vitam diagnosis of paratuberculosis is performed either directly by detection of the causative agent using faecal culture or PCR, or indirectly by measurement of immune reactions like antibodies against Mycobacterium avium subsp. paratuberculosis (MAP) in blood or milk or cell mediated immunity. The outcome of diagnostic tests is strongly related to the pathogenesis of the disease.

After oral infection in the first months of life, the organisms are believed to penetrate the mucosa of the small intestine into the lymphoid system via M cells (12). The bacterium is than taken up by macrophages, initiating a cellular response. MAP is able to survive and replicate in macrophages by inhibition of bactericidal mechanisms, species of macrophages, initiating a cellular response. MAP is able to produce gamma interferon (γIFN) is one of the earliest detectable reactions to MAP infection. Cell mediated immune functions are necessary to contain the intracellular infection. However, shedding of the organisms, although often in low numbers, is already measurable in young cattle. Antibody immune responses develop as the disease progresses. The humoral response is not protective and does not stop the progression of MAP infection and pathology (12). Clinical disease is characterized by non-specific symptoms like chronic emaciation and reduced productivity, not in all species accompanied by non-treatable diarrhoea. The animals die from mal-absorption and nutrient deficiency.

Direct detection of the organism

Faecal culture

Although labour intensive and time consuming, faecal culture is still the most reliable method for intra vitam diagnosis of paratuberculosis. The diagnostic sensitivity (Se) of the method depends on the disease state of the infected animals. Specificity (Sp) is considered 100%.

In routine diagnosis, cultivation on solid media is most common, although also automated liquid cultivation systems have become available in the last years (3, 6). Cultivation of MAP from faecal or organ samples requires a decontamination step for the samples in order to inactivate other bacteria, yeasts and fungal present in high amounts especially in the faecal content. Worldwide, several methods for decontamination are used (8, 20).

Different media have been shown to be useful for primary isolation of MAP. Similar to isolation of mycobacteria of the M. tuberculosis-complex, combination of different types of media seems to be most effective (5). In contrast to other mycobacteria, MAP is not able to take up iron in sufficient amounts in vitro. Therefore, growth of MAP is dependent on the addition of mycobactin to the culture media, a siderophore, which enables iron uptake from the media. This feature of the organism is used for phenotypic characterization of MAP colonies. Since the media used for cultural isolation of MAP are not selective, identification of colonies suspected to be MAP is necessary. This can be done by determination of mycobactin dependent growth and also by MAP specific PCR (see below).

The detection rate of faecal culture as well as direct PCR is determined by biological and methodological factors. In latently infected animals shedding of MAP is often low and occurs intermittently. The organisms form aggregates in faeces, furthermore, especially in low shedders they are unevenly distributed. The detection rate of the method itself depends on the decontamination procedure, the type and quality of the culture medium and the number of culture tubes. Depending on feeding of the animals, contamination of cultures with yeasts and fungi is one problem of MAP cultivation, which has not completely been overcome even with high quality media containing very effective antibiotics and antymycotics.

Contamination rates of 10 – 20% have been reported (15).

So far, automated liquid cultivation systems prove advantageous in comparison to cultivation on solid media, although they are also labour intensive and sensitivity is not markedly enhanced. However, cultivation time can be significantly reduced (24).

PCR

Different target sequences have been identified for the molecular biological identification of MAP. The most common target is the insertion sequence IS900, occurring in 14 – 20 copies in the MAP genome. Careful selection of primer sequences is necessary for this target, because IS900-like sequences have also been found in mycobacteria other than MAP. Certain IS900 primer pairs can be recommended (14). During recent years, other very specific targets have been published, like IS57, locus 255, ISMap02 and others (1, 17, 21).

Direct identification of MAP in faecal or organ samples by PCR would largely reduce the detection time of MAP infection. So far, Se of this method is limited by the effectiveness of DNA extraction. Large differences exist between commercially available DNA extraction kits for faeces. In general, the detection rate of direct PCR is related to the amount of MAP in the faecal samples, with 80 – 100% positive results in samples...
with high bacterial load assuming a high analytic sensitivity of the PCR itself, and decreasing rates in samples with lower bacterial load.

**Indirect measurement of immune reactions**

**Serology**

At present, because of its speed, relatively low cost and the potential for high throughput analysis, antibody detection by ELISA is considered the method of choice for the diagnosis of paratuberculosis. Worldwide, agar gel immunodiffusion test (AGID) and complement fixation test (CFT), which used to be the traditional methods for the diagnosis of the disease, are loosing importance.

However, the number of commercial ELISA tests used in routine diagnosis is limited. They are mainly based on complex antigen preparations of MAP, like cytoplasmatic proteins, whole cell antigens or cell wall components, respectively. False positive reactions which have often been reported using the traditional methods for the diagnosis of the disease, are loosing importance.

Positive reactions which have often been reported using the traditional methods for the diagnosis of the disease, are loosing importance.

ELISA is considered the method of choice for the diagnosis of paratuberculosis. For certain ELISA tests good correlations of the results of blood and milk have been reported (25).

ELISA (former CSL), Se values between 6.9 % (13) and 88.2 % (18) have been published. These differences are due on the one hand to the kind of gold standard and its methodologically determined Se, on the other hand to the composition of the animal population studied. McKenna et al. (13) proved, that Se values of the ELISA tested were lower when cultural detection of MAP in organ samples was used as a gold standard instead of faecal culture.

Furthermore, Se of ELISA tests is clearly dependent on the disease state of the infected animals. The detection rate of antibodies was much higher in clinically ill than in latently infected dairy cattle (4, 11). In addition, an increase in the detection rate with increasing intensity of shedding was shown (2). However, faecal culture seems to be much more sensitive than ELISA especially in latently infected cattle.

For different paratuberculosis ELISA, variation in the Sp between herds or between geographically separated animal populations has been reported (9, 11, 16). As reasons for this, environmental factors, as well as the presence of cross reacting antigens are discussed. Infections with corynebacteria, nocardia or other mycobacteria have been shown to induce cross reactions with MAP antigens (26).

**Determination of cell mediated immunity**

Determination of cellular reactions is considered the only way for diagnosis of paratuberculosis in animals of young age. The skin test exploiting a delayed type hypersensitivity reaction to Johnin, a purified protein derivative of MAP, used to be a wide spread diagnostic method for paratuberculosis in the past. Because specificity and sensitivity of the test proved to be insufficient, it is no longer used in a large scale.

In recent years, attempts were made to adapt the γIFN test, which has originally been developed for the diagnosis of bovine tuberculosis, also for the diagnosis of paratuberculosis.

This two-step in-vitro test is based on the ability of specifically sensitized T lymphocytes to release γIFN upon re-stimulation with specific antigens. For the diagnosis of paratuberculosis, whole blood is incubated for 24 hours at 37°C in the presence of Johnin, bovine or avian PPD or buffer. The amount of γIFN released in the supernatant is measured by ELISA. Animals are considered paratuberculous positive when the level of γIFN production in the Johnin-stimulated samples exceeds the level of the other samples. However, the test is not suitable to predict animals which will eventually develop disease but it seems useful for the identification of animals which have been exposed to MAP earlier in their lives (7). So far, specificity of the test seems not to be sufficient because it is dependent on different factors. Variations between antigen batches and among herds have been reported (10). Data on the sensitivity of the method are not consistent. Test performance is influenced by the temperature at which blood samples were transported and stored and by the time delay between blood collection and processing (19). All these drawbacks prevent the large scale use of the test.

**Concepts and limitations of paratuberculosis diagnosis**

Up to now, none of the diagnostic procedures available enables a reliable diagnosis of paratuberculosis on the individual animal level. Especially in herds with low disease prevalence, the positive and even more, the negative predictive values of the tests are insufficient. Sensitivity and accuracy of diagnosis can only be increased by repeated testing. Because sensitivity and specificity of the tests depend on the animal population studied, prevalence estimations are always inaccurate.

Determination of the herd status is also complicated. Antibody detection in individual serum or milk samples of the whole herd seems to be a cost effective way for screening. However, small herds with low disease prevalence may not be detected. Moreover, in large herds, determination of freedom from disease is very difficult when tests with Sp < 100 % are applied. Under these circumstances, the probability of false positive results is rising with increasing herd size. Therefore, non-suspect status can only be confirmed by repeated culture of faecal samples.

Different strategies have been tested to reduce the number of samples for screening and monitoring like antibody detection in bulk milk samples, culture of pooled faecal samples or detection of MAP in samples from the farm environment. Unfortunately, it became obvious from field studies that herds with low apparent prevalence will not be identified using these methods and therefore, monitoring of the progress of control programmes will not be possible.

**Future of diagnosis**

Worldwide, enormous research efforts are undertaken to improve existing and to develop novel diagnostic methods. Tests that enable specific diagnosis of the disease in young animals and accurate identification of individual infected adult animals are urgently needed. Detection of faecal shedders could be accelerated by more rapid detection systems for faecal culture and more efficient methods for DNA extraction from faeces. Research is going on to identify new MAP specific antigens with potential to improve antibody detection or measurement of cell mediated immunity. Even completely new diagnostic approaches based on biomarkers should be taken into consideration.

Despite all these problems, control of paratuberculosis is possible already now, provided that regular diagnosis is combined with strict sanitary measures and removal of infected animals.

**REFERENCES**


Good Veterinary Practice to improve bovine udder health: do’s, don’ts, and opportunities

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ABSTRACT
Udder health is considered as an important part of the work of dairy cattle practitioners. There are differences among countries in emphasis on problem solving or on preventive herd health programs. In all countries veterinary practitioners are, to some extent, involved in diagnosis, treatment, and preventive measures on udder health. Important aspects on these subjects are discussed, from the perspective of different parts of the world, leading to practical advices for practitioners, described as “do’s, don’ts, and opportunities”.

KEY WORDS
Mastitis, Practitioner, GVP, Diagnosis, Treatment

INTRODUCTION
In developed countries all over the world, mastitis is the most important health problem of dairy cows. In addition to the economic consequences of mastitis (46), suboptimal udder health reduces the quality of milk (28), increases the risk of antibiotic residues (40), decreases work satisfaction of farmers (22) and impairs dairy cow welfare (30). Dutch dairy farmers have a high regard for veterinarians’ knowledge and advice and consider their own private practitioner as the most important source of information (22). In many countries, the provision of udder health programs to dairy farms remains an untapped area of veterinary work. In one U.S. study, only 24% of farmers indicated that they planned milk quality programs with their herd veterinarian (38). Practitioners generally consider udder health as an important part of their work, although their role differs among countries. In most countries solving and preventing herd level problems is the main activity of veterinarians. This paper will discuss the role of veterinary practitioners in improving bovine udder health.

Timely Diagnosis
Early diagnosis results in early commencement of treatment. Clinical diagnosis is based on the accuracy and experience of the milker and large differences exist among them (23). Forestripping is an important part of udder preparation (36), and is very helpful in detection of mastitis. Forestripping is practiced by 86% of farmers in Wisconsin (38) by 35% of farmers in the Netherlands, but by only 2% in New Zealand (27). Given the same knowledge is available internationally, these differences are striking, and regional decision making not based on evidence based medicine principles, is likely responsible (25).

Somatic cell count (SCC) is widely used to detect mastitis (45). The California Mastitis Test (CMT) is an inexpensive and easy test for detection of quarters with high SCC. While the test characteristics are far from perfect (41), the CMT is very practical and has proven its value over many years. Bacteriological culture of milk is necessary to reach a definitive diagnosis of the causative pathogen. Selective therapy, that is not using antibiotics in some clinical mastitis cases from which no bacteria or Gram-negative bacteria are recovered is practiced in some regions. In these herds, milk samples are cultured before treatment to save costs and to prevent unnecessary use of antibiotics. Time is an important factor here, and on-farm culture systems reduce the interval between diagnosis and treatment (8). The identification of pathogens before treatment may guide selection of antibiotics and duration of treatment, but the selection of antibiotics based on sensitivity patterns appears poorly correlated with outcomes (15).

Do’s
1) Early and sensitive diagnosis is a crucial part of mastitis management, with clinical diagnosis as an important starting point. High sensitivity of clinical mastitis diagnosis requires training and adequate light intensity. For example, if you are unable to read a newspaper in the milking parlour, you either need to wear glasses during milking or it is too dark.
2) Stimulate an accurate and timely recording of clinical mastitis cases. These data are a very valuable monitoring tool. Encourage the recording of severity scores for each clinical case as this distribution can be an important indicator of the timeliness of diagnosis.
3) Evaluate the bacteriological status of a herd on a regular basis. Ideally all clinical mastitis cases are cultured to optimize treatment of individual cows. Alternatively, collect samples of all cases of clinical mastitis, label and freeze them. Samples can then be cultured as required. The distribution of bacterial pathogens should be assessed at least annually and as a
minimum, quarter samples from at least 10 high SCC cows and 10 clinical mastitis cows should be cultured.

Don’ts
1) It is unwise to use a single SCC result as a source for diagnosis at cow level, due to daily variability (Figure 1). At the herd level, distributions of single data points are very informative (45).

2) Don’t ignore clinical symptoms as even the smallest clot is a signal that something is wrong. Record it and take appropriate action.

3) Take care in using antibiotic sensitivity patterns to predict cure. The relation between in vitro and in vivo findings is sometimes weak, especially in (chronic) Staph. aureus infections (15).

Opportunities
1) Undertake an annual review of the udder health status of herds and set goals for the next year. Estimates of the economic impact of mastitis on the herd can be used to motivate and to prioritize. Set realistic goals for SCC and clinical mastitis based on the results of the previous year. Follow this up with an action plan that includes timelines and assigns responsibility. Review all bacteriological results from across the year. Often these data of individual cows are available, but they are rarely organized and analysed at the herd level.

2) Simple methods like CMT can be helpful and are often underutilized. Sometimes farmers have simply never learned to perform it correctly, which can result in distrust in the technique. Simple instruction cards (Figure 2) are available in many countries (18) and are an important aid for teaching.

3) Explain the limitations, but also the value of SCC in mastitis diagnosis. Despite its variability, SCC data (especially subsequent data of the same cow) are very informative on the udder health status. Simple plots like the ones in Figure 1, representing data of cows with different SCC patterns, can be very informative in determining the incidence of infection and help define the most prevalent pathogen types in the herd (13).

Treatment
Treatment of mastitis cases never solves udder health problems, but merely aids in control. Incorrect therapy may result in disappointing cure-rates (48), can be a source of new infections (24) and some data suggests high usage of antibiotics, especially penicillin and pirlimycin, is associated with prevalence of resistance (35). Correct antimicrobial therapy is important, but cow and pathogen factors also affect outcomes, as has been described for Strep. uberis (29) and Staph. aureus (2).

The first step to optimize treatments is making rational decisions and developing standard herd-level treatment protocols for clinical mastitis and for dry cow treatment. On-farm protocols may use clinical signs such as general impression of the cow, fever, colour and texture of the milk, to categorise cases and select case-specific therapy. No more than three of four different treatments should be advised, to minimize errors due to excess complexity. Levels of severity might be:

– Local only, e.g. clots in milk
– Diseased cow (fever)
– Very sick cow

The specific treatments recommended depend on available products, price and quality of these products, and earlier culture results. Although there is some evidence that in vitro sensitivity testing is a predictor of therapy outcome (33) other studies have not confirmed this finding (15). The farmers experience needs to be considered, to ensure good compliance with the protocol.

Decisions about dry-cow therapy need to be made at the cow level. Dry cow therapy aims to cure existing infections and to prevent new infections. ‘Selective’ dry cow therapy (i.e. treatment of a subset of cows based on elevated SCC and/or a history of clinical mastitis) seems economically attractive (17). However, as the preventative benefits are lost in the untreated cows, there is an increased risk of new infections over the dry period, which can lead to more clinical mastitis cases during the dry period (44) as well as during the subsequent lactation (10). Use of an internal teat-sealant improves prevention of new infections during the dry period of cows (39), as well as in heifers (34). The cure-rate of intramammary infections depends on therapy, pathogen and cow factors (2). Historically, most attention has been directed at therapy. Cure-rates, however, are highly dependent on the causative pathogen: Strep. agalactiae for instance is easier to treat (50) than Staph. aureus (47). Also, the ‘cow’ effect seems underestimated. Factors like age, SCC and number of quarters infected play an important role, as well as bacterial colony counts in milk, and duration of infection (2).

Do’s
1) Ensure cows treated with antibiotics are identified. Commonly antibiotics present in bulk tank milk are due to a dry cow treatment being put in the wrong cow or a clinical mastitis cow being milked within her withdrawal time. A standard rule should be to first mark the cow, and then treat her.

2) Develop a standard treatment schedule for clinical mastitis and dry cow treatments. Most farmers appreciate that

Figure 2. Instructive pictures to explain how to – correctly – execute the CMT. 1. Work with clean material. 2. Express a few squirts of milk and discard. 3. Express two squirts of milk from each quarter into separate compartments. 4. Poor excess of milk out, until the indicated level is reached. 5. Add an equal volume of test liquid and rotate for a minute. 6. Read the result by checking thickening of the liquid (See colour picture at the B3 page).
type of advice (22). To develop such a protocol, try to find out what they are doing already, instead of telling them what they ought to do. This will enable you to learn a lot about how farmers treat their cows. Make a clear, simple and readable schedule, and ensure it hangs in a highly visible place.

3) When subclinical infections have to be treated during lactation, attention should be given to suitability of a cow to be treated instead of focusing on suitability of treatments only. For *Staph. aureus*, cure-rate data have been evaluated in detail (47, 48). Treatment of young animals is often justified, while treatment of chronic infections in older animals often leads to unnecessary and inefficient use of antibiotics. For other pathogens comparable analyses are not available to the authors’ knowledge, but a comparable approach seems obvious.

**Don’ts**

1) Don’t change a treatment if it doesn’t seem to work after one day. Duration of therapy is an important determinant of cure (2). If the optimal treatment has been chosen, persevere with it, rather than changing therapy every second day.

2) Don’t give high SCC or antibiotic milk to calves. Discard milk from sick, high SCC, or antibiotic-treated cows is often used as an economical alternative to milk replacer. This poses a health risk to calves. If calves are housed in groups, cross suckling of teats may spread bacteria introduced in discarded milk. Milk from cows with Mycoplasma mastitis can cause pneumonia, otitis media or arthritis in calves (5). A strong association between high SCC and antibodies to *Mycobacterium avium ssp. paratuberculosis* has been demonstrated highlighting a potentially increased risk of transmission of this pathogen when milk with high SCC is fed to calves (1).

3) Milk letdown is a complex mechanism that should not be disturbed by unexpected actions (36). Although never scientifically proven, it seems likely that stress may have negative effects on milk quality due to depressed host resistance of cows. Hence the milking routine should be quiet, careful and consistent. Don’t inject cows in the milking parlour, ensure cows can be restrained for treatment after milking outside the parlour (18).

**Opportunities**

1) Audit the medicine stock of your clients regularly. Warn your client of your intention, which hopefully will inspire a rationalisation of the medicines or at least an understanding that monitoring is occurring. The first inspection may reveal bottles suitable for the museum. Minimising the costs due to expired medicines can be achieved by explaining basic principles like ‘first in first out’.

2) Evaluate results of treatment. In most dairies individual SCC are available on a regular basis. A standard treatment schedule should be evaluated at least annually. Evaluation should be done by gauging the farmers’ satisfaction and by evaluating SCC patterns after treatments and after calving. Additionally, results of bacteriological cultures (before and after treatment) may be evaluated. Be aware of the earlier discussed ‘cow effect’; it is not only the therapy that is being evaluated. Other outcomes, like days shipping milk or culling within 60 days of the case can be calculated and seem to be received well by farmers.

3) Check how much drug remains in intramammary tubes after treatment. Simply taking tubes out of the trash and check how much antibiotic is left in them can lead to surprising results.

**Prevention**

Although much time is spent by clinicians on diagnosis and advice on treatment, more effect on udder health is to be expected from preventive measures. A whole herd approach has proven to be successful for subclinical as well as clinical mastitis (11). The well known five point plan consists of: milking technique and milking machine; postmilking teat disinfection; treating clinical mastitis; drying off all cows with antibiotics; and culling chronically infected cows. The schedule has been updated in recent years as the National Mastitis Council 10 point plan, which adds: establishments of goals for udder health; maintenance of a clean, dry and comfortable environment; good record keeping; maintenance of biosecurity; and regular monitoring of udder health status. It is beyond the scope of this paper to discuss all possible preventive measures in detail, the key topics of milking, environment, and host resistance will be discussed.

The milking-procedure has a big influence on udder health and directing or participating in training of the milkers can be an important role for the veterinary practitioner. In larger herds, frequency of training milkers has been related to both efficiency of milking and the rate of clinical mastitis (38). Milking starts in the cow collecting yard. With twice-daily milking, cows should have at most one hour waiting time at each milking (18). Cows entering the milking parlour should be as clean as possible. Pretreatment is crucial for milk let down, and 60 – 90 seconds should elapse between the beginning of pre-treatment and cluster attachment (36). Prevent blind-milking. Many automatic detaching devices are activated when milk-flow falls below 200 grams/min or less. With normal variation this may lead to over milking. Detaching clusters at 400 grams/min or even higher flow rates improves udder health (3). Scoring teat condition gives a good impression of the functionality of the milking machine and procedure. Many articles have been published on this subject, recently summarized by Ohnstad et al. (31).

The effect of cow comfort and housing hygiene on udder health has been proven in several studies (20). A significant relation exists between SCC and leg and udder hygiene scores (43). In an English study performed in herds with low bulk milk SCC, the frequency of mucking out straw yards and the percentage of cows leaking milk outside the parlour were correlated with clinical mastitis (32). When cows are overstocked (>100%), they are more likely to be displaced from stalls, there is more competition and lying time for individual cows is reduced (6).

Host resistance, is crucial in maintaining udder health and will be covered in detail by others at this meeting (4, 9). Apart from external factors like stress, and other diseases such as BVD or lameness, nutrition influences host resistance, especially during the dry period. Although first test day milk yield and the fat to protein ratio were found to be more reliable indicators of disease than body condition score (14), these data are not available during the dry period. In a study in 52 UK herds, routine body condition scoring at drying off was found to be associated with a reduced rate of clinical mastitis (12). Thus both, milk yield and body condition score should be monitored. The primary goal is to prevent negative energy balance in transition cows, as well as deficiencies of vitamins or minerals, specifically vitamin E and A (37), Se (16) and Cu (42).

**Do’s**

1) Prevention is crucial, put it on the agenda. Even if farmers don’t experience a problem, but it is obvious that they take unnecessary risks, tell them. In a Dutch study only 24% of practitioners said they took action in such a situation, so there seems something to gain here (19). If farmers accept your advice, you prevent problems and that is what you are an advisor for. If they don’t accept your advice, it may be that later on you are happy that you warned them.

2) Communication is not simple, especially if farmers aren’t experiencing a problem. Many practitioners tend to focus on explaining the technical background of their advice, trying to
convince clients of the importance of it (26). This is the correct approach for ‘assimilators’, people who learn by sorting information logically. This, however, is only one of four learning styles described by Kolb (21). Kolbs’ theory holds that an individuals’ ability to learn will be enhanced by strategies that conform to the individuals’ preferred learning style. If you really want to change things, offer the knowledge in a different way to different people, try different approaches.

2) Don’t underestimate the knowledge of your clients, but don’t overestimate it either. Although some things look logical to you as an advisor, they may not be so obvious from the perspective of the farmer. For you it may seem logical that a teat dip container is cleaned daily. If you don’t talk about it, some people will just not think about it. Some farmers also lack comparison, they see their own farm and herd on a day to day basis, and sometimes fail to see disorders.

3) Don’t give too much advice. Focus on the issue that is most important to the farmer at that time. A farmer can only handle a limited number of recommendations at the same time. Be sure to select the most important points and try to make them executed, don’t try to change the world in one day.

Opportunities

1) There are many ways to approach udder health on farms, study-groups are one of them. Problem solving generally is best done on a one to one basis, on farm. Talking about prevention, further improvements or ways to make management easier, however, can also be discussed in study groups. Positive results were experienced in on-farm study groups in Denmark (49) and in the Netherlands (25). One of the key-factors in this is that farmers can exchange knowledge and experiences, often accepting advice from colleagues earlier than from an advisor.

2) A visit to the farm during milking-time is critical to see what really happens, and what the effect of that management is. You can judge teats (31), monitor milking procedure, check on the effect of post milking teat disinfection, and so on. For example, teat spraying is often ineffective with only partial coverage of the teat occurring. By wrapping a paper towel round the teat, you can visualize the effect of this action in a very evident way (18).

3) Following the idea of the NMC udder health awards, the Dutch udder health program (www.ugcn.nl) awarded farmers on udder health at a national base. Some local practitioners in the Netherlands did the same thing and gave a prize to their client with the best udder health. Doing that has a number of potential advantages, of which a positive marketing effect may be one.

Discussion

Mastitis is a multifactorial disease, and management can go wrong in many places. To manage the disease, a check-list is required. For years that has been the five-point plan, nowadays the NMC ten-point plan. Nutrition should always be an important part of such a check-list. To really improve udder health a plan should be made. A unified approach is required within a practice so that all clients receive a consistent advice from all representatives of the practice. That requires planning, often unrelated with technical issues, and more with organisational aspects. If there is disagreement among colleagues about technical issues, these need to be resolved via discussion and a consensus reached. If colleagues can’t agree, how can consistent, reliable and believable advice be given to your clients?

Some farmers indeed are not motivated to improve udder health, even after different communication approaches have been tried. But these approaches have to be tried, to make most of your practice. As an advisor, you need to find the right moment to raise udder health issues (19). If you wait until they discover it themselves, little will happen and your main work will be to control damage. Good veterinary practice for udder health is doing the right things when asked, reaching good diagnoses on a cow or herd level, providing the optimal treatment, restricting unnecessary antibiotic use, and giving advice on how to prevent problems reoccurring. Good veterinary practice, also involves showing possible improvements to farmers that they aren’t (yet) aware of. Discuss different opportunities to improve management and take action to get mastitis on the agenda. That is the more pro-active approach, which improves udder health and increases your business.

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How transcriptomic studies may help to improve the control of bovine diseases: an example with calf pneumonia and endotoxemia

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ABSTRACT
Global change in genes expression induced by pathological processes can now be analysed in cattle by new tools called microarrays. These transcriptomic studies may help to better understand the pathophysiological mechanisms responsible for the disease and may therefore contribute to develop more efficient preventive and curative strategies. An example is given with a model of calf pneumonia and endotoxemia.

KEYWORDS
Microarray, transcriptomic, pneumonia, endotoxemia, bovine

INTRODUCTION
In cattle production, a wide variety of acute and chronic pathologies are economically important for production and profitability. Mastitis and bovine respiratory complex are two of the most important problems facing respectively milk and beef industry. To prevent the economic losses associated with these diseases, we must enhance the understanding of their respective physiopathology.

Different models have been developed for many infectious diseases and these models have been used to investigate host–pathogen interactions and immune responses in cattle. However a paucity of research tools in cattle has limited the molecular characterisation of disease pathogenesis and host response to pathogens (25). With the sequencing of the bovine genome, a diversity of biological and molecular techniques hold substantial promise for elucidating new molecular mechanisms involved in disease pathogenesis.

Gene expression, transcriptomic studies and microarrays
A variety of tools have been developed over the last decade to facilitate large-scale analyses of gene expression at the level of individual cells, tissues, or whole organisms. The most commonly used tools at the present time include oligonucleotide microarrays (7), cDNA microarrays (15), and serial analysis of gene expression (21).

Microarrays (biopuces) have become an important research tool for life science researchers to investigate normal physiology (4) and disease pathogenesis (26). cDNA microarrays are able of profiling gene expression patterns (ie transcriptomic analysis) of tens and thousands of genes in a single experiment and it has been validated by the scientific community (4). With only a few exceptions, every cell of a bovine contains a full set of 30 chromosomes and identical genes. Only a fraction of these genes are turned on, however, and it is the subset that is “expressed” that confers unique properties to each cell type. “Gene expression” is the term used to describe the transcription of the information contained within the DNA, the repository of genetic information into messenger RNA (mRNA) molecules that are then translated into the proteins that perform most of the critical functions of cells. Gene expression is a highly complex and tightly regulated process that allows a cell to respond dynamically both to environmental stimuli and to its own changing needs (14). Microarray is a powerful screening tool to identify the key gene expression patterns that are influenced by environment, disease, treatment or any process of tissue development. Compared to PCR technology, the power of this technology derives from its ability to measure simultaneously mRNA levels across thousands of genes and therefore to be able to identify specific profiles of expression and interactions between genes (19).

Therefore, microarrays should facilitate the identification of host responses that are conserved among pathogens and help identify those responses unique to each pathogen.

To date, relatively small number of bovine microarray studies have been published during the last years. These investigations used a variety of approaches to analyze gene expression in a wide variety of tissues, including PBMC (5, 6, 18), macrophages (23), placenta (1), mammary gland (17, 18), and liver cells (9). Microarray analyses of gene expression were used primarily to address a variety of questions relating to normal physiological processes, such as cell differentiation, pregnancy, lactation, and parturition.

The purpose of the present study was to identify the gene expression profile induced by a therapeutic test during Mannheimia haemolytica-induced pneumonia and endotoxemia in calves.

Gene expression profile induced by a therapeutic test during Mannheimia haemolytica-induced pneumonia and endotoxemia in calves
Bovine respiratory disease complex remains the most economically important disease affecting feedlot cattle (8). The most common bacterial agent isolated from fatal cases of this complex is Mannheimia haemolytica serotype A1. During the infection, Mannheimia haemolytica releases a wide variety of virulence factors including leukotoxin A (LktA) and endotoxin (LPS) which induce the secretion of different pro-inflammatory cytokines and chemotactic proteins. These are implicated in the attraction of neutrophils and in the shock observed in severe cases of mannheimiosis (27).

Because of the implication of the inflammatory response in the early stage of the disease, the use of non-steroidal anti-inflammatory drugs (NSAIDs) of second generation has been recommended in the treatment of this complex, in addition to the antibiotherapy (3, 11, 12).

An example of the use of microarrays in bovine medicine is the study of the host response to a NSAID treatment in Mannheimia haemolytica-induced pneumonia and endotoxemia (22). Briefly, 12 Holstein calves were inoculated with a large dose of Mannheimia haemolytica and toxins. One hour later, similar clinical signs (depression, tachypnea) were observed in all calves. At this time, 6 calves were treated by intravenous route with a commercial NSAID whereas the six remaining calves received a placebo. The clinical parameters were measured at 3 and 7 hours post-inoculation (PI).

At 7 hours PI, a significant aggravation of the disease score was observed in the control group while the clinical signs were stabilised in the treated group. The main difference observed at T7 between the two groups was due to the signs of severe...
depression and dyspnea in the control group. Associated to these clinical observations, pulmonary lesions were consistently less severe in the treated group.

During the same experiment, venous blood was collected at T+3 and T+7 to perform a microarray study. After euthanasia (T+7), liver samples from each calf were also sampled to study differential gene expression between control and NSAIDs-treated calves. To minimise technical variability, the successive RNA processing steps (RNA extraction, probe labelling and chip hybridisation) were performed simultaneously for control and treated calves samples. The hybridisation of RNA samples was made on an Affymetrix GeneChip Bovine Genome Array. Data were extracted using Affymetrix Microarray Analysis Suite (MAS) 5 software and filtered to exclude genes that were not expressed, i.e. taken to be equivalent to a MAS5 absent call. Of the 23,000 oligonucleotide probe pair sets contained on this bovine microarray, 14 genes were identified as differentially highly regulated (fold change > 4) between NSAIDs-treated calves and controls in nucleated blood cells at T7. These variations notably concerned several genes implicated in the inflammatory response, ie CXC-chemokine type 6 (CXCL6), T cell receptor (BVG3.1, BVd1.17), or Interferon-stimulated protein (ISG15). At the same time, gene expression in liver cells showed a high significant difference for 19 genes. In these, 5 were up-regulated in the NSAIDs group whereas 14 were down regulated. The treatment was associated with a significant decrease of many inflammatory gene’s expression (CXCL1, CXCL2, CXCL6, CCL2, CCL20, IL6, ILS and selectin) and also genes implicated in the coagulation like plasminogen activator inhibitor-1 and -2. Cyclooxygenase 2 (COX2) expression was also strongly down-regulated in the liver cells of treated calves.

In mouse and rat models, inflammatory genes have been implicated in the chemoattraction of neutrophils in the liver and in the initiation of pneumonia and endotoxemic shock (10, 13, 20). These reductions could explain at least in part the differences observed between the two groups in the clinical and pathological pictures. Moreover, plasminogen activator inhibitor-1 and -2 are implicated in the disseminated intravascular coagulation (DIC) occurring in gram-negative endotoxemia. The PAI-1 and 2 inhibit the activation of the plasminogen into plasmin, an important enzyme present in blood that degrades notably fibrin clots. Indeed, enhancing mammary-specific transcripts. Physiol. Genomics, 2003. 16. 467–470.

CONCLUSION
In this experiment the use of a specific microarray has allowed to better understand the molecular mechanisms mediating therapeutic effects of a NSAID in an experimental model of pneumonia and endotoxemia. Such transcriptomic study could therefore contribute to improve the treatment or prevention of a wide variety of diseases in cattle.

REFERENCES


**The multifactorial approach to fertility problems in dairy herds**

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**ABSTRACT**

Routine health reports based on epidemiological models are today a common tool used by farmers, veterinarians and nutritionists. The multifactorial approach to fertility problems, which includes routine monitoring and causal analysis, is described. The risk factors evaluated in practice and presently discussed are calving diseases, unobserved heat, negative energy balance in early lactation, the lengths of the rest period and the dry period respectively, body condition score, summer effects, high somatic cell counts, and the cycles’ distribution. The author believes that future improvement of fertility in practice through data analysis will be possible in three main fields a) improvement of the quality of data through automation; b) development of multidisciplinary models including economical evaluations and c) improvement of analytic methods applied to small herds.

**KEY WORDS**

Herd Health, Fertility, Dairy cows

Feeding for efficient milk production leads in modern dairy practice. Efforts to maintain production and fertility at optimal levels under given market, husbandry and feeding conditions, often fail. Yet, financial losses for an “open day” are estimated in various studies to be 2.5 to 5.0 US$.

The “Double Blind” (PG) versus the “Multifactorial Approach” to fertility problems

Like most production and infectious diseases and traits fertility problems are multifactorial as illustrated in Figure 1 that evaluates risk factors responsible for poor fertility in 144 Israeli herds in 1996.

**Monitoring fertility**

As in other areas of herd health, we monitor fertility periodically; data are exported from the farm’s computer. The monitoring report alerts against any fall from preset targets, and is short, concise, and issued at regular times. Targets (the best quartile of Israeli herds, updated every year) are used as a challenge for farmers. Monitoring fertility of our Sample Herd is in Table 1.

Causal analysis of infertility

We evaluate routinely factors contributing to lower fertility indices (not pregnant to first service, open ≥150 days from calving) either too short or too long; **SUMMER**=Summer calvings; **BCS**=Either too fat or too thin at calving; **DRY**=With dry periods either too short or too long

If fertility is a multifactorial entity and involves various disciplines, a “**multifactorial approach**” is called for. This approach is in contrast to others advocated elsewhere (2); in fact, the choice is between “The Double Blind (PG) versus the Multifactorial Approach to fertility problems”. Details of the multifactorial approach and the routine veterinary work in dairy herds advocated by the author, and practiced in Israel since the early 1990th are found elsewhere (5), some major points will be described presently. The data are, unless otherwise stated, from 3620 lactations of primiparous cows and 5757 lactations of multiparous cows, all calving in the period 01/95-06/98 in 7 herds from the author’s own practice (Nir-Galon Set) and from Hachaklait Herd Health reports issued in 2007 (Herd Health Set). All the results presented are the outcome of logistic or linear regression models. If not stated otherwise, effects of herds, years, parity and season are allowed for in all models, only results with statistical significance of p<0.05 are shown.

**PPUD**=Calving diseases; **ANEST**=Unobserved heat; **YIELD**=High yield (FCM) before service; **REST**=Rest period, **SUMMER**=Summer calvings; **BCS**=Either too fat or too thin at calving; **DRY**=With dry periods either too short or too long

![Figure 1. Factors responsible for lower fertility (% 144 Israeli herds In 1996)](image-url)
Pre service unobserved heat

Pre service unobserved heat has an adverse effect on fertility in most herds. Unobserved heat can result from poor management, nutritional factors, various calving diseases, feet problems, and the “cow factors”. We found risk of recurrence for inactive ovaries to be 1.8 (4).

We evaluated the various effects on unobserved heat and on ovarian inactivity respectively (Table 4). Respective rates of unobserved heat and ovarian inactivity were 36.2% and 10.1% for primiparous and 42.5% and 10.3% for multiparous cows.

Based on our studies we summed the epidemiology of inactive ovaries in Figure 2.

**Negative energy balance (NEB)**

Negative energy balance (NEB) after calving due to rising production and the increasing risk for a “negative selection” associated with it, is present a major risk for the industry. The effects of a NEB on fertility can be either direct on the pregnancy rate, or indirect through its effect on anestrus.

A change in the body score, which reflects fat mobilization to get energy balance, is the “Gold Standard” used to evaluate NEB in the field. In our data set mean losses of BCS from calving to 40 – 60 DIM were 0.57±0.44 u BCS for first, and 0.64±0.49 u BCS for older lactations respectively. Cows of higher parities, those with longer dry period, heavier at calving, after calving diseases, and with higher peaks lost more BCS before AI.

We compared the associations of fertility indices with some measures we use in the field for evaluation of NEB (Table 5). Respective rates of unobserved heat, failure to conceive to first AI service, and being open >150 DIM were 37.3%, 61.6%, and 26.5%. The associations between losses of BCS from calving to AI, and the ratio between the fat to protein ratios in the test

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### Table 1. Reproduction Monitoring Report, Sample Herd. Calving period 11/06-10/07

<table>
<thead>
<tr>
<th>Reproduction Factor</th>
<th>Primipara 177</th>
<th>Multipara 356</th>
<th>Heifers 222</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. % Not inseminated by 150 DIM</td>
<td>5.1 (10.0)</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>c. Loss of BCS from calving to 1st service, n</td>
<td>161</td>
<td>292</td>
<td></td>
</tr>
<tr>
<td>% lost ≥0.5 u</td>
<td>45.3 (40.0)</td>
<td>54.8 (40.0)</td>
<td></td>
</tr>
<tr>
<td>d. % Unobserved heat</td>
<td>17.1 (10.5)</td>
<td>20.8 (26.6)</td>
<td>27.8 (31.3)</td>
</tr>
<tr>
<td>e. % Inactive ovaries</td>
<td>2.3 (0.5)</td>
<td>10.1 (5.9)</td>
<td>11.5 (6.9)</td>
</tr>
<tr>
<td>f. Mean rest period (days)</td>
<td>14.8</td>
<td>112.0</td>
<td>105.0</td>
</tr>
<tr>
<td>g. % Pregnant to 1st service</td>
<td>68.9 (69.5)</td>
<td>37.5 (47.4)</td>
<td>32.9 (38.2)</td>
</tr>
<tr>
<td>h. % Open &gt;150 DIM</td>
<td>5.4 (1.0)</td>
<td>45.8 (31.6)</td>
<td>46.2 (36.7)</td>
</tr>
<tr>
<td>i. Mean open days (150 days limit)*</td>
<td>132 (117)</td>
<td>129 (116)</td>
<td></td>
</tr>
<tr>
<td>j. Cycles distribution (% in days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) Total</td>
<td>92</td>
<td>290</td>
<td>430</td>
</tr>
<tr>
<td>2) Short cycles, 5-17 d</td>
<td>10 (3)</td>
<td>4 (3)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>3) Medium cycles, 18-24 d</td>
<td>54 (77)</td>
<td>64 (72)</td>
<td>59 (66)</td>
</tr>
<tr>
<td>4) Long cycles, 25-36 d</td>
<td>9 (6)</td>
<td>10 (9)</td>
<td>13 (12)</td>
</tr>
<tr>
<td>5) Double cycles, 36-60 d</td>
<td>27 (14)</td>
<td>22 (16)</td>
<td>24 (17)</td>
</tr>
</tbody>
</table>

### Table 2. Factors responsible for lower fertility in second lactation cows, Sample Herd

<table>
<thead>
<tr>
<th>Factor</th>
<th>Value</th>
<th>Second lactation</th>
<th>Primipara 46.7</th>
<th>Multi parata 129</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n 121</td>
<td>% pregnant 33.1</td>
<td>% pregnant 33.1</td>
<td>% pregnant 33.1</td>
</tr>
<tr>
<td></td>
<td>With-out</td>
<td>With-out</td>
<td>With-out</td>
<td>With-out</td>
</tr>
<tr>
<td>Unobserved heat</td>
<td>32 (89)</td>
<td>62.5 (40.9*)</td>
<td>141 (122***)</td>
<td></td>
</tr>
<tr>
<td>Short rest periods</td>
<td>39 (82)</td>
<td>28.6 (57.7*)</td>
<td>112 (136)***</td>
<td></td>
</tr>
<tr>
<td>Summer calvings</td>
<td>50 (71)</td>
<td>62.0 (35.7*)</td>
<td>133 (122*)</td>
<td></td>
</tr>
<tr>
<td>Low BCS at calving</td>
<td>44 (76)</td>
<td>22.7 (39.5†)</td>
<td>139 (123†)</td>
<td></td>
</tr>
<tr>
<td>Lost ≥0.5 u BCS in the dry period</td>
<td>28 (88)</td>
<td>17.9 (38.6*)</td>
<td>139 (123*)</td>
<td></td>
</tr>
<tr>
<td>Negative Energy Balance at calving</td>
<td>1,293 (70)</td>
<td>25.0 (40.0*)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.1; *p<0.05; **p<0.01; †Lowest (shortest) or highest (longest) thirds
days following and preceding the first AI respectively proved to be the strongest and similar (7).

**Cycles' distribution**

Pregnancy rates are affected by the length of the cycle (3) those after regular (medium) cycles are higher. The “cycles” in our analysis are the inter-inseminations interval, their classification and the respective goals (the means of the best quarters of the Israeli herds) are in our reports (Table 1); the high rates of medium (“regular”) cycles are to an extent a manifestation of the common use of pedometers in those herds.

Studying the effects of various factors on the first two cycles, we established, somewhat in contrast to traditional explanations, valid statistical associations of long & double cycles with the long dry periods, calving diseases, negative energy balance after calving, unobserved heat, parity, season, and location of cows. Table 6 and describes the distribution of the cycles in one herd (IH#2).

Applying a logistic regression model we were able to establish (Figure 3) that in herd IL#2 most of the double cycles were due to “cow” factors and not to poor heat detection.

As the sensitivity and accuracy of heat detection by pedometers, and by all other methods of heat detection, are largely affected by the level of the threshold, it is possible to apply different thresholds to various groups of cows.

**The voluntary rest period**

Mostly, but not always, first service pregnancy rate improves with time from calving. This effect is in contrast to that on the open period, odds ratio not being pregnant to first service was 0.9 (p<0.01) and an estimate of an additional 5.1 days open

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### Table 3. The association of fertility traits with calving diseases and traits (3620 and 5757 lactations of first and second or more lactations respectively in 7 herds for cows calving 01/95 through 06/98).

<table>
<thead>
<tr>
<th>Lactation</th>
<th>Rate (%)</th>
<th>Unobserved heat</th>
<th>Inactive ovaries</th>
<th>Not pregnant to first AI</th>
<th>Open &gt;150 days</th>
<th>Rest Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>6.0</td>
<td>1.6**</td>
<td>2.3**</td>
<td>1.3*</td>
<td>1.4*</td>
<td>1.4*</td>
</tr>
<tr>
<td>2nd</td>
<td>1.6**</td>
<td>1.4*</td>
<td>2.3**</td>
<td>1.3*</td>
<td>1.4*</td>
<td>1.4*</td>
</tr>
<tr>
<td>1st</td>
<td>3.9</td>
<td>0.6**</td>
<td>4.6**</td>
<td>3.0*</td>
<td>2.8**</td>
<td>2.8**</td>
</tr>
<tr>
<td>2nd</td>
<td>3.9</td>
<td>0.6**</td>
<td>4.6**</td>
<td>3.0*</td>
<td>2.8**</td>
<td>2.8**</td>
</tr>
<tr>
<td>1st</td>
<td>0.4</td>
<td>5.3**</td>
<td>3.0*</td>
<td>2.8**</td>
<td>2.9**</td>
<td>2.9**</td>
</tr>
<tr>
<td>2nd</td>
<td>0.4</td>
<td>5.3**</td>
<td>3.0*</td>
<td>2.8**</td>
<td>2.9**</td>
<td>2.9**</td>
</tr>
<tr>
<td>1st</td>
<td>17.7</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
</tr>
<tr>
<td>2nd</td>
<td>17.7</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
</tr>
<tr>
<td>1st</td>
<td>31.3</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
</tr>
<tr>
<td>2nd</td>
<td>31.3</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
</tr>
<tr>
<td>1st</td>
<td>0.9</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
</tr>
<tr>
<td>2nd</td>
<td>0.9</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
</tr>
<tr>
<td>1st</td>
<td>6.9</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
</tr>
<tr>
<td>2nd</td>
<td>6.9</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
<td>1.5**</td>
</tr>
</tbody>
</table>

†p<0.1 *p<0.05 **p<0.01; TWIN=Multiple births; STILL=Stillbirth; MF=Milk fever; PRO=Prolapsed uterus; DA=Displaced abomasum; RP=Retained placenta; MET=Primary metritis; KET=ketosis; “Odds ratio suffering from the trait for a cow “with factor”. Estimates of additional days of Rest Period for a cow “with factor”. Effects of herds, years and summer were included; Effects of herds, years, parity and summer were included.

### Table 4. Factors responsible for unobserved heat and inactive ovaries in 3761 lactations (6 herds calving period 01/95-05/98)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Rate (%)</th>
<th>Unobserved heat</th>
<th>Inactive ovaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous cows (n=1530)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer calvings</td>
<td>30.7</td>
<td>0.4**</td>
<td></td>
</tr>
<tr>
<td>BCS at calving, units</td>
<td>3.41±0.33</td>
<td>1.3*</td>
<td></td>
</tr>
<tr>
<td>Postparturient diseases</td>
<td>56.3</td>
<td>0.7*</td>
<td></td>
</tr>
<tr>
<td>Multiparous cows (n=2231)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer calvings</td>
<td>28.4</td>
<td>1.2*</td>
<td></td>
</tr>
<tr>
<td>BCS at drying off</td>
<td>3.33±0.48</td>
<td>0.1**</td>
<td></td>
</tr>
<tr>
<td>BCS*BSC at drying off</td>
<td>1.5**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lost ≥0.5 units BCS in the dry period</td>
<td>27.7</td>
<td>1.3*</td>
<td></td>
</tr>
<tr>
<td>Gained ≥0.25 units BCS in the dry period</td>
<td>21.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daydry&lt;60 days</td>
<td>15.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daydry &gt;75 days</td>
<td>9.8</td>
<td>1.4*</td>
<td></td>
</tr>
<tr>
<td>Postparturient diseases</td>
<td>35.1</td>
<td>1.8**</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 *p<0.01

---

![Figure 2. The epidemiology of inactive ovaries](image)
(p<0.01) were associated with each additional 10 days of rest. The models also allowed for the effects of calving diseases and unobserved heat. Estimates for additional days of rest associated with various risk factors are presented in Table 7.

Recommendations for the optimal rest period for cows in specific farms are debatable; few economical models had been suggested, taking into account milk yields and persistencies, feed costs, and prices of replacements and calves.

Body condition score
We found that cows calving in a higher body condition had a better fertility. Odds ratios were also adjusted to the effects of peak yield and postparturient diseases (6). No association could be established between pregnancy rate to first service and body condition score at calving, but open period was shorter (6.3 days) for each additional 1.0 unit BCSC in primiparous cows. The only association established in multiparous cows was with inactive ovaries (odds ratio of 0.4).

The effects of body condition at calving on fertility traits in our data were mainly in the first three months after calving, and they diminished with time. The results suggest that a low BCSC is a determinant of reduced fertility mainly by delaying the onset of ovarian activity.

We used data from 4578 lactations of multiparous cows in 57 herds of the Herd Health Set (calving in 1996) to evaluate the effects of changes in BCSC during the dry period on fertility. Effects of herds, parity, season, BCSC at drying off, birth of twins, and length of dry period were included in all models. Mean of BCSC at drying off was 3.29±0.5 units SD. Respective rates of cows loosing ≥0.5 units or gaining ≥0.25 units in the dry period were 25.9% and 24.3%. Odds ratios of cows suffering from unobserved heat were 0.8 and 1.2 for cows gaining or loosing BCSC in the dry period respectively compared to those with no such changes. No associations could be established between failure to conceive to first AI service and BCSC changes, while risk being open >150 DIM was 1.3 as higher for cows loosing BCSC compared to those with no changes.

The length of the dry period
In contrast to the strong association we established previously between production and the length of the dry period, we failed to do it with the fertility variables. The only valid statistical association we established was that between unobserved heat and the length of the dry period, odds ratio of cows suffering from unobserved heat was 1.1 more for each 10 days of dry period.

Summer calvings
The negative effect of the summer on fertility under the Israeli conditions could be heavy. Although this factor reflects any effects associated with the summer, we assume that climatic effects (heat stress) are the main ones. We found from data of 109 herds in 1993, transferring 1% of the cows in the Israeli National Herd from winter to summer calving was associated with additional 17 days open per cow in the country.

Results in the individual farm should be interpreted in the light of the following considerations: a) Quotas. Many farms with extra production potential, direct cows to calve in the summer due to the season differential pricing of milk. Additional income should be weighed against loss of milk; b) Financial returns from investment in better housing, shading and cooling systems.

High somatic cell counts
It had been suggested that the phenotypic unfavorable correlated changes in lactation mean somatic cell score and conception rate at first service are associated with the genetic improvement of mature equivalent milk yield (1). Furthermore, the effects of clinical mastitis and that of high somatic cell counts (HSCC) on fertility were investigated in Israel (6). We analyzed the effects of HSCC on some fertility traits in the Nir-Galon Set. A first lactation cow with SCC >200,000 and second or more lactations cows with SCC >400,000 in at least two out of the six first milk tests were defined...
as having HSCC. Rate of HSCC was 34.3%. Odds ratios of cows being open >150 DIM was 1.20 compared to those with low SCC. The strong association we established between HSCC and calving diseases called for confounding them in all models. We later expanded the models and incorporated them in the routine herd health reports. The rates of herds with statistical significant associations between some fertility traits and HSCC in a sample of 149 herds of the Herd Health Set are described in Table 8.

**“Common” factors**

“Common” factors are the sum of the residuals, and represent unknown factors not included in the models; special designated investigations must be carried out to reveal them. Factors claimed to lower fertility include a) nutritional factors such increased protein intake, excess of rumen degradable protein, unbalanced minerals and microelements feeding and others; b) infectious diseases such as leptospirosis, IBR, BVD; and c) toxic factors such as estrogens, nitrites, and gossypol.

From manual observations to automation

More automation will lead to better data, both in quantity and in quality. Many milking systems have already automated components that replace, partly or completely, the need for manual observations (milk recording, milk conductivity, and pedometers). The future introduction of on-line progesterone analysis will allow us to cross the line from mechanical to physiological heat detection.

Body condition scoring (BCS) of dairy cows in various stages of the lactation is the most important tool used to evaluate energy balance of cows over the lactation in the field. The two major handicaps of BCS are its low objectivity and resolution (0.25 units in a scale of 1 to 5). AfiScale® is an automated scale, which is an integrated part of the AfiMilk® system. We use body weight (BW) data derived from the AfiScale® in our models, the results show that BW can replace BCS in the models evaluating the effects of NEB, not only when differences between BW in the various stages of lactation are calculated, but also when stand as a single measurement.

On-line analysis of milk fat and protein is now possible and will allow for better diagnosis, retrospective analysis and decision-making.

**CONCLUSIONS**

Routine health reports based on epidemiological models are today a common tool used by farmers, veterinarians and nutritionists in Israel and in some other countries. Though experts prepare the reports, their improving quality is the result of routine practice evolved through understanding of the multifactorial nature of modern veterinary issues. Through their postgraduate training, most practicing veterinarians are capable of reading the reports, interpreting them and implementing the conclusions in their practice. The author believes that future improvement of fertility in practice through data analysis will be possible in three main fields a) improvement of the quality of data through automation; b) development of multidisciplinary models including economical evaluations and c) improvement of analytic methods applied to small herds.

**REFERENCES**


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**Table 7.** Estimates of additional days in the rest period (3761 lactations of cows calving in 6 herds in the period 01/95-05/98) associated with various factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Primiparous cows (n=1530)</th>
<th>Multiparous cows (n=2231)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate/Mean Estimate</td>
<td>Rate/Mean Estimate</td>
</tr>
<tr>
<td>Summer calvingsc</td>
<td>30.7</td>
<td>28.4</td>
</tr>
<tr>
<td>BCS at calving, units</td>
<td>3.41±0.33</td>
<td>3.15±0.46</td>
</tr>
<tr>
<td>Postparturient diseases</td>
<td>56.3</td>
<td>35.1</td>
</tr>
<tr>
<td>BCS lost between calving and AI</td>
<td>0.58±0.44</td>
<td>0.64±0.49</td>
</tr>
<tr>
<td>Unobserved heat (active ovaries)</td>
<td>26.1</td>
<td>9.1**</td>
</tr>
<tr>
<td>Unobserved heat (inactive ovaries)</td>
<td>10.1</td>
<td>33.0**</td>
</tr>
</tbody>
</table>

**Table 8.** Herds (%) with statistical significant associations (p<0.1) between fertility traits and HSCC (39,343 lactations of cows calving in 149 herds in the period 10/06-02/07)

<table>
<thead>
<tr>
<th></th>
<th>1st lactation</th>
<th>2nd lactation</th>
<th>≥3rd lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy to first AI</td>
<td>8.1%</td>
<td>6.7%</td>
<td>9.4%</td>
</tr>
<tr>
<td>Pregnancy to first AI</td>
<td>10.7%</td>
<td>10.7%</td>
<td>18.8%</td>
</tr>
<tr>
<td>Open days</td>
<td>13.1%</td>
<td>14.1%</td>
<td>19.2%</td>
</tr>
</tbody>
</table>

**Definitions of HSCC:** SCC > 150,000 in the milk test preceding the first AI service; SCC >150,000 in either of the milk tests preceding or following the first AI service respectively; A first lactation cow with SCC >200,000 and second or more lactations cows with SCC >400,000 in at least two out of the six first milk tests.
ABSTRACT
Perinatal calf mortality rates are still unacceptably high ranging from 2 to 10% internationally, and are increasing, on dairy farms. In a recent study in a pasture-based system (Ireland) the risk factors traditionally associated with perinatal mortality were still associated with such loss (4.3%). Management strategies at the herd level and management procedures at the animal level can be implemented to improve perinatal welfare. The key features of successful newborn dairy calf management are ensuring heifers and cows are moved in time to calve in suitable maternity housing, discreet calving supervision and appropriate timing of any necessary calving assistance, immediate parturientevaluation of at-risk newborn calves followed by aggressive resuscitation, strategic navel antisepsis, early detection (and treatment) of perinatal problems and prompt movement of the newborn calf to hygienic calf housing. Veterinarian-led producer implementation of active management of calving and newborn calf care can improve perinatal welfare and health.

KEYWORDS
Perinatal calf mortality, dairy, management.

INTRODUCTION
The 3 Rules of Spring: ‘If it’s in, pull it out’, ‘If it’s out, push it in’ and ‘If it’s down, give it calcium’ (Irish veterinary practitioner).

Table 1. Prevalence of perinatal calf mortality in dairy heifers and cows internationally (16).

<table>
<thead>
<tr>
<th>Country</th>
<th>Breed of dam</th>
<th>Heifers (%)</th>
<th>Heifers and cows (%)</th>
<th>Definition of calf mortality</th>
<th>Management system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>Holstein-Friesian</td>
<td>10.8</td>
<td>5.1</td>
<td>Death within 48 hours of a singleton calving</td>
<td>Pastoral</td>
</tr>
<tr>
<td>Canada</td>
<td>Holstein-Friesian</td>
<td>9.0</td>
<td>9.6a</td>
<td>Dead at birth</td>
<td>Confinement</td>
</tr>
<tr>
<td>Denmark</td>
<td>Holstein-Friesian</td>
<td>9.0</td>
<td>NRb</td>
<td>Death within 24 hours of calving</td>
<td>Confinement</td>
</tr>
<tr>
<td>India</td>
<td>Jersey</td>
<td>NR</td>
<td>3.8</td>
<td>Foetal death</td>
<td>Pastoral</td>
</tr>
<tr>
<td>Israel</td>
<td>Holstein-Friesian</td>
<td>7.2</td>
<td>5.0</td>
<td>Death within 24 hours of calving</td>
<td>Confinement</td>
</tr>
<tr>
<td>Iran</td>
<td>Holstein-Friesian</td>
<td>4.3</td>
<td>3.5</td>
<td>Death within 1 hour of calving</td>
<td>Confinement</td>
</tr>
<tr>
<td>France</td>
<td>Holstein-Friesian &amp; Normande</td>
<td>NR</td>
<td>7.4</td>
<td>Death within 24 hours of a singleton calving</td>
<td>Pastoral</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>Holstein-Friesian</td>
<td>11.4</td>
<td>6.9</td>
<td>Death within 24 hours of a singleton calving</td>
<td>Confinement</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Holstein-Friesian, Jersey and their crosses</td>
<td>7.4</td>
<td>7.2</td>
<td>Death within 48 hours of calving excluding inductions</td>
<td>Pastoral</td>
</tr>
<tr>
<td>Norway</td>
<td>Norwegian Red</td>
<td>3.0</td>
<td>2.0</td>
<td>Death within 24 hours of calving</td>
<td>Confinement</td>
</tr>
<tr>
<td>Sweden</td>
<td>Swedish Red</td>
<td>3.6</td>
<td>2.5a</td>
<td>Death within 24 hours of a singleton calving</td>
<td>Confinement</td>
</tr>
<tr>
<td>UK</td>
<td>Holstein-Friesian</td>
<td>10.9</td>
<td>5.3</td>
<td>Death within 48 hours of a singleton calving</td>
<td>Pastoral</td>
</tr>
<tr>
<td>USA</td>
<td>Holstein-Friesian</td>
<td>12.1</td>
<td>8.0</td>
<td>Death at birth</td>
<td>Confinement</td>
</tr>
</tbody>
</table>

a cows only, b not recorded

Perinatal mortality (PM) may be defined as calf death prior to, during or within 48 hours of calving, following a gestation period of at least 260 days, irrespective of the cause of death or the circumstances of the calving. The majority of PM occurs within one hour of calving (75%) with the remainder occurring either pre- (10%) or post-partum (15%) (10). Some 90% of calves, which die in the perinatal period, were alive at the start of calving and so much of this loss is preventable.

Recent studies in Denmark, The Netherlands, North America and Sweden indicate that the prevalence of bovine PM is increasing, particularly in Holstein primiparae (17,20). Currently, the prevalence of bovine PM in cows and heifers varies between 2 and 10% across dairy industries internationally (Table 1). These average figures obscure the fact that PM follows a right skewed distribution where most herds have none or minimal losses but some herds have high (25%) mortality (16).

Traditionally, over 50% of PM has been directly attributed to dystocia (9). Other significant risk factors which have been associated with bovine PM include age at first calving, primiparity, foetal gender, gestation length and season of calving. However, there is now some evidence to suggest that an increasing proportion of PM occurs at unassisted calvings (idiopathic stillbirth or weak calf syndrome) where placental dysfunction and low birth weight

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may be causative factors (1,19). This raises the questions as to whether the risk factors conventionally associated with PM may be of greater or lesser importance now. Towards this end a European working group has been established to coordinate research into bovine stillbirth (11) part of which involves eliciting feedback from different countries on the current epidemiology of PM.

**European perinatal calf mortality status – a snapshot**

The salient findings from a questionnaire survey conducted of delegates in a recent workshop of the annual European Society of Domestic Animal Reproduction meeting in Celle, Germany in 2007 are shown in Table 2. The majority of respondents believed that PM is increasing but the majority were not clear as to why this is occurring. There was a strong view that some herds are

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>How are stillbirths recorded in your country (n=12)?</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>National breeding or milk recording organisation</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other-abattoirs</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not recorded</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has perinatal calf loss increased in your country (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t know</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Why do you think this is (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larger herds/less labour</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selection for increased milk production</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other-mineral deficiencies, reduced use of vets</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t know</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are some herds more affected than others (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Why do you think this is (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experience of herd personnel</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd size/labour availability and skill</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow breed</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of beef sires</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other-seasonal calving</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t know</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What is the average calf loss rate from birth to 24hrs in your country (n=21)?</td>
<td>2-15%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What are the most common causes of stillbirth (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dystocia</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth at unassisted calving</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other-prolonged calving, short calving but calf dead</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do farmers in your country commonly have stillborn calves necropsied (n=21)?</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What are the common lesions found following necropsy (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoxia</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital defects</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead in uterus</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other-poisons, thyroid haemorrhage, cerebral oedema.</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No specific lesions</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are mechanical calf pullers/calving jacks widely used by farmers (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What are the main underlying factors associated with stillbirths (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problems with big calves from individual sires</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heifers too small/fat at calving</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calving problems (uterine torsion, twins, milk fever, etc..)</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal calves just not surviving a normal calving</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other-stress at calving</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How can farmers best prevent stillbirths (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select sires for smaller calf size</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have heifers large enough at calving</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improve their calving techniques</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provide better nutrition during late pregnancy</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Involve their vet more</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improve their calf resuscitation techniques</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other-better calving surveillance, induce calvings</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What can veterinary practitioners do to prevent stillbirths (n=21)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educate clients, e.g. sire selection, heifer condition</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other-examine herd calving management, conduct more necropsies, assist more rapidly</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Responses from delegates in a European workshop on perinatal calf mortality.
more affected than others and this was primarily attributed to herd personnel experience and availability. Mechanical calving aids are widely used by farmers in most countries (though not officially in some) and the most common causes of PM are still dystocia and eutocic stillbirth with the former contributing the most common lesions at necropsy; though necropsies are not commonly conducted in most countries. Respondents believed that farmers can best prevent PM by judicious choice of sires and by correct heifer size at calving while they believed that veterinary practitioners should focus their efforts on educating their clients in these and other aspects of calving management. These responses indicate that more widespread application of existing knowledge by veterinarians and their clients may contribute substantially to a reduction in PM. These additional comments demonstrate the wisdom of experienced practitioners; ‘many farmers do not understand the power of calving jacks...also many vets!’, ‘it is easier to change younger farmers’ ideas/protocols’, ‘unclear stillbirth cases are increasing’, ‘think as a mother...let women do the calvings!’.

**Perinatal mortality in a pasture-based management system**

Many of the recently published studies on bovine PM have been conducted in confinement systems where nutrition, housing, genetics, disease risk and stock management are quite unlike the pasture-based management systems. Hence, a study was carried out recently to benchmark the national prevalence of PM (calf death within 24 hours of calving) in heifers and cows on commercial dairy farms in pasture-based management systems in Ireland and to determine the current significance of putative exposures and attributes (16). A total of 182,026 records of full-term calvings from Holstein-Friesian dams served by AI sires of seven breeds in herds of 20 calvings or more per year were available from the Irish national breeding database over four years (2002-2005). The prevalence of PM was 4.29% (7.7% in primiparae and 3.5% in pluriparae). The likelihood of PM increased between 2002 and 2005. There was an interaction (P<0.001) between the effect of calving assistance and parity with the effect of dystocia on PM being greater in primiparae. The odds of PM were greater in male (OR=1.12, P<0.001) and in twin calves (OR=5.70-13.36, P<0.001) and in dams that had PM at the previous calving (OR= 4.21, P<0.001). The logit of the probability of PM increased by 0.099 per unit increase in sire predicted transmitting ability (PTA) for direct PM. The probability of PM increased at an increasing rate in primiparae as animals calved at a younger age relative to the median age at first calving. The only herd-level factor examined, herd size did not affect the odds of PM. These data indicate that the prevalence of PM in this cattle population is similar to that in other pasture-based dairy systems worldwide. The putative exposures and attributes traditionally associated with PM were associated with PM in this dairy cow population. The practical implication of these results is that as many of the significant risk factors are largely not under management control (year of calving, month of calving, twin calving, primiparity, previous PM, and foetal gender) herdowners must focus on the significant determinants under their control [age at first calving, sire genetic merit for direct PM (Figure 1) and both the extent of calving supervision and the degree of assistance].

**Management to prevent perinatal mortality**

Management of the newborn dairy calf is best achieved through implementation of simple protocols which document the correct strategies to be followed at the herd level and the correct procedures to be carried out at the individual animal level (13). These protocols cover management of the prepartum cow, management of calving (monitoring of eutocia and detection and management of dystocia) and newborn calf care. Discussion with producers about newborn calf problems or care represents a contact moment which veterinarians should utilize to expand their role in veterinarian-led dairy herd reproductive management (12).

**Management of the cow at calving**

Phenotypic dystocia rates are increasing internationally (14) with currently 40% of heifers and 20% of cows assisted on US dairies (5). Recent results suggest that it is less detrimental (reduced assistance, dystocia and stillbirth rates) to move animals into the maternity unit which have already commenced calving (stage two) than it is to move animals which are about to start calving (stage one) (4). Addressing the question of whether to intervene during calving, intervention is recommended in cases of: foeto-pelvic incompatibility (FPI), maldisposition, twinning, uterine inertia and vulvar or cervical stenosis. Addressing the question of when to intervene, early intervention is recommended during stage one for uterine inertia, and during stage two, for maldisposition and twinning. Delayed intervention is recommended, during stage two, for FPI and cases of vulvar or cervical stenosis (15). The importance of progress, rather than clock-watching during stage two is emphasised, as the onset of stage two is usually unknown, however this may change with developments in calving monitors (3) or possibly with nutritional strategies (6). When the dam is first detected in stage two an exploratory examination should be conducted. This includes cow health (milk fever, mastitis), the integrity and contents of the amniotic sac, the disposition, vigour and size of the calf, the
degree of dilation of the vagina and vulva and an assessment of how long the cow has been in stage two of calving. A vital calf will have strong interdigital, bulbar, lingual, swallowing and anal reflexes. With increasing degree of acidoses, failure to show the interdigital reflex will precede failure to show the bulbar and swallowing reflexes. If the amnion or foetal legs are dry and cold, the cow has been calving at least 30 to 60 minutes. If indentations from the calf’s incisors are visible on the lower surface of a swollen upturned purple tongue, the calf has been stuck at the vulva for at least 3 hours. Signs of progress during stage two include a recumbent dam straining intermittently but strongly, with occasional breaks while she stands up and lies down again and progressive emergence of the foetal legs and head through the vulva. It is normal for the greatest delay in delivery of the foetus to occur once the muzzle and forehead have emerged, but the eyes are not yet visible. Once progress is normal, discrete monitoring without disturbance every 30 minutes, or continuously if patience can be assured, is recommended. Intervention should not be carried out before the calf’s muzzle has emerged and not before the calf’s fetlocks are visible. As a general rule, if ropes have to be placed on the calf’s legs in the vagina, intervention is too early. When progress ceases over 30 minutes or the calf begins to exhibit signs of reduced vigour (such as capital or lingual oedema, buccal or lingual cyanosis, scleral haemorrhages or reduced responsiveness to stimulation) intervention should be conducted. When severe acidosis can be traced back to stage two of relatively short duration, rapid improvement can be achieved by resuscitative care. When acidosis exists over a longer period, as in delayed assistance, the efficacy of supportive care is lower as hypoxic lesions such as meningeal, subepicardial and subpleural haemorrhages may develop (7). It has been suggested that the stress of a prolonged delivery, rather than the type of assistance may ultimately be responsible for reduced calf vigor following dystocia.

**Perinatal calf evaluation**

Calves assisted compared to unassisted or pulled out by strong compared to mild traction have increased respiratory-metabolic acidosis and take longer to achieve sternal recumbence (7,18). The vigour of the calf can be assessed immediately after calving by its reflexes and the time it takes to head-right, achieve sternal recumbence, attempt to stand and to stand (3, 5, 20 and 60 minutes, respectively) (7,21). If the calf exhibits subsidiary abdominal breathing, has poor reflexes or it takes more than 15 minutes to achieve sternal recumbence the prognosis is poor (18).

**Calf resuscitation**

Resuscitation can commence while the calf is still in the birth canal and continues until the vital signs have normalised (e.g. posture, activity, respiratory function, rectal temperature) or until a heart beat is undetectable with a stethoscope. Once the calf’s thorax has emerged from the cow the calf can breathe even if it remains in situ due to hip-lock. Thus, resuscitation can begin during a problem calving by stimulation of the calf’s nasal receptors with straw or a finger (or an intranasal tube if oxygen therapy is available). Immediately after birth the calf should be briefly suspended upside-down. This procedure facilitates postural drainage of pulmonary fluids and has a positive impact on pulmonary gas exchange, correction of mixed acidosis and subsequent absorption of colostral immunoglobulins (22). Clearance of the airways can begin with pharyngeal and nasal suctioning using an aspirator pump (8). Though only a small volume of fluid (<10ml) is generally removed, the procedure significantly benefits pulmonary gas exchange and acid-base balance (23).

Hypothermic stimulation has become the most common technique used to resuscitate calves by pouring cold water down the calf’s ear or over the head or whole body to induce a gasp reflex. Recent research indicates that it has a beneficial effect on pulmonary gas exchange and acid-base balance in calves (23). Once a patent airway has been established and breathing stimulation commenced, the calf should be placed in sternal recumbence in the ‘dog sitting’ posture with lateral support. This has a positive impact on physiological adaptation mechanisms, prevents hypostatic congestion in the dependant lung of lateral recumbence and facilitates attempts at positive pressure ventilation (22). Compressed air devices (e.g. Ambu bag, H-K Calf Resuscitator), when used correctly (usually necessitating two people to operate), are clinically effective in newborn calves even without intubation (8).

Studies in neonatal calves indicate oxygen therapy can improve perinatal survival. For herd staff use, industrial oxygen can be administered with a face mask or intranasal tube while experienced veterinary practitioners can administer oxygen via a cuffed endotracheal tube. A high flow rate (25L/min) is used along with sealing of the mouth, nostrils and oesophagus to ensure immediate lung inflation in cases of partial atelectasis. In cases where the calf is breathing but dyspnoeic, a lower flow rate (5L/min) can be used for insufflation until eupnoea returns. The clinical benefits of pharmacological respiratory stimulants, such as doxapram and etamphylline, in newborn calves are inconclusive, hence their use is often discouraged. However, they are widely used and studies have shown positive effects on acid-base balance, hence, they should be considered for use in dyspnoic neonatal calves. Following successful perinatal resuscitation some calves develop secondary acidosis within 24 hours of birth with a poor suck reflex, tachypnoea, tachycardia, weakness, depression and hypothermia. Correction of postnatal metabolic acidosis can be effectively achieved with drip or bolus intravenous infusion of sodium bicarbonate instituted after resuscitation and repeated as necessary (2).

**REFERENCES**

Developing an evidence-based diagnostic approach for perinatal mortality in cattle

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ABSTRACT
The significance of perinatal mortality in cattle is considered worldwide together with infectious and non-infectious aetiologies. The diagnostic approach for sporadic perinatal mortality and the consequences of focusing diagnostic methods purely on infectious causes are assessed; in laboratories where infectious diseases are screened for almost exclusively the diagnostic rate is around 20% compared to approximately 45% where pathological examination of the fetus is the basis for diagnosis. The efficiency of various diagnostic methods for identifying infectious causes is reviewed from the recent literature. The diagnostic approach to investigating bovine perinatal mortality is compared to that used in human cases. In humans, the overall diagnostic rate is over 60% of which half the cases have some infectious aetiology attributed to them.

KEYWORDS
Perinatal mortality, bovine, laboratory diagnosis

INTRODUCTION
Bovine perinatal mortality and abortion are important components of reproductive failure and associated economic losses worldwide. In the United States (US), a viable fetus is worth around $200 (1) and in the United Kingdom (UK) about £140 (2). In countries with important beef and dairy industries, an overall loss of 10% of potentially viable fetuses with a value of around $160 – 200 or £85 – 140 each is significant: in Argentina this equates to an annual loss of approximately $165 million, in the US it is $840 million, and in the UK £22 million within the dairy industry alone. When compiling these figures no account has been made of other related costs.

For all bovine perinatal mortality, occurring at any stage of pregnancy, approximately 40% has been associated with infectious agents (3). For full-term fetuses it increases generally with the degree of calving difficulty; for calving cattle experiencing serious difficulty or requiring caesarean section to overcome severe dystocia the fetal mortality rate may be as high as 52% (2). Other contributing risk factors include birth of a non-viable fetus after an earlier pregnancy (4), increasing parity and twin pregnancy (5), dietary factors (6) and myocardial degeneration associated with vitamin E/selenium deficiency (7).

Reproductive failure in cattle is not only a problem for countries with intensive livestock farming. The Food and Agriculture Organisation have started a five-year research programme to devise an integrated approach to improve small scale market oriented dairy systems in developing countries. There are six specific objectives: to identify and prioritize the constraints and opportunities in selected dairy farms; determine the most important limiting factors to profitability; develop effective intervention strategies and assess their economic impact; develop methods for recording economic performance, and encourage farmers to adopt proven methods and strategies. Specific issues related to reproductive efficiency include fertility parameters such as extended calving interval and calf mortality, management of natural breeding, and diseases of reproduction such as brucellosis, campylobacteriosis, trichomoniasis and infectious pustular vulvo-vaginitis (8). In these small herds the average perinatal mortality rate is around 4%, excluding those born prematurely (9).
Because of the diverse nature of the aetiologic factors associated with perinatal mortality, the diagnostic approach to sporadic cases or epidemics is important. For example, to rely on laboratory investigations focused primarily on infectious agents will more than halve the chances of making a diagnosis since 60% of cases have a non-infectious origin. This paper considers the accuracy of laboratory methods used to identify causes of perinatal mortality in cattle as a basis for developing an evidence-based diagnostic approach for this syndrome.

### Diagnostic rates for perinatal mortality

Generally, the diagnostic rate is significantly higher for infectious than for non-infectious or management-related causes, with exceptions (see Table 1). All the papers listed below carried out a pathological examination of fetuses and used diagnostic microbiological techniques to support a diagnosis.

In contrast, the overall diagnostic rate for a laboratory diagnostic service focusing almost exclusively on infectious causes of perinatal mortality was 18%; of 4732 cases examined 15% had infectious and 3% non-infectious aetiology (VIDA II 2006), a diagnostic rate below other equivalent laboratories. Using a similar diagnostic approach in the Republic of Ireland, of 2220 cases of perinatal mortality investigated in 2006 the overall diagnostic rate was 22% with 21% associated with infectious agents (14).

The context within which different veterinary diagnostic services operate is relevant to the data presented above. In Great Britain, perinatal mortality is investigated usually as part of an infectious disease profile affecting herd profitability. For example, the disease spectrum associated with Bovine Viral Diarrhoea virus (BVDv) includes neonatal and concurrent respiratory infections, mucosal disease, and reproductive failure through abortion and birth of non-viable calves: effective control measures can be devised to control infection generally, such as vaccination, which will also improve reproductive efficiency. The relatively low cost of such a focused diagnostic protocol - in the UK around £24.50 - is attractive to the farmer. However, in countries where there are broader disease categories and husbandry-related causes of fetal mortality, such a narrow diagnostic approach is difficult to justify and implement.

### Efficiency of diagnostic methods

#### Virus isolation and/or antigen detection

Bovine epithelial cell lines, particularly Madin Darby kidney and turbinate cells, are used routinely for both BVDV (15) and bovine herpesvirus (b-HV1; b-HV4) (16, 17) isolation from clinical material, which is considered to be definitive for confirming fetal infection. For this method to succeed there must always be viable viral particles present in the field material that can replicate in culture. However, concurrent bacterial contamination of aborted tissues or autolysis, especially placenta, can make viral isolation impossible (18). Whilst immunohistochemistry has a very high concordance with viral isolation for detecting BVDVs in field infections (15) it is less robust for detecting b-HV1 antigen (16). Success using this method does not rely on the virus retaining its infectiousness but is affected adversely if the virus is damaged. Therefore, other methods have been investigated such as polymerase chain reaction after reverse transcription (RT-PCR) or semi-nesting (SN-PCR) to detect viral genome fragments that correlate well with virus isolation.

However, aborted fetal tissue has often undergone moderate to severe autolysis in utero before its delivery and there has been speculation as to its influence on detecting virus or viral fragments in these cases. Recently, Benson and others (2002) studied the impact of autolysis on the three methods described above for identifying viral antigen in porcine reproductive and respiratory syndrome virus (PRRSV) transplacental infection in fetuses. Autolysis reduced virus isolation rates to 47% and 7% after 24 and 72 hrs storage at 25°C respectively; by comparison, such conditions had little impact on the expected 94% sensitivity of RT-PCR for detecting viral infection in either pooled fetal fluid or tissue samples. Immunohistochemistry gave similar results to those for viral isolation but the best results were tissue specific e.g. thymus. Autolysis for 48 hrs also reduced by 25% the sensitivity of immunohistochemistry for identifying rinderpest virus: the best results obtained were also tissue specific, in tonsils (20). For comparison, Gruber and others (21) detected BVDv RNA by RT-PCR from all the non-fixed brain tissue they examined after 10 days autolysis. Results of this type of analysis are summarised in Table 2.

Whilst laboratory techniques vary in their ability to detect viral antigen there is another confounding issue: the gestational age of the aborted fetus. If fetalpatic infection occurs before the fetus has developed immunoocompetence around 120 days gestation, then antigen detection by the appropriate method should be successful. However, if infection triggers an effective fetal immune response in mid or late pregnancy then no live virus will survive and be found when a dead fetus is delivered subsequently.

### Microorganism culture and isolation

For some infections, this remains the standard definitive diagnostic method for zoonotic diseases such as brucellosis and salmonellosis.

The perineal region or the hind gut is the environmental or ecological niche for the majority of opportunistic bacteria isolated from aborted bovine fetuses (see Table 3). The Gram +ve and some Gram –ve organisms involved are intracellular and depend on host cells to provide the crucial nutritive resources essential for their survival and replication. Extracellular pathogens, such as Campylobacter fetus subsp. venerealis and Escherichia coli, adhere to host cell surface membranes and depend on the availability of nutrients present in the ecosystem adjacent to mucosal surfaces for their survival. Thus, a labile equilibrium exists between the normal function and nutritive needs of host cells and those of the pathogen.

All microorganisms grow within specific pH ranges and have a preferred requirement for oxygen (aerobic or anaerobic). They also require iron and small organic molecules and amino acids – such as tryptophan – to be absorbed from their habitat since they have

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**Table 1.** Diagnostic rate for perinatal mortality, including both abortions and stillbirths, in cattle over a 35 year period

<table>
<thead>
<tr>
<th>Reference</th>
<th>Infectious</th>
<th>Diagnostics (%)</th>
<th>Overall diagnostic Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirkbride and others (1973)</td>
<td>30</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Agerholm and others (1993)</td>
<td>13</td>
<td>59</td>
<td>72</td>
</tr>
<tr>
<td>Alves and others (1996)</td>
<td>32</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Campero and others (203)</td>
<td>34</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>Khodakaram-Tafi &amp; Ikede (205)</td>
<td>35</td>
<td>9</td>
<td>44</td>
</tr>
</tbody>
</table>

**Table 2**

K hodakaram-Tafi & Ikede (205) 35 9 44
Campero and others (203) 34 11 45
Agerholm and others (1993) 13 59 72
Kirkbride and others (1973) 30 5 35

**Table 3**

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lost the ability to synthesise these directly (24). Thus, microbiological culture of relatively fresh aborted tissues such as stomach contents or lung may yield a useful bacterial profile that could account for a particular perinatal fatality.

Autolysis has a profound adverse effect for obtaining an accurate bacterial profile in cases of fetal perinatal death. The lowering of tissue pH, host cell degradation with destruction of cytoplasmic organelles and change in gas partial pressures within the micromilieu of pathogens suggests that bacterial culture from autolytic material will be unrewarding. This is one reason why extracellular Gram –ve organisms are isolated frequently from this type of material. Fairbrother (25) investigated the effect of tissue autolysis on culture of *Leptospira interrogans* var *pomona* and found no viable organisms present in kidney tissue stored at 20°C for 24 hrs prior to inoculation although they could be identified for up to 38 days after being placed in urine. Soluble leptospiral antigens could be identified in only 13 – 20% of autolysed tissue samples and he considered that enzyme-linked immunosorbent assay (ELISA) systems could be useful for detecting them in aborted fetuses.

**Identification of protozoa or their antigens**

Bovine fetuses infected with *Neospora caninum* have tachyzoites and tissue cysts present. These may be identified in haematoxylin and eosin stained thin tissue sections of myocardium or brain but immunohistochemical staining is a much more reliable method. It is very difficult to distinguish between groups of tachyzoites and tissue cysts. Attempts to isolate viable *N. caninum* by bioassay have been mostly unsuccessful. Dubey and Schaere (26) reviewed the sensitivity and specificity of PCR for detecting *N. caninum* against several protozoans, described in 25 papers published between 1996 – 2002; where the analytical sensitivities was given, the various PCR methods could detect between 1 – 10 tachyzoites in up to 5 mg brain tissue examined. Fortunately, both *Toxoplasma gondii* and *Sarcocystis cruzi* are rarely associated with bovine abortions.

For venereal infections which cause infertility and abortion, such as *Trichomonas fetus* where the diagnosis is made primarily using preputial washings, a simple PCR assay compares advantageously to the classical culture method (27).

**Maternal serology as a diagnostic tool**

Whilst serology is a valuable diagnostic tool of illness or abortion it has serious limitations and must be used in conjunction with other diagnostic tools such as clinical and pathological examination. It provides retrospective evidence of prior infection or disease. When lesions suggest a specific disease may have been present a positive serological diagnosis can be achieved only if two paired serum samples are obtained from the same animal; the first is collected at the first sign of disease and the second up to four weeks later. Negative paired sample titres eliminate the microorganism in question: positive sample titres are of no diagnostic value unless the second sample, titrated simultaneously with the first, has a serum neutralising antibody titre at least fourfold higher than the first or the O.D value is at least 0.2 O.D units higher. Interpretation of results must be done with caution because those microorganisms associated with abortion are common and fetal infection may have occurred weeks or months after the dam was infected initially. A positive seroconversion indicates a particular organism could be involved but does not prove it.

Of particular significance may be results involving BVDv infection, where the bulk milk antibody titre is high suggesting that acute active infection is present but the cow which has aborted has a consistently negative titre suggesting she may be persistently infected. This may be confirmed using immunofluorescence assays from the buffy coat of a heparinised blood sample (28).

Single serum samples are of value only as a means of eliminating specific infections. They are used in serological surveys (29). However, they may prove useful if used in conjunction with other similar tests such as bulk milk antibody, that allow a comparison of titres to be made between the animal under investigation and the infectious disease status of its herd mates. Sero-diagnosis has been used successfully to eradicate *Brucella abortus* infection from cattle in many parts of the world. Standardization of the various methods is based on use

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**Table 2. Sensitivity of different laboratory techniques for detecting viral antigens in autolysed and/or aborted fetal material**

<table>
<thead>
<tr>
<th>Antigen detection method</th>
<th>Sensitivity</th>
<th>Virus</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation</td>
<td>*</td>
<td>BVDv</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>***</td>
<td>bHV-1</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>ditto</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td>***</td>
<td>bHV-5</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>b-HV4</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>PRRSV</td>
<td>(19)</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>***</td>
<td>BVDv</td>
<td>(21)</td>
</tr>
<tr>
<td>SN-PCR</td>
<td>***</td>
<td>b-HV1</td>
<td>(23)</td>
</tr>
<tr>
<td></td>
<td>***</td>
<td>ditto</td>
<td>(18)</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>***</td>
<td>PRRSV</td>
<td>(19)</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>***</td>
<td>BVDv</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>b-HV1</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>b-HV4</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>PRRSV</td>
<td>(19)</td>
</tr>
</tbody>
</table>

**Table 3. Opportunistic bacteria isolated from cases of bovine abortion and perinatal mortality**

<table>
<thead>
<tr>
<th>Gram +ve</th>
<th>Opportunistic bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcanobacterium pyogenes</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td><em>Fusobacterium necrophorum</em></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td><em>Histophilus somnii</em></td>
</tr>
<tr>
<td>β-haemolytic Streptococci</td>
<td><em>Leptospira borgpetersenii</em></td>
</tr>
<tr>
<td>Others:</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>Mycoplasma bovigenitalium</td>
<td><em>Salmonella dublin</em></td>
</tr>
<tr>
<td>Ureaplasma diversum</td>
<td><em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>Others:</td>
<td><em>Yersinia pseudo-tuberculosis</em></td>
</tr>
</tbody>
</table>

**Table 88. XXV. Jubilee World Buiatrics Congress 2008**
of strongly and weakly positive and negative standard serum issued by the Veterinary Laboratories Agency, Weybridge, UK. Diagnosis is made using agglutination and complement fixation tests on the visual reaction patterns obtained. More recently, a second generation competitive enzyme immunoassay has been developed to detect the re-polysaccharide of smooth lipopolsaccharide derived from B. abortus $\text{Si1193}$ which does not react with protein A/G; with a 100% sensitivity and specificity, test harmonisation in the future should be possible (30).

The microscopic agglutination test (MAT) is the method used most widely to detect Leptospira antibodies in cattle serum. Cross-agglutinin absorption studies showed moderate antigenic similarity between organisms within the same serogroup. This fact is used currently to detect prior infection of cattle by any member of the Sejroe serogroup which includes L. interrogans var hardjo (LIH), DNA homology studies have shown that L. borrepietersemi (LB) serovars are different from the Sejroe subgroup. However, the same LIH antigen is used in most MAT to identify all potentially pathogen leptospires in cattle. Recently, Hotka et al. (31) compared the cross-reactivity of serovar LIH antigen with LB in the MAT; LIH missed only 1.6% of LB positive sera. However, if the LB antigen was used in the MAT, 58% of LIH positive sera failed to react. Unless veterinary diagnostic laboratories know which specific leptospiral serovars are associated with cattle infections in their own countries /locations, poor laboratory techniques could lower the efficiency of cattle disease surveillance and control measures for bovine leptospirosis. When used for diagnosis of perinatal mortality in cattle a single serum sample, taken at the time of abortion or calving, is sufficient in unvaccinated cattle; MAT titres >1/400 indicate prior infection of the dam up to three months before sampling. Titres lower than this indicates that either the animal could have been vaccinated several weeks before parturition or else the cow was infected at some time in the past. In northern Spain, L. interrogans var bratislava infection is endemic and 9% of bovine abortions have been associated with this infection when MAT titres were set at >1/300 (32).

In their most comprehensive review of serological methods for diagnosis of N. caninum in cattle, Ghalmi et al. (33) considered 47 research groups describing 73 serological investigations; the definitive diagnostic method using indirect immunofluorescence (IFAT) was examined in 27 studies, ELISA in 46, and a direct agglutination test in just two. All the assays were based on tachyzoite antigens. Several papers compared IFAT against the other methods: the reviewers found a kappa value of 0.97 when comparing IFAT with ELISA overall, the problem with ELISA being variation in laboratory techniques. Whilst there is no question of the association between known seropositive cows and the relative high risk of these pregnant cattle aborting, serology is not the definitive diagnostic method for individual cases: PCR is the method of choice to detect N. caninum infection of fetal or neonatal tissue. However, serological tests are very useful to describe the potential weight of infection within herds and in wider epidemiological studies, despite some antigen homology existing between N. caninum, Sarcocystis cruzi and Toxoplasma gondii. Worldwide, there are ten serological diagnostic test kits for neosporosis available commercially (26).

Fetal serology as a diagnostic tool
The fetus develops immunocompetence at approximately 120 days gestation and is able to produce virus specific antigen IgG from that time onwards. For more complex micro-organisms such as B. abortus and pathogenic Campylobacter spp, fetal antibodies can be detected from about 180 days gestation. Where the fetus is alive, antibodies can be detected in both allantiot and amniotic fluid (34): in aborted dead fetuses sero-sanguineous transudates found in the pleural and pericardial cavities contain immunoglobulins that reflect the humoral fetal response to infection. However, autolysis causes protein degradation that reduces significantly the sensitivity of fetal antibody detection for diagnosis. Other reasons include a short interval between fetal infection and death, as occurs with bHV-1, and lack of fetal immunocompetence for the presenting antigens. Thus, a negative serological result does not exclude the risk of a particular infection. Western blot assay techniques increase the sensitivity and specificity of this diagnostic tool. In an old study before neosporosis had been identified as a possible bovine abortifacient agent, Murray (35) examined 136 aborted fetuses and non-specific immunoglobulins were present in 35: of these, 13 were seropositive for BV Dv antibodies, 5 indicated bHV-1 had challenged the fetus, and 7 were positive for L. hardjo infection. A diagnosis was not made using these results unless they complemented any fetal histopathological findings.

When used for the diagnosis of fetal neosporosis infection care should be taken when interpreting a positive result. The vast majority of N. caninum-infected fetuses develop normally and are not stillborn; therefore specific antibodies present in an aborted fetus do not indicate that an abortion was caused primarily by this pathogen. The correct conclusion has to be that its dam was infected prior to the abortion occurring (26).

Comparison with medical diagnostic units for sexually transmitted diseases
During the year 2000, approximately 49 million tests were carried out annually for Chlamydia and gonorrhoea in humans in the United States; in Public Health laboratories, over 200,000 tests for syphilis and 54,000 tests for genital herpes infection were performed. Of this total, around 63% of all tests were DNA probes. For clinical diagnosis of genital herpes, a non-specific infection of the genital tract where sero-diagnosis is useful but of low sensitivity, only 12% of all tests carried out were serological using mostly non-specific herpesvirus assays (36).

In humans, it is estimated that up to 50% of all conceptions miscarry. Approximately 10 – 15% of these are recognised clinically and around 4% are associated with anatomical defects. From a social point of view, recurrent miscarriage presents some of the most difficult diagnostic challenges: it affects 1 – 3% of couples trying to conceive and no diagnosis is reached in about half the cases investigated. In a recent study, Laury et al. (37) found that of 146 cases of intrauterine fetal mortality 36% were associated with chromosomal abnormalities, and 55% presented with chorioamnionitis of which 42 had acute and 10 chronic inflammation present; bacterial or viral infections were associated with only 14 (33%) of these cases. Similar proportions of infectious and non-infectious causes of abortion have been found following investigation of bovine abortions (10, 35, 12). Generally over the past 10 years, the numbers of pathological examinations carried out in human medicine have fallen but diagnoses associated with perinatal mortality rely still on pathological examination of the fetus and placenta. This conclusion was typified by Laury et al. (37) who identified the cause of fetal loss in 69% of cases examined. This should be the long term objective in farm animal veterinary diagnosis of perinatal mortality.

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