Paratuberculosis – potentials and limits of control programs

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ABSTRACT

Paratuberculosis (Johne’s disease) is one of the most important diseases in ruminants today. Its distribution is worldwide and the disease is causing severe financial losses among cattle producers in some countries. Paratuberculosis is untreatable, diagnosis limited to the early stages of the infection and control of the disease is difficult.

Examples of control and surveillance programs for paratuberculosis are presented in this paper. Additionally the Austrian regulation on monitoring and abatement of clinical paratuberculosis in ruminants (Paratuberculosis-Regulation) will be described and discussed.

KEYWORDS

Paratuberculosis, national surveillance programs, Paratuberculosis-Regulation, Austria

INTRODUCTION

Paratuberculosis (Johne’s disease) is widely distributed throughout the world and is caused by Mycobacterium avium subspecies paratuberculosis (MAP). The disease mainly affects domestic and wild ruminants (7,8) but MAP can also be found in numerous monogastric animals (5,9,15).

Young ruminants are most susceptible to MAP infection, but animals can become infected throughout lifetime. The bacterium is excreted with faeces, the most important source of infection, but is as well shedded in milk and ejaculate (9). The transmission mainly takes place in calves via faecal-oral route by contamination of feed and udder, or by intake of infectious milk and colostrum. The incubation period may range from two to ten years (17).

Subclinically infected ruminants show a reduced feed efficiency, milk production and fertility, a decreased slaughter weight at culling and an increased incidence of mastitis (13). They may shed small numbers of the organism in faeces and may have detectable antibodies in this stage of the infection (23). Typical for clinical infection with MAP are weight loss despite normal appetite and intermittent diarrhoea. ELISA and faecal culture often show positive results in this stage of the infection (9,11,23).

The advanced clinical disease is characterised by cachexia, severe diarrhoea and intermandibular edema, death occurs as result of dehydration and cachexia (23).

The economic losses due to the clinical disease can be considerable but indirect costs and losses because of subclinical infections are even more devastating (11).

Diagnosis of subclinically infected cattle is challenging. Direct methods such as the examination of Ziehl-Neelsen stained smears, faecal culture and PCR (Polymerase Chain Reaction) and indirect methods to measure humoral immune response as Agar Gel Immunodiffusion (AGID), Complement Fixation Test (CFT) and the Enzyme Linked Immunosorbent Assay (ELISA) can be used. For increasing sensitivity and specificity the combination of direct and indirect methods and repeated testing is recommended (20).

Paratuberculosis is difficult to diagnose and untreatable. Therefore control and reduction in MAP positive herds and prevention of spreading the disease to negative herds is most important.

Different programs to control paratuberculosis in domestic ruminants from all over the world should be presented and discussed in this paper. Of course these programs only represent examples and this manuscript does not claim to be complete.

Examples for control programs against paratuberculosis

USA

Different voluntary programs have been implemented in the United States for a long time. Uniform program standards for a voluntary bovine Johne’s disease control program have been released in the United States in 2006. The program is based on the 3 elements: education, management and herd testing to provide minimum standards for the control of paratuberculosis throughout the country.

The education element serves as the entry level for participating in the voluntary program and provides producers with basic information about the disease as well as management and control strategies. Prior to herd testing and classification a herd management plan to prevent introduction of paratuberculosis into the herd and to reduce transmission of the disease has to be developed together with a certificated veterinarian or animal health official. Herds participating in the program are tested by faecal or tissue culture, PCR or histology of tissues. Additionally ELISA, environmental sampling or pooled faecal samples can be performed as screening tests. Herds are than classified as level A (no positive animals) to level D (at least one MAP positive animal or more than 15 % ELISA positive animals). For renewal and advancement of a herd, tests must be repeated within 14 months and management plans must be updated (2).

Australia

Australia has probably implemented the most sophisticated control program for paratuberculosis in all species of ruminants around the world. There exist numerous different programs which are described at http://www.animalhealthaustralia.com in all details. Only the main ideas and actions of the superordinated national program should be described within this paper.

The Australian National Johne’s Disease Control Program (NJDCP) is a cooperative program which involves different control strategies for paratuberculosis from veterinarians, livestock industries, and government. The NJDCP aims to control the disease in Australia and limit its spread between areas and properties as well as to reduce the impact in affected herds. The national program is coordinating projects in market assurance, disease control, research, communication and information, training and diagnostic methods. Market assurance programs are a key strategy in the control of MAP in Australia. These programs are voluntary and enable farmers to identify and promote their paratuberculosis status. If a herd is found to be MAP-positive, disease control programs which vary from state to state can be implemented. The NJDCP also provides standard definitions and rules for bovine and ovine paratuberculosis as well as standard diagnostic procedures.

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Japan

Since 1971 paratuberculosis is a notifiable disease in Japan, followed by culling of the infected animal and compensation by the government. Because of a low rate of about 200 reported cases every year, an active surveillance program has been started in Japan in 1997. In this active surveillance all cattle have to be tested at least once in every five years by ELISA, faecal culture or microscopically in faeces of animals showing clinical signs of paratuberculosis. Affected farms are monitored and retested for a certain period.

Since the start of this surveillance program the number of reported annual cases varies around 1000 every year. The nationwide annual detection rate (detected animals/tested animals) is stable at a level of 0.1 to 0.2 % (16).

Sweden

Sweden is the country with the most rigorous control program against paratuberculosis. The incidence of the disease in Sweden is very low with only 53 infected cattle herds between 1993 and 2005 (26). The disease is compulsory notifiable in all animals, followed by stamping out of infected herds. Furthermore all imported ruminants have to be tested for MAP during quarantine. Farmers are forced to import semen and embryos instead of living animals and purchase of imported animals to dairy herds is prohibited (12).

Denmark

A voluntary control program for paratuberculosis was established in Denmark in 2006. Approximately 24 % of the dairy farms are participating in this program until today. All lactating cows of participating farms are tested four times a year by milk ELISA, results are used for risk-based management of infected animals. Risk assessment is also used to identify crucial areas of transmission within the farms and to increase herd management. The control program is expected to last 6 to 8 years, a surveillance component may be added to the program and is currently under development as next step (19).

The Netherlands

In 1998 the Dutch national voluntary paratuberculosis program was started. It consisted of the program "non-suspect status paratuberculosis," which had the aim to prevent free herds from the disease and the program "assisting infected herds" for eradication of paratuberculosis in infected herds (6).

The national control program had 10 herd status levels. Status 5-10 indicated, with different degrees of certainty - the higher the status, the higher the likelihood, that a farm is free of paratuberculosis. Status 1-4 was for infected herds or herds with an unknown status, and status 5 for herds which were free of classical paratuberculosis. To reach status 6 all animals aged 3 years or older had to be ELISA negative. A herd could proceed from status 6 to 10 over a minimum period of 4 years if faecal samples of all animals with a minimum age of 2 years were negative.

If a farm with a status of 5 or above, purchased a cow from a lower ranked farm the herd was classified as the farm of origin of the purchased animal, if the animal wasn’t removed from the herd within 7 days.

In the first year of the program 1839 dairy farms participated in the program, the majority were classified as status 2 (600), status 4 (282) and status 6 (773) (6).

Recently this program was changed and today farms are classified into 3 different statuses only. Status A for farms without ELISA-positive animals, status B for farms with positive cattle if positive animals are removed from the herd within 6 weeks and status C if positive animals stay in the herd. Farms with status A have to be retested by blood- or milk-ELISA every two years, B and C farms have to be retested every year. After each test the status of the farm has to be adapted according to the latest results (http://www.gddeventer.com/rund).

Germany

In Germany paratuberculosis is rated as a notifiable disease, cases have to be reported periodically to the OIE (World Organisation for Animal Health), but notification do not cause any official actions. Voluntary control programs are existing in several federal states and costs for participating farms are covered by the government to certain extend in some of them. In 2005 guidelines for the management of paratuberculosis in ruminants were released by the German Ministry of Food, Agriculture and Consumer Protection. Aims of the guidelines are the standardisation of control programs in Germany, reduction of clinical cases, prevention of transmission of paratuberculosis to free herds and the decrease of the seroprevalence. According to the guidelines positive farms should be rated as status 1 if the hygienic measurements of the guidelines are implemented on the farm and regularly controlled by state veterinarians. A farm can reach status 2, if additionally to status 1 all animals are tested for paratuberculosis by ELISA or PCR once a year. For the next step, status 3, all serologically positive animals should be tested for MAP by culture or PCR 3 times in an interval of 6 months. All MAP positive animals should be slaughtered, negative individuals have to be retested for MAP shedding every year. It is recommended that offspring of positive animals is separated and tested for paratuberculosis at an age of 2 years. After a herd has been tested for 5 years without any positive results it can reach status 4 which is called “MAP-unsuspicous” (3).

How many regional control programs and farms are following these guidelines in Germany is not known.


The Austrian Paratuberculosis-Regulation

Due to a documented increase of MAP in Austria (4) and the ongoing discussion about a possible link between paratuberculosis and Crohns disease in humans (18,21) the Regulation of the Austrian Federal Ministry of Health and Women on monitoring and abatement of clinical paratuberculosis in ruminants (Paratuberculosis-Regulation) came in force in April 2006 (1).

The regulation affects cattle, sheep, goats and farmed deer. Animals showing clinical signs of paratuberculosis have to be notified to the district veterinarian and separated. Slaughtering and use of milk is prohibited. The major symptoms leading to the suspicion of clinical paratuberculosis are listed in the regulation (chronic diarrhoea despite normal appetite, emaciation, oedema, decreased milk yield…). Blood and faeces of suspicious animals are taken by the district veterinarian and sent to the National Reference Laboratory for Paratuberculosis of the Austrian Agency for Health and Food Safety.

If during slaughtering an individual is showing severe emaciation which could have been caused by MAP, organ samples have to be sent to the reference laboratory for testing by PCR. The same actions have to be taken if signs of clinical paratuberculosis occur in animals that die or are culled.

Samples of suspicious animals are tested and adjudged by ELISA and if necessary also tested with PCR by the National Reference Laboratory for Paratuberculosis. Clinically ill, MAP positive animals have to be culled within 3 days. Furthermore, hygienic precautions listed in the Parataberculosis-Regulation have to be performed. Compensation for culled animals, depending on age and value of the animal, is paid by the government. Meat of slaughtered animals found MAP positive is declared unfit and has to be disposed.

Whenever an animal showing clinical signs or emaciation is diagnosed as MAP positive the farm of origin has to be placed
under the control of the district veterinarian for further cases of clinical paratuberculosis.

Due to the difficulties in the diagnosis of subclinically infected animals, ruminants with a positive ELISA or PCR result for MAP not showing any clinical signs are not affected by the regulation.

More details about the Austrian regulation can be found at Khol et al. (14).

Discussion and Conclusion

Many different countries have established voluntary programs to control paratuberculosis and prevent further spreading of the disease. Some of these programs have been running for a long time or are still running. This helped to reduce clinical disease and spreading of infection for participating farms or areas but due to their voluntarily character could not be applied to the entire livestock population (17, 25). In other countries compulsory registration for paratuberculosis is performed, infected animals have to be reported but are causing no consequences.

In some other countries strict control and stamping out programs for paratuberculosis are in action. Beside Sweden, Austria is the second European country to declare clinical paratuberculosis a notifiable disease. The Austrian Paratuberculosis-Regulation could be an important step in the fight against this disease, although, due to insufficient way of diagnosing subclinically infected animals, only ruminants showing clinical paratuberculosis are affected by the law. It has been reported that animals showing clinical signs of paratuberculosis signify only the tip of the iceberg and that there is always a significantly higher number of subclinically infected animals (22). But on the other hand a severely affected animal can shed high amounts of MAP to the environment and some so called “super-shedders” can even excrete more bacteria with faeces than 20,000 low shedding animals (24). The reduction of these “super-shedders” in livestock can cause a significant decrease of MAP in farms and environment leading to a smaller amount of MAP intake to the food chain and might help to prevent further spreading of the disease.

The attendance of veterinarians and animal owners always is crucial in disease control programs. Regulation of an animal disease introduces social and economic disincentives for farmers and veterinarians to notify the authorities. One incentive for farmers to participate in the program could be a paid compensation for culled animals by the state. Industrial and public pressure as well as economic impact of the disease could work as an additional force and overbalance possible disadvantages for the farmer by being known to have MAP positive animals.

Another big advantage of implementing a national control program for paratuberculosis is that consequently the disease is recognized as important by farmers, veterinarians and consumers. The introduction of the Paratuberculosis-Regulation in April 2006 in Austria caused many discussions about the disease, control strategies of MAP in livestock and the usability of the regulation. Within the first year since the implementation of the regulation in Austria samples from 103 farms were sent to the national reference laboratory for paratuberculosis. Clinically suspicious cases were confirmed in 36 (35 %) of this samples (10).

The future will show which program can best fulfil the expectations of farmers, veterinarians and consumers and is a suitable tool for the reduction of paratuberculosis. For sure a lot of information and awareness training is necessary to persuade those who are keeping and working with ruminants to follow guidelines of regulations and participate in control programs.

Paratuberculosis is a worldwide problem that cannot be solved by one country alone. As a first step in a supraregional control program it should be discussed how clinical ill animals could be eliminated from livestock. Beside all differences in actions and opinions in the fight against paratuberculosis this could be considered as a “minimum level” which could be achieved in reasonable time and would be accepted by all participants. Brought but tangible discussion should be organised as a following step to find an international agreement on livestock trading and prevention of spreading of paratuberculosis to free herds and regions. Additionally to the various scientific efforts concerning this important disease the focus of the discussions should be brought to usable measurements that can already be taken today to prevent further increase of paratuberculosis in ruminants.

REFERENCES


Pregnancy-associated glycoproteins and their importance in monitoring the progress of bovine pregnancy

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ABSTRACT
Pregnancy diagnosis is an important part in reproduction management of ruminants. In the last years, a large polymorphic family of placenta-expressed proteins has been discovered in ruminant species, which have been used for pregnancy diagnosis. Members of this family, called the pregnancy-associated glycoproteins (PAG), are synthesized in the binucleate cells of the ruminant’s trophoectoderm. Part of them are released into the maternal blood circulation that is hence used for their assay by different laboratory techniques. Due to the large variety both in the PAG molecules that are expressed and in the posttranslational processes of PAG, different immunosystems show different efficiencies in quantifying the PAG released in blood. The sensitivity (92 to 100%) and specificity of the PAG radioimmunoassay for pregnancy diagnosis are both very high. The assay of PAG can also provide useful information for researchers working in programs focused on the study of embryonic/fetal mortalities, embryo biotechnology (embryo transfer, in vitro fertilization, and cloning), nutrition and infectious diseases in animals leading to pathologies affecting the pregnancy.

KEYWORDS
Pregnancy-associated glycoproteins, pregnancy diagnosis, ruminant, markers.

INTRODUCTION
Characterized for the first time in the early eighties, the pregnancy-associated glycoproteins (PAG; also called pregnancy-specific protein B (PSPB) or pregnancy-specific protein 60) constitute a large family of glycoproteins expressed in the outer epithelial layer (chorion/trophoectoderm) of the placenta in eutherian species. They are synthesized by the mononucleate and binucleate trophoblastic cells, some of them being secreted into the maternal blood from the moment when the conceptus becomes more closely attached to the uterine wall and placenta formation begins (30).

Using different chromatographic procedures, some members of the PAG family have been isolated from the cotyledons of cow, ewe, goat, bison, buffalo, moose, and elk (reviewed by Sousa et al. (20)). Purified and semipurified preparations have also been used to immunize rabbits; the antisera (AS) thus obtained have led to the development of homologous (15, 18, 31) and heterologous radioimmunoassay (RIA) (1, 17) and sandwich enzyme-linked immunosorbant assay (ELISA) systems (6).

Screening of placental libraries with nucleic acid probes has identified many cDNA sequences, which code for 22 distinct PAG molecules (boPAG-1 to boPAG-22). These investigations have also shown that the various PAG cDNAs are not expressed uniformly throughout the pregnancy (5). Some are expressed during the early stages alone whereas others are observed at different specific stages of pregnancy. Using cDNA microarray analysis, Usihzawa et al. (23) have shown that several PAG molecules are expressed very early in the placental tissue (boPAG-4, -5, and -6 at day 7; boPAG-2, -8, -11, -16, -17 between days 7 and 14; and boPAG-1, -2, -5 to -7, -9 to 13, -15 to -17, -19, -21 between days 14 and 21). In the later stages of pregnancy, at least some of these boPAG molecules reach
detectable concentrations in the maternal circulation, thus being used for pregnancy diagnosis and the subsequent follow-up of placental secretory function, from the 28th to the 30th days or until parturition (18, 21).

The first PAG purified from bovine fetal cotyledons has been named boPAG-1 or boPAG56kDa (29). As shown by Xie et al. (27), this protein belongs to a family of proteolytic enzymes known as aspartic proteases, with more than 50% amino acid sequence identity to pepsin, cathepsin D, and cathepsin E. However, because of key mutations close to the active site, this molecule is catalytically inactive.

More recently, Klisch et al. (10) have isolated 3 additional PAG molecules from bovine cotyledons: boPAG56kDa, boPAG67kDa, and boPAG57kDa. The glycosylation pattern of PAG changes during different periods of pregnancy (11). Therefore, it has been hypothesized that PAG may have different carbohydrate-mediated functions at various stages of the pregnancy (12). The variable degree of glycosylation in the different PAG molecules has also been claimed to be an important factor regulating the plasma half-life of these proteins, including their peripheral concentrations (10). No precise function has been experimentally assigned yet for molecules from the boPAG-1 group (26). However, their high level of expression in early gestation (23) points to a fundamental role for these molecules in embryo implantation and placentogenesis (8).

1. PAG detection by RIA and ELISA

1.1. The classical PAG-RIA-497

In 1992, Zoli et al. (31) described the validation of a homologous PAG-RIA using boPAG67kDa as the standard, tracer, and immunogen for antiseraum production (AS8497). This RIA (RIA-497) can detect PAG in the maternal circulation of some cows at around Day 28 of pregnancy but in all cows from the 30th to the 35th days of pregnancy (concentrations higher than 0.5–0.8 ng/ml). In the early and middle stages of gestation, their concentrations increase slowly and gradually, remaining lower than 160 ng/ml until the 240th Day of pregnancy. Around parturition, the PAG concentrations increase rapidly to reach peak values of 1,000 to 5,000 ng/ml a few days before delivery. PAG concentrations decrease steadily in the postpartum period, reaching undetectable levels around day 100 postpartum. The relatively long interval needed for PAG to be cleared from the maternal circulation can be explained by the very high concentrations present in maternal blood at parturition and the long half-life of this glycoprotein, estimated to be 7.4 to 9 days (9).

1.2. Alternative PAG-RIA

Improving the existing PAG-RIA methods by testing new AS has been attempted because of the temporal expression of different, as yet unpurified, PAG molecules during early pregnancy. Thus, several authors have reported a distinct ability of different AS to detect PAG epitopes during early pregnancy in cattle. Perényi et al. (17), for the first time in 2002, compared the use of anti-boPAG67kDa AS with new ones raised against PAG molecules from caprine placenta (PAG55+62kDa and PAG56+59kDa) and boPAG65kDa and boPAG75kDa, respectively. As described by Zoli et al. (31), concentrations of PAG are higher in the maternal than in the fetal serum, suggesting that this glycoprotein is directed preferentially into the maternal system.

Concentrations of PAG in the maternal plasma or sera are correlated with the placental mass (24), which in turn is related to the stage of pregnancy (25). Although the fetal number has also been proposed to influence the PAG concentrations from Day 30 (16) or from Day 180 until term (24), this parameter cannot be used for the prediction of twin pregnancies.

Investigations carried out in peripartum animals suggest a positive influence of both maternal environment and fetal genotype (race and sex) on the peripheral blood concentrations of PAG molecules. Mean peripartum PAG concentrations are higher in Hereford cows than in Holstein cows or heifers carrying purebred Holstein fetuses (31).

As described by Lopez-Gatius et al. (13), other parameters such as the sire’s genotype are also correlated with the PAG concentrations. Interestingly, the same authors have described that the risk of fetal loss in the warm season was 10 and 6.8 times more likely in cows with low (<2.5 ng/ml) or high (>4.0 ng/ml) PAG concentrations, respectively, measured at day 35 post-AI (14).

Another original approach, recently developed by Lopez-Gatius et al. (14), determines the plasma levels of bovine PAG (measured by RIA-497 and RIA-706) and P4 in Holstein Friesian dairy cows. Cows are followed by venipuncture on Days 35, 42, 49, 56, and 63. The results show an interaction between milk production and PAG levels at day 63 using both the RIA systems. PAG concentrations decreased when the daily milk production increased, whereas the P4 levels remained unaffected by milk production. It remains to be determined whether the PAG decrease is due to an increased traffic of PAG to the mammary gland or due to their increased clearance as a consequence of higher metabolic activity.

3. Pregnancy diagnosis

The measurement of PAG concentrations in the maternal blood helps in the confirmation of pregnancy (pregnancy diagnosis) and in following the trophoblastic function in both research programs and veterinary practice. In cattle, the concentrations of PAG can be measured from Days 28 (21, 22) to 30 (7, 18) after breeding or AI, with a sensitivity of 92%. Sensitivity increases at Days 37–38 to reach 99–100% (Table 1). The specificity calculated for different previous reports ranged from 80 to 90%, mostly because of naturally occurring embryonic mortality.

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The measurement of PAG has been further developed for routine analysis in veterinary laboratories. The leading country for pregnancy diagnosis is France, with almost 100,000 pregnancy diagnoses per year, followed by Belgium (6,000) and other European countries such as Italy, Spain, and Scotland.

4. Clinical application of PAG-RIA for pregnancy follow-up

In the cow, the embryonic period extends from conception to the end of the differentiation stage (around day 42), whereas the fetal period extends from day 42 to parturition. Most pregnancy losses occur during the early pregnancy period, although the incidence of early fetal loss is increasing under the current intensive management systems, especially used for dairy-cattle production. In this context, the assay of PAG can provide very useful information for researchers working in programs that are focused on embryonic and fetal mortalities, infectious diseases, and embryo biotechnology (embryo transfer, in vitro fertilization, and somatic nuclear transfer). Some aspects of the PAG concentrations under different physiopathological conditions are described below.

4.1. Study of infectious embryonic/fetal mortalities

Measurement of PAG/PSPB concentrations after infectious (Actinomyces pyogenes) abortion in cattle was first described by Semambo et al. (19). These authors reported that sequential monitoring of PSPB enables the identification of embryonic death, when a continuous fall in its plasma concentration is observed. Sequential measurement of PAG in goats also facilitated the determination of the onset of disturbance in trophoblastic activity, associated with the death of one or multiple fetuses (28). Therefore, the systematic application of PAG-RIA in herds with a high rate of pregnancy failure could help pinpoint the phenomena that might be implicated in triggering these events.

4.2. Study of noninfectious embryonic/fetal mortalities

The simultaneous measurement of both P4 and PAG concentrations in the maternal peripheral blood has been very useful in the elucidation of some physiopathological conditions related to noninfectious pregnancy failure in ruminant species. In this approach, the P4 level provides the information regarding the corpus luteum functioning, whereas PAG levels reflect the trophoblastic secretory function of the developing conceptus (21) (Figure 1).

4.3. The use of embryo biotechnology and the follow-up of placental secretory function

In nuclear-transfer programs, even if the majority of calves are of normal size, a few among them can develop morphological abnormalities such as edema of the umbilical cord with placental hypertrophy. As early as in 1996, Ectors et al. (3) described higher concentrations of PAG in the maternal blood of recipient heifers carrying cloned embryos/fetuses. Abnormally high PAG concentrations in the maternal circulation were observed concurrent with an abnormal amount of trophoblast tissues, as observed in hydatiform molar pregnancy (3). Similarly, Patel et al. (16) described higher PSPB concentrations in the maternal circulation of a cow that gave birth to a schistosomus reflexus calf.

More recently, by analyzing serial PAG determinations (RIA-497, RIA-706, and RIA-708) and successive ultrasound fetal measurements, Chavatte-Palmer et al. (2) showed that cattle recipients carrying somatic clones showed alterations both in the rate of their development and in their PAG concentrations. Between days 34–36 and 50 of pregnancy, the concentrations determined by RIA-497 and RIA-706 were higher in cows carrying clone pregnancies than in the controls (in vitro-fertilized embryos). However, in clone pregnancies, the PAG concentrations determined by both the aforementioned PAG-RIA systems were lower in cows that had

### Table 1. Diagnosis of pregnancy in cattle by PAG measurement.

<table>
<thead>
<tr>
<th>Day of gestation</th>
<th>N</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>References</th>
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<tr>
<td>29–30</td>
<td>138</td>
<td>92.0</td>
<td>82.6</td>
<td>81.6</td>
<td>92.5</td>
<td>Szenci et al. (22)</td>
</tr>
<tr>
<td>35</td>
<td>430</td>
<td>98.9</td>
<td>87.5</td>
<td>93.0</td>
<td>97.9</td>
<td>Zoli et al. (31)</td>
</tr>
<tr>
<td>37–38</td>
<td>125</td>
<td>100</td>
<td>84.0</td>
<td>83.5</td>
<td>100</td>
<td>Szenci et al. (22)</td>
</tr>
</tbody>
</table>

PPV: positive predictive value; NPV: negative predictive value.

**Figure 1.** Plasma PAG and P4 concentrations in four cows in which an initial positive (P) ultrasonographic pregnancy diagnosis was followed by a negative (N) pregnancy diagnosis based on PAG/P4 concentrations or by direct ultrasonographic observation of fetal death. Day 0 corresponds to the day of the first artificial insemination. Arrows indicate the probable time of embryo/fetus death. The dotted line represents the concentration of progesterone. The black continuous line represents the PAG concentrations. FM implies fetal death. (Adapted from Szenci et al. (21)).
experienced early pregnancy loss (between days 35 and 90) compared to cows that continued the pregnancy until a later date.

CONCLUSION
On the basis of the results from international collaborative studies, it has been shown that PAG levels are good indicators of fetal-placental well-being and that alterations of PAG levels can precede pregnancy failure.

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Recent trends in the molecular diagnosis of emerging and reemerging transboundary animal diseases and in the detection of food- and waterborne infections

A review from the OIE Collaborating Centre for Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine

Sándor Belák*, Karl Ståhl

Abstract

Transboundary animal diseases (TADs) are highly pathogenic infectious maladies that migrate across boundaries between regions or countries, causing very high economic and socioeconomic losses. These diseases are fought at the international level by international organisations such as the World Organisation for Animal Health (OIE), supported by international expertise. OIE Collaborating Centres are centres of expertise in a specific designated sphere of competence, with a mandate to provide their expertise internationally. In this short review, some of the recent activities and achievements of the OIE Collaborating Centre for Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine, at the National Veterinary Institute (SVA), are summarised.

Keywords

Transboundary animal diseases, diagnosis, OIE Collaborating Centre, PCR, molecular epidemiology

Background

Transboundary Animal Diseases (TADs) is a term commonly used for highly pathogenic infectious diseases that migrate across the borders between countries, or spread over whole continents, causing major losses and emergencies in large geographic areas. TADs are regularly emerging and re-emerging, with disease outbreaks such as rinderpest, foot-and-mouth disease, bluetongue, African horse sickness, highly pathogenic avian influenza, Newcastle disease, classical swine fever and African swine fever causing very high economic and socioeconomic losses in many regions of the world. Considering the global importance, international organisations such as the World Organisation for Animal Health (OIE, previously known as Office International des Epizooties), the Food and Agricultural Organisation (FAO) and the International Atomic Energy Agency (IAEA) are fighting the TADs at the international level. Simultaneously, the EU, the USDA and other international and national programmes and grants are supporting the work for the improved detection and control of TADs. As an example, in 1994 the FAO established an Emergency Prevention System (EMPRES) for Transboundary Animal and Plant Pests and Diseases in order to minimize the risk of such emergencies developing. In the “Animal Diseases” component of EMPRES major TADs are targeted, including rinderpest and other epidemic animal diseases, such as contagious bovine pleuropneumonia, foot-and-mouth disease, bluetongue, African horse sickness, highly pathogenic avian influenza, Newcastle disease, classical swine fever and African swine fever causing very high economic and socioeconomic losses in many regions of the world. 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as those utilized in the LightCycler® (Roche), dye-labelled oligonucleotide ligation (DOL) and SYBR Green, showed high sensitivity and specificity for the detection of viral pathogens in particular, but also bacteria. These methods provided novel, powerful and rapid means for virus detection and identification and greatly reduced hands-on time compared to previous PCR assays, where the products were run on agarose gels and stained for visualisation. The single-run amplification on the 96-well microtitre plate format allowed the easy automation of the diagnostic amplification procedures. The diagnostic work was further automated by using robotics for nucleic acid extraction and pipetting. Compared to the previous gel-based (“classical”) amplification assays, the real-time PCR techniques have a further advantage: they allow the quantitative assessment of the targeted viral genomes. This can be very important in viral diagnosis, such as in the case of diagnosis of Postweaning Multisystemic Wasting Syndrome (PMWS), where the number of viral particles or the viral loads of porcine circovirus type 2 (PCV2) have to be determined (see listed reviews).

In the diagnostic laboratories the TaqMan and MB are the most commonly used real-time PCR methods today, but other approaches, like the PriProET system, and LUX PCR are also frequently applied. Recently further real-time PCR methods, such as the LATE PCR are also considered as robust, reliable assays for improved detection of various viruses.

**Sensitivity and specificity of the real-time PCR assays, detection range**

Some of the real-time PCR assays are able to detect as few as ten genome copies of the viruses, which indicates very high analytical sensitivity. Concerning specificity, the majority of the assays is able to detect and amplify entirely the selected target nucleic acids and no cross-reactivity is disturbing the diagnosis. On the other hand, “wide spectrum”, “pan-” or “general” PCR assays are also in use. Such methods are for example the “pan-pesti” PCR assays, which amplify very conservative regions of the pestivirus genomes (e.g., selected regions from the 5’NCR), and are able to amplify all tested pestiviruses, such as bovine viral diarrhoea virus (BVDV), classical swine fever virus (CSFV) and Border disease virus (BDV). A diagnostic laboratory can work very effectively when using an arsenal of wide-range assays for preliminary screening of the samples and highly specific PCR assays for the exact identification of the detected pathogens.

**Molecular epidemiology**

The PCR products can be investigated and analysed by several means. The nucleic acid composition of the products can be determined through nucleotide sequencing. The obtained nucleic acid sequences can be analysed and compared with each other and with previously described sequences, obtained from large international databases, such as the GenBank. The rapid phylogenetic identification and tracing of viruses is termed “molecular epidemiology”. Molecular epidemiological studies were conducted, for example, when genetic variants of classical swine fever virus (CSFV) were identified in several countries of Central Europe and when it was hypothesised that EU and US genotypes of the porcine respiratory and reproductive syndrome virus (PRRSV) evolved from a common ancestor, which is suspected to originate from Eastern Europe [12, 15]. Also, a molecular epidemiological approach was implemented as part of the Swedish BVD-control programme as a tool to facilitate the identification and tracing of routes of transmission of bovine viral diarrhoea (BVD) between herds [14].

**Recent achievements at our OIE CC**

In the following paragraphs examples are given for the illustration of several new technical approaches, developed for the improved diagnosis of TADs in various host species and for improved food safety.

**Development of novel TaqMan® and Primer-Probe Energy Transfer assays for the improved, universal detection of Hepatitis E virus**

Hepatitis E virus (HEV) is an important cause of food- and waterborne diseases in countries with poor sanitation, but recently it is getting more frequent also in regions of the world where the health services are of high standard. Previously the disease cases were observed in connection to travelling, recently zoonotic transmission is also suspected, i.e., a direct route of infection, from animals to humans. For the improved detection of the virus, two real-time PCR methods were developed and compared: a TaqMan® and Primer-Probe Energy Transfer (PriProET) assay. These robust, highly sensitive methods provide valuable diagnostic tools to investigate zoonotic transmission, to detect the virus in the food chain. They are used in research related to the potential of hepatitis E virus to cross the species barrier. By using the two novel PCR assays a broad range of viruses were detected, representing all the four genotypes of HEV. On comparison, the TaqMan® assay showed higher fluorescence values for positive samples. On the other hand, the PriProET better tolerated the point mutations in the target nucleic acids. Thus, the PriProET provides a more powerful tool to detect new variants of HEV. The two real-time PCR assays are useful novel tools for virus detection and for molecular epidemiology. In addition, the assays provide novel tools to study the biology of the viruses, including the transmission between various species and the zoonotic aspects of HEV infections [further details see in Ref. 8].

**Development of a real-time PCR assay based on primer-probe energy transfer for the detection of swine vesicular disease virus**

Based on primer-probe energy transfer (PriProET), we developed a real-time PCR assay to detect swine vesicular disease virus (SVDV). The assay was highly sensitive with a detection limit corresponding to five copies of viral genome equivalents, and had a high specificity demonstrated by testing of heterologous viruses. A major advantage of the PriProET chemistry is tolerance toward mutations in the probe region. Melting curve analysis directly after PCR, with determination of probe melting point, confirmed specific hybridisation of the SVDV strains. Eight of twenty SVDV strains tested, revealed shifted melting points that indicated mutations in the probe region, which were confirmed by nucleotide sequencing. With the PriProET system there is a chance to identify phylogenetically divergent strains of SVDV, which may appear negative in other probe-based real-time PCR assays. Moreover, any difference in melting points may provide an indication of divergence in the probe region. The described SVDV PriProET assay, with high sensitivity, specificity, and tolerance toward mutations in the probe region, provides a powerful tool for the improved and rapid detection of SVDV. Furthermore, it allows a reduced turnaround time and the use of high-throughput, automated technology [further details see in Ref. 9].

**Simple and rapid detection of swine vesicular disease virus with a one-step reverse transcriptase loop-mediated isothermal amplification assay**

A one-step reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) assay was developed recently for the improved detection of swine vesicular disease virus (SVDV). The assay provided a wide SVDV detection range, since all the 28 tested isolates of this virus were tested positive. Simultaneously, it yielded high specificity; because all tested heterologous viruses gave negative results, such as foot-and-mouth disease virus (FMDV) and vesicular stomatitis virus (VSV). Since SVDV, FMDV and VSV cause very similar symptoms, the highly specific detection and identification of SVDV is very important. By testing RNA from clinical samples including nasal swabs, serum and faeces, the performance of the RT-LAMP was...
compared to a real-time PCR assay. When testing nasal swabs and serum, the sensitivity of the assays was approximately equivalent. Interestingly, by testing faecal samples the RT-LAMP assay performed better. According to our hypothesis, inhibitory substances probably less influenced the RT-LAMP assay and this could be the reason of the better performance. The RT-LAMP assay has several strong features, which prove the applicability as a powerful new tool of SVDV detection: i) it is an isothermal amplification method, which does not require costly PCR machines, just a simple thermoblock; ii) it is rapid, because results are obtained within 30-60 minutes; iii) it is highly specific and sensitive, as described above; iv) the test reading is simple, since the results are visualised either by gel- electrophoresis or by the naked eye through the addition of SYBR Green (Figure 1). Since the RT-LAMP can easily be performed in modestly equipped field laboratories and it can be adapted to mobile diagnostic units, it provides novel tool for the “front line” diagnosis of swine vesicular disease, an important TAD [further details see in Ref. 6]. Considering the above-listed advantages of the RT-LAMP technology, we are adapting this method to the improved diagnosis of a range of viral diseases in various host species, including cattle.

**Subtyping and pathotyping of avian influenza viruses with a one-step real-time SYBR Green RT-PCR assay**

For the rapid subtyping and pathotyping of avian influenza viruses (AIV) a one-step real-time SYBR Green RT-PCR assay was developed. Primers were selected to target highly conserved nucleotide stretches that flank the cleavage site of the haemagglutinin (HA) gene of AIVs. By sequencing the amplified PCR products, both the subtype and in case of H5 subtypes even the pathotype of the detected AIV can rapidly be identified. By testing 27 strains of AIV and nine heterologous pathogens, including influenza B and C, and various avian viruses, the specificity of the assay was confirmed. Since the subtype and pathotype determination were completed within approximately six hours, the SYBR Green RT-PCR assay provides a powerful new tool in the arsenal of influenza diagnostics [further details see in Ref.16].

**Padlock probes for broad-range detection and subtyping of avian influenza viruses**

Using padlock-probe chemistry for multiplexed preamplification and microarray for detection, we developed an assay for the simultaneous detection and subtyping of avian influenza viruses (AIV). The assay has the outstanding feature to identify both the hemagglutinin (HA) and the neuraminidase (NA) surface antigens of AIV from a single reaction. We tested 77 influenza strains, representing the entire assortment of HA and NA, and 100% (77/77) of the samples were identified as AIV and 97% (75/77) were correctly subtyped. The specificity of the assay was determined testing heterologous pathogens. The results indicate that the assay is a useful and robust tool for high throughput rapid detection and typing of AIVs, with advantages compared to conventional methods [further details see in Ref. 7].

A simple magnetic bead-based microarray for detection and discrimination of pestiviruses

A novel assay was developed for the rapid detection and discrimination of pestiviruses, i.e., BVDV types 1 and 2, CSFV and BDV, by using magnetic bead detection of PCR products on microarrays. After amplification, the PCR products are hybridized onto an array, followed by visualization with streptavidin-coated magnetic beads. The simple set-up allows visualization of results on the array either with the naked eye or a microscope, and makes this novel assay suitable for use in a modestly equipped laboratory.

A panel of pestiviruses comprising members of all the four accepted species was used to evaluate the assay. Other post-PCR detection methods (e.g., gel electrophoresis and suspension hybridization) were used as comparisons for the determination of the detection sensitivity of the assay. The results clearly indicate that the assay provides a novel, robust and highly sensitive and specific method for the improved detection and discrimination of viral pathogens. Considering the simplicity of the assay, and the very simple detection procedure in particular, this magnetic bead-based assay offers a powerful and novel technology for molecular diagnostics in virology [further details see in Ref. 10].

**Detection of an emerging pestivirus in cattle and further characterization by means of molecular diagnostics and reverse genetics**

During a study on Bovine Viral Diarrhoea (BVD) epidemiology in Thailand, by using indirect antibody ELISA, an antigen ELISA and PCR, a pestivirus was detected in heat-inactivated serum sample of a calf. The PCR product was sequenced and the comparative nucleotide sequence analysis showed that this virus was closely related to a recently described atypical pestivirus (D32/00_“HoBi”) that was first isolated from a batch of foetal calf serum collected in Brazil (Figure 2). It was also demonstrated that the Thailand virus (called Th/04_KhonKaen, or TKK) was circulating in the herd [13]. The study was the first to report a natural infection in cattle with a virus from this group of atypical pestiviruses. The data suggested that these viruses might be spread in cattle populations in various regions of the world. If so, these atypical bovine pestiviruses can have important implications for BVD control and for the biosafety of vaccines and other biological products, produced with foetal calf serum. To study these
important issues, we needed a “live” virus, which can be replicated and studied from various aspects. Since the serum was inactivated, virus isolation was not possible. Thus, we applied methods of reverse genetics, in order to “reconstruct” the inactivated virus from the inactivated serum sample. By using transfection of the viral nucleic acids, the virus was reconstructed and re-gained its capacity to grow in cell cultures. Full-genome characterization of the new virus was performed after recuperation of the inactivated virus through transfection. This characterization, and phylogenetic analysis based on the full genome sequence, demonstrated that the virus was closely related to BVDV, suggesting that TKK and other HoBi-like pestiviruses constitute a third genotype of BVDV, i.e. BVDV type 3 (BVDV-3) [for further details see Ref. 11].

In summary, the listed results provide a brief view on the development and application of novel, biotechnology–based methods in the diagnosis and characterisation of emerging and re-emerging animal diseases, including TADs. Furthermore, the applicability of novel methods for the improved detection of food-, and waterborne pathogens was also shortly touched. This review is providing a brief summary of the activities of our OIE CC in the biotechnology-based diagnosis of infectious diseases in veterinary medicine and in food safety.

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Association between passive transfer and antimicrobial resistance in the enteric flora of calves

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ABSTRACT
Failure of passive transfer of immunity and hypogammaglobulinemia in calves remain very common on US dairy farms, leading to increased incidence and severity of calfhood infectious diseases. These diseases correspondingly result in increased therapy with antimicrobial drugs, which is likely to contribute to the high level of antimicrobial resistance among both the normal enteric flora and the pathogens of young calves. We conducted a series of field trials to determine the relationship between failure of passive transfer and antimicrobial treatment and antimicrobial resistance. The results indicated that failure of passive transfer remains among the strongest indicators of the risk of infectious disease, antimicrobial treatments and death. Farms varied widely in the success of passive transfer management. In addition, some farms administered antimicrobial treatments to a very high proportion of low risk (eugammaglobulinemic) calves. The intestinal flora of calves treated with antimicrobial drugs exhibited increased antimicrobial resistance and calf flora generally was more resistant than that of adults, even on farms that used no antimicrobial drugs.

KEYWORDS
Passive immunity; antimicrobial resistance; morbidity and mortality; overuse of antimicrobial drugs; bovine/cattle/calves

The strong association between acquisition of colostral immunoglobulin and reductions in incidence and severity of infectious calfhood diseases has been documented for decades.1-3 In a recent series of 3 field trial on calf ranches including 273 calves that were monitored from day of arrival onto the calf ranch as day-old calves until 28 days of age, mortality was 40% for calves with failure of passive transfer (FPT) (<350mg/dl serum IgG at 24 hours of age). Calves with partial failure of passive transfer (P-FPT = 351-999 mg/dl serum IgG) had a 12% mortality rate, compared with 2% in the calves with adequate passive transfer (APT = ≥1000 mg/dl serum) (p < 0.0001). Calves were monitored daily for health, attitude and appetite by a veterinarian blinded to the calves colostrum status. Antimicrobial treatments were administered on 21%, 11% and 5% of FPT, P-FPT and APT calf-days, respectively. Diarrhea was noted on 15.4%, 9.6% and 5.6% of FPT, P-FPT and APT calf-days, respectively (p < 0.0001). Respiratory disease, including nasal discharge, coughing and signs of pneumonia, was observed on 6%, 4% and 2.5% of FPT, P-FPT and APT calf-days, respectively. Lastly, there was also an effect of passive transfer status on weight gain in the calves: Calves with FPT gained 0.39 kg/d, markedly less than the 0.51 kg/d gained by the APT calves (ANOVA, controlling for birth weight and location, P<0.01). External stressors may greatly increase the mortality risk of FPT; in another study of 180 calves conducted in the summer of 2006 during a period of extreme heat stress, FPT calves experienced a mortality rate of almost 50% (Figure 1).

Calves typically possess an enteric flora with a markedly higher degree of multiple antimicrobial resistance compared to adult cattle.4-8 Enteric flora of neonatal animals exhibiting a higher degree of antimicrobial resistance than older animals is not unique to cattle and similar patterns have been documented in many other species.8,9 At least in calves, this seems likely to be due in part to the relatively high incidence of infectious diseases requiring antimicrobial therapy compared to older animals on the farm, resulting in increased selection pressure for resistance in the flora of young calves.1 Interestingly, a similar pattern albeit less marked tendency is clearly evident in organic farms where no antimicrobial therapy is used, indicating the existence of factors other than local antimicrobial drugs use.10 Nevertheless, the increased resistance of calf enteric flora on conventional farms compared to organic farms indicates that calf antimicrobial therapy does result in a selective effect. As the mobile resistance elements carried by the normal enteric flora of cattle are deemed to represent some risk to public health due to the potential for their transfer to zoonotic pathogens and as a similar risk undoubtedly exists for resistance transfer to bovine pathogens, we examined to interactions between passive transfer, the incidence of infectious diseases requiring antimicrobial therapy, and the effects on the antimicrobial resistance of the normal E. coli flora of dairy calves.

Management systems have been developed that ensure adequate colostral immunoglobulin absorption by a high proportion of calves even in modern dairy cattle (where natural suckling systems frequently fail to achieve this). In addition, oral supplements are now available that contain an adequate immunoglobulin mass to replace colostrums when it is unavailable.11 Nevertheless, failure of passive transfer remains a common phenomenon across the USA, with >40% of heifer calves obtaining <1000 mg/ml IgG.12 Recently, Berge et al. demonstrated an overall rate of failure of passive transfer (Serum IgG ≤ 1000 mg/dl) of 45% based on a study of over 700 calves on 33 California farms (Figure 2). Factors that contribute to increased failure of passive transfer include 1) use of bottle feeding systems, which generally fail to result in an adequate
volume of dairy colostrums ingestion, 2) use of colostrum pools, which typically have lower immunoglobulin concentration due to the effect of those cows which produce high volume colostrums, 3) when calves are force fed colostrum, use of an inadequate volume of colostrum that fails to contain an adequate immunoglobulin mass, and 4) failure to feed colostrum soon enough after birth.

Even calves with excellent passive transfer are typically affected by one or more episodes of diarrheal disease during the first month of life. The calf scour complex includes diverse viral, bacterial and protozoal agents, most of which are not effectively treated by antimicrobial drugs. Nevertheless, it is a not uncommon practice on large U.S. dairy farms to both include one or more antimicrobial drugs in the milk diet for at least part of the first month of life and also to include antimicrobial drugs as part of a routine therapeutic regimen for calf scour. Despite the overall dearth of studies demonstrating efficacy of either prophylactic or therapeutic antimicrobial treatments, particularly for calf diarrheal disease.

It is also the common practice for veterinarians to provide large dairies with specific treatment protocols, including indications for treatment, specific drugs, doses, and treatment durations. However, calf crew personnel, in an attempt to minimize calf losses and to conscientiously perform their duties, frequently may be inclined to treat more scouring calves than truly need or would benefit from drug therapy. A US national study in 1994 revealed that 25% of heifers on dairies were treated with injectable antimicrobials and 30% were given oral antimicrobials during their first few weeks of life. Even calves with excellent passive transfer are typically affected by one or more episodes of diarrheal disease during the first month of life. The calf scour complex includes diverse viral, bacterial and protozoal agents, most of which are not effectively treated by antimicrobial drugs. Nevertheless, it is a not uncommon practice on large U.S. dairy farms to both include one or more antimicrobial drugs in the milk diet for at least part of the first month of life and also to include antimicrobial drugs as part of a routine therapeutic regimen for calf scour. Despite the overall dearth of studies demonstrating efficacy of either prophylactic or therapeutic antimicrobial treatments, particularly for calf diarrheal disease.

For example, we monitored antimicrobial treatments of calves during the first month of life on a Washington state dairy. This dairy had an effective colostrum management system in place that resulted in relatively few calves with failure of passive transfer (7% with serum IgG <1000 mg/ml). The calves were fed pasteurized whole milk for the first month of life and as expected, calf health on this dairy was generally good and calf mortality was consistently <5%. In this farm, calf health protocols set up by the herd veterinarian included antimicrobial drug supplementation of the milk diet during the first two weeks of life with tetracycline and neomycin. In addition, calves observed with severe watery diarrhea received antimicrobial drug treatments. Monitoring of calf health records demonstrated that >90% of the calves received primary treatment with sulfafoxazole/trimethoprim and penicillin G for 4 days and 69% of these calves received follow-up treatment with lincomycin/spectinomycin (LS50) for 5 days and penicillin G for a further 3 days. In addition, more than half of the calves that did not receive the primary treatment nevertheless received the LS50 regimen. The treatments were typically initiated at approximately 10-20 days of age in response to undifferentiated calf diarrheal disease.

In response, we provided this farm with a modified protocol designed to reduce prophylactic and therapeutic antimicrobial therapy, and performed a field trial to test the new protocol. The results of the field trial failed to detect any beneficial effect of the milk antimicrobials but rather revealed a strong tendency towards adverse health effects: Calves receiving the supplement during the first 2 weeks of life were 1.3 times more likely to scour than calves not receiving antimicrobials in the milk (p=0.06). Cessation of routine use of antimicrobial supplementation of milk diets for calves in the first two weeks of life was therefore expected to be either neutral or beneficial for calf health while estimated to save approximately $1.50/calf in drug costs, not including the labor costs associated with administering the medication. The revised protocol also modified the therapeutic plan for uncomplicated diarrhea, and antimicrobial therapy was instituted only when diarrhea was complicated by an indication of more severe illness such as complete anorexia or severe depression and this targeted treatment strategy saved the dairy $5/calf. Rectal temperatures were checked on all calves with diarrhea and only those calves with a temperature over 39.5°C were treated with antimicrobials. The temperature checks revealed a strong correlation between the presence of fever and anorexia or depression. Institution of this revised protocol resulted in a marked decrease in the percentage of calves treated with oral or parenteral antimicrobial drugs, while the mortality rate remained unchanged. The revised therapeutic protocol was determined to result in similar or improved health status with significant reductions in drug and labor costs.

Antimicrobial therapy has detectable effects at both the herd and the individual animal level. The clearest demonstration of the herd effects can be seen in the comparison of organic herds on which no antimicrobial drugs are used with conventional herds. For example, Figure 3 shows the differing frequencies of multiple antimicrobial resistance in the enteric E. coli flora of calves and adult cattle on northwestern U.S. farms. Similarly, direct comparison of the antimicrobial resistance of the E. coli flora of calves that had received antimicrobial therapy within 5 days demonstrated a significantly more resistant flora in the treated calves.

In summary, the results of these field trials indicate 1) that passive transfer failure of immunity in calves remains very common on dairy farms, despite the existence of management systems demonstrated to greatly reduce its incidence, 2) that as expected, excess morbidity and mortality is associated with failure of passive transfer, 3) that even in the presence of adequate passive transfer and the resultant low risk of mortality due to infectious diseases, some farms use antimicrobial drugs in excessive amounts, including routine in-feed supplementation and individual calf oral or parenteral treatments, and 4) that antimicrobial therapy of calves, including that required to increase survival of high risk failure of passive transfer calves and also excessive and unnecessary treatment of low risk calves, has the direct result of increasing
the antimicrobial resistance of the enteric flora of these calves, increasing the risk of transfer of resistance to both calf pathogens and zoonotic agents.

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Farm management: The “invisible cow” in herd health practices?

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ABSTRACT

Dairy farm management comprises external farm and internal cow management.

External management deals with eight operational farm functions, which are controlled by the farmer while internal management is involved with a number of biological systems, which are under the control of the autonomic nervous system.

The management activities of the internal biological systems are not visible to the naked eye, as might be some unobserved or neglected external management activities. Together they have been labelled as “the invisible cow”.

Veterinary food animal practitioners need a deep insight into the interrelated management activities of external farm functions and internal biological systems in addition to their clinical skills.

External management manipulates internal management.

Management of the biological systems inside the cow determines cow and herd performances.

Manipulation of external management activities will only result in a more consistently healthy and productive herd performance when there is a thorough understanding of the impact of the visible and ‘seemingly invisible’ external management practices and environmental conditions upon the functioning of operational biological systems and processes inside the cow.

Herd performance can be analyzed via either an outside-inside or an inside-outside approach.

The inside-outside approach addresses management activities of 1) of internal biological systems and 2) external operational farm functions.

The concept of the inside-outside analytical herd performance approach can be easily incorporated into veterinary management advisory services.

To make the veterinary food animal practitioner a better advisory partner for the dairy farmer, veterinary and agricultural academic organizations should offer food animal veterinarians master’s courses in Herd Health and Production Management, under mutual responsibility.

KEY WORDS

Management, invisible cow, inside outside approach, veterinary services and education.

INTRODUCTION

Due to the fact that dairy herds increase in size and that dairy farmers have to fulfill consumer demands and government regulations they are forced to manage their farms more efficiently, economically and innovatively. This puts a heavy load on management capabilities. Dairy farm management comprises external farm and internal cow management. Both external and internal management activities have the ultimate goal of bringing the genetic makeup of the cow to full expression for health and reproductive performance, and milk production. The question is whether veterinarians are able to adequately support dairy farmers’ in their daily management, because limited attention is paid to the ins-and-outs of farm management in the veterinary curriculum.

The objectives of this presentation are to emphasize the importance of management in relation to health and reproduction performance and milk production. This will be done by first studying the characteristics of external farm management and internal cow management and to identify the relationships between their components. Subsequently, the concept of the “invisible cow” in management will be presented. Special attention will be paid to the contrast between

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the outside-inside and the inside-outside approaches to herd performance analysis. Finally, the implementation of the inside-outside analytical herd performance approach in veterinary management advisory services will be discussed.

CHARACTERISTICS OF EXTERNAL FARM AND INTERNAL COW MANAGEMENT

External dairy farm management deals with the regulation, manipulation, and control of eight interrelated operational farm functions, each with its own specific processes (1). These are crop/fodder production, nutrition, replacement rearing, health care, reproduction, milk production, buildings/labor/equipment, and financing/budgeting or cash management (Figure 1, left). External management also includes manipulation of environmental conditions related to these functions. Each of the eight operational functions is made up of a number of components. For example, health care comprises infectious and metabolic diseases.

Figure 1. External (left) and internal (right) operational functions controlled by management of the farmer and the cow respectively

Internal cow management is involved with the regulation of the functioning of a number of biological systems inside the body such as: the intermediary metabolic, immunologic, neuro-endocrine systems, and others (Figure 1, right). They are managed by the autonomic nervous system via homeostatic and homeorrhetic control mechanisms in a very coordinated manner (2).

Figure 2. Some interrelationships between external dairy farm functions

The management of each of the eight operational dairy farm functions must always be monitored in conjunction with other external functions and never in isolation. For example, management of reproduction is closely related to nutrition, labor, equipment, and housing as well as to health care and milk production (Figure 2). Farm advisors therefore should not think about management of reproduction, nutrition, milk production, etc., as exclusive or distinct concepts, but rather as integrated management activities. Changes in management activities in one operational function always have direct or indirect effects on other functions. The same applies to operational management activities inside the body. In addition, external management activities continuously influence internal operational management activities (Figure 1). For example, the external operational farm function of crop production may influence intermediary metabolism through nutritional management in many ways. Subsequently, intermediary metabolism influences external performances of health, reproduction, milk production, growth of replacements, etc.

This means that nutritionists not only need to delve continuously into the depths of biochemistry but also to make a concerted effort to understand the basics of immunology, while immunologists need to become comfortable with the fundamentals of nutrition (4,5).

THE “INVISIBLE COW”

Management activities are either visible or invisible. Management can be compared to an iceberg of which only a small part is visible (Figure 3). For dairy farms we label the invisible part of management activities as the “invisible cow.” It comprises a number of components: 1) all managerial activities regulating biological functions inside the cow; 2) managerial activities regulating external operational farm functions and environmental conditions that are not observed or are neglected by the veterinary farm advisor; and 3) managerial activities that might not be
Examples of invisible management activities regulating operational body functions:
Examples of invisible internal management activities are the autonomic regulation of coordinated gene expression that controls cow performance, management activities of reproductive processes and many other biological systems. Internal management of biological systems can become indirectly visible in the form of health and reproductive performance and milk processes and many other biological systems. Internal example of seemingly invisible (unobserved) management activities regulating operational body functions:

Examples of seemingly invisible (unobserved) management activities that regulate external operational farm functions:

Examples of external management activities that might not be reported to the advisor:

Temporary loss of motivation by the farmer/herd manager who, for unknown reasons, neglects recommendations given, even when a positive outcome is ensured.

Incorrect answers by the farmer to advisor questions whereby the latter may come to the wrong conclusions.

Withholding of information by off-farm companies or by the farmer about activities that the veterinarian or nutritionist has no knowledge of or no opportunity to observe. For example, an unknown change in the composition of feed components in concentrates by the feed company.

Invisible and seemingly invisible managerial practices may play an essential role in dairy herd performances. If not taken into account during a farm inspection they may be responsible for unsuccessful advisory service outcomes. One of the advisor’s most important, yet least recognized roles is therefore to help make the invisible visible for the farmer, thereby enhancing the level of clarity and understanding of actual herd performance. To do this veterinary farm advisors have to become detectives during a farm inspection and users of their own observational “laboratory,” which includes all their physical senses. In addition, advisors should train themselves to ask crucial questions and to listen very closely to the answers given by the farmer.

The advisor should also realize that farmers often don’t have an idea on what is happening within and between the biological systems inside the cow and that these biological systems have their own rhythm. They might also be unaware of the fact that the functioning of the internal biological systems determines the level of herd performance. In turn, the cow does not understand what the farmer wants or cannot fulfill the farmer’s demands because the farmer speaks the language of profits instead of the "biological language" used by the cow. Increases in herd size and adoption of automation also increase the distance between the farmer and the cow.

FARM ADVISORY SUPPORT SERVICES

OUTSIDE-INSIDE AND INSIDE-OUTSIDE HERD PERFORMANCE ANALYSIS APPROACHES

Herd management can either be analyzed from the outside or the inside of the cow. Both approaches will be discussed in more detail in the next paragraphs.

Outside-Inside Herd Performance Analysis Approach

Traditional outside-inside analysis approaches commonly start with a farm/herd inspection thereby searching for risk factors related to actual management practices and environmental conditions (Figure 4, left). A retrospective monitoring, evaluation, and analysis of health, reproductive, and milk production data then follow this. Examples include analysis, and interpretation of herd milk production test data, somatic cell counts, body condition scores, reproductive data, laboratory test results, environmental conditions, and many others. Trends in outcomes of these activities are used as clues to solve problems by curative actions.

Within this approach treatments and adjustments are often restricted to a rearrangement of some external management activities, merely reacting to inadequate management in one or more operational farm functions. However, rearrangement of external management activities only lead to restoration of disturbed herd performances when they are causally related to processes within internal management systems such as intermediary metabolism or immunologic processes, etc. It should be stressed that herd performances are ultimately the result of management activities of internal biological system, which are continuously influenced by external management practices and environmental conditions. External management...
is only a steering wheel for internal management of the cow. If external management activities do not line up with management activities inside the cow such as intermediary metabolism and immunological processes, the measures taken will most often not result in desired improvements in herd performance. Treatments or adjustments of disturbances that are only taken at the level of external management can be characterized as making change by hoping for positive outcome.

A diagnosis of a disturbance can therefore never be considered as an endpoint but instead as a starting point to search for underlying causal management activities within internal operating biologic systems that contribute to disease and production inefficiencies. A crucial question is whether the management advisory services offered by the food animal veterinary practitioner is only directed at management activities of external operational farm functions and not towards creating optimal conditions for proper autonomic management of operational systems inside the cow.

Inside-Outside Herd Performance Analysis Approach

The "inside-outside" herd performance analysis approach, studies performance and performance-related problems from the inside of the cow instead of from the outside (Figure 4, right). This concept requires an in-depth understanding of the functioning and management of metabolic, neuro-endocrine, and immunologic operational systems inside the cow. Likewise, an appreciation is required of the limits of physiologic adaptations to physical and pathological stress.

The inside-outside approach starts with recapitulating available knowledge about the functioning of internal operational biological systems and continues with a search for relevant indicators of disturbed operational biological systems and processes by the farm advisor. The details of intermediary metabolism are central to understanding the processes in other internal systems and the external operational functions. For example, the advisor must understand how the Krebs cycle functions within the mitochondria of body cells and rumen bacteria and how it is influenced by external managerial activities such as nutritional management or stress caused by changing environmental conditions. Likewise, immunologic (udder defense) and reproductive (anovulatory) events are intertwined with intermediary metabolism and influenced by external operational functions.

If indicators of disturbed operational biological systems and processes are found, they are matched with actual external operational management activities and (adverse) environmental conditions that could be responsible. The matching activities will often lead to a diagnosis of one or more suboptimal performances. Recommendations then have to be formulated for adjusting external managerial practices and environmental conditions. Treatments and adjustments should not only be directed at curing disturbances in one operational farm function but also at correcting disturbances in other external functions. In addition, connections with potentially underlying causes in internal operational systems have to be examined and if found adjusted via related external functions.

This means that in case of clinical mastitis, as a component of the operational function of health care, treatments should always be followed with a thorough examination of other external operational functions such as nutritional management, labor, buildings, and equipment etc. Subsequently an examination of internal operational biological systems that are causally related to the disturbed intramammary infection resistance should be performed. This may lead to detection of a negative energy status, a disturbed calcium metabolism or other disturbances within internal systems. It is at the level of internal operational body systems where actual interactions with external managerial activities and environmental conditions take place. It is also the place where preventive and curative activities should start. In so doing, existing problems are then addressed at their roots and in a holistic manner.

The inside-outside approach cannot only be used to solve but also to prevent problems and to predict what will happen if recommendations are not implemented. The approach is therefore a double-edged sword that may cut in two directions.

THE INSIDE-OUTSIDE VETERINARY FARM MANAGEMENT ADVISORY SERVICE MODEL

The inside-outside herd performance analysis approach can be easily incorporated into an overall veterinary farm management advisory service-model (Figure 5). The model connects interactions between the cow, farmer, and farm advisor. Because the cow is at the center of the model the first step is to retrieve data on the indicators of “invisible” internal management activities that form the backbone of the advisors’ analytical approach. Data collection on indicators seems simple, yet it is often the hardest step. If indicated, laboratory tests should be conducted to become better informed and thus achieve a better understanding about the actual state of performance of the internal operational systems.

The second step is to find out what management practices of external operational functions are known and executed by the
farmer. It also requires detecting, as much as possible, those practices that are “unknown” and thus “seemingly invisible” and not executed by the farmer. Effort should be made to make them visible by interviewing the farmer in combination with a thorough farm and data inspection.

The third step includes matching the internal and external information sources together. This includes indicators of internal management activities, inadequate external farm management practices, and adverse environmental conditions. Everything that has been observed and heard must be evaluated while contemplating processes that are taking place inside the cow. It should then be determined which of these biological processes are affected and how the invisible processes might influence and improve external cow and herd performance. After matching all relevant factors, the results are translated into understandable recommendations for the farmer. The implementation of recommendations depends heavily on the readiness of the dairy farm management team to make changes to their management practices so as to improve operational functions inside the cow and subsequent herd performance as a whole. To stimulate cooperation of the farmer goals must be set with the aim to evaluate progress regularly and to show improvements.

Example of inside-outside advisory services

Figure 6 presents an example of the inside-outside approach, which deals with heat stress. Each consists of three stages: 1) physiological changes, (patho) physiological developments and adaptations of the cow to events over time; 2) the actual management activities of the farmer to respond positively or negatively to these events; and 3) responses of the farm advisor after matching the various findings.

![Figure 6. Practical example of interactions between the cow, farmer, and farm advisor during heat stress](http://photos/natural/iceberg.asp)

Traditional services have a common way of approaching problems namely reducing the number of cows that deviate from preset targets in order to obtain a healthier herd. In contrast, the inside-outside approach is directed at obtaining a healthy herd by optimizing operational biological systems inside the cow. The starting point is not the detection of external risk factors that might be related to a problem but the search for indicators of underlying disturbed operational systems at the organ, tissue, and even molecular levels.

DISCUSSION

Many food animal veterinary practitioners complain that owners of large dairy herds are not making full use of their services suggesting that a gap exists between what veterinary advisors are currently offering and what large dairy farmers need. To close the growing gap between food animal practitioners and farmers veterinary farm advisors should improve their insights into the breadth and depth of complex management activities related to external operational functions and internal biological systems. Daily external operational management practices of the farmer, the farm environmental conditions, and the management of the cow’s internal operational biological systems determine the profitability of a dairy enterprise. They also should renew or update their knowledge in biochemistry, immunology, endocrinology and other relevant disciplines and effects of external management practices and environmental conditions on these functions. The holistic inside-outside advisory service model presented in this paper is an important tool that can be used to accomplish this. A holistic approach to dairy herd veterinary management advisory services includes evaluation of all farm management practices and environmental conditions in connection with biological systems inside the cow (4,5).

In our opinion, the management-oriented education of the veterinarian has to be strengthened within the actual overwhelming disease-oriented education. This strengthening will provide the food animal veterinary practitioner more insight into how individual cow and herd performances are influenced by managerial practices and environmental conditions. Special attention should be given to nutrition and related interrelationships with biochemistry, immunology and other physiologic functions.

Veterinary and agricultural academic organizations should discuss the possibility of offering veterinary students that are interested in becoming food animal practitioners, joint master’s courses in farm management and related subjects.

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