Department Clinical Research and Veterinary Public Health
der Vetsuisse-Fakultät Universität Bern
(Leitung: Prof. Dr. T. Leeb)

Veterinary Public Health-Institut
Leitung: Prof. Dr. Gertraud Schüpbach

Arbeit unter wissenschaftlicher Betreuung von
Dr. H. Schwermer
und PD Dr. M. Reist

Beurteilung des Potenzials der Überwachung von Antibiotika-Resistenzen, Tierseuchen und Zoonosen mittels Tankmilchproben

Inaugural-Dissertation

zur Erlangung der Doktorwürde
der Vetsuisse-Fakultät Universität Bern

vorgelegt von

Antonella Reber
Basel Stadt, BS und Diemtigen, BE

2013

Von der Vetsuisse-Fakultät Universität Bern auf Antrag
von Prof. Dr. Gertraud Schüpbach als Dissertation genehmigt.

Bern,

Der Dekan der
Vetsuisse-Fakultät
Universität Bern

A Luigina et Martin

„On ne va jamais aussi loin que lorsqu'on ne sait pas où l'on va. "
Christophe Colomb (1451-1506)

Assessment of the capacity for the monitoring of antibiotic resistance, epizootics and zoonoses by bulk milk samples

Table of contents

Chapter 1 ***page 5***

General Introduction

Chapter 2 ***page 10***

A review of the capacity for the monitoring of epizootics, zoonoses and other pathogens by bulk-tank milk samples

Chapter 3 ***page 35***

Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhinotracheitis and bovine enzootic leucosis in Switzerland

Chapter 4 ***page 45***

Public health prioritisation of zoonotic pathogens that can be transmitted to humans by raw milk or milk

Chapter 5 ***page 58***

Calculation of costs and benefits of surveillance systems for listeriosis and salmonellosis utilizing bulk-tank milk samples

Chapter 6 ***page 74***

Potential of bulk-tank milk samples for antibiotic resistance monitoring

Chapter 7 ***page 83***

Discussion and Conclusion

Acknowledgement ***page 86***

Chapter 1

General Introduction

The most important livestock species in Switzerland is cattle representing 33% of the whole agricultural production value. The most relevant production branch in cattle is milk production representing 21% of the whole agricultural production value[1]. High quality milk products are the most important of the few Swiss agricultural products capable of competing on export markets. In the European countries and in Switzerland the importance of good health status of livestock is of major importance in order to permit free trade of animals and animal products. In order to maintain the status of disease freedom after eradication of diseases, continuing surveillance is of critical importance. To demonstrate disease freedom to trading partners, this surveillance has to be performed in accordance with international agreements and regulations. Conducting these programmes annually generates considerable costs for the national veterinary authorities, even in the absence of the respective pathogen. Until recently active surveillance or eradication programmes such as the surveillance programme of infectious bovine rhinotracheitis (IBR) and enzootic bovine leucosis (EBL), or the eradication programme of bovine viral diarrhoea (BVD), are conducted utilising individual blood or tissue samples. Surveillance programmes of zoonoses or of antibiotic resistance are conducted through the sampling of animal products or from samples taken in slaughterhouses. Individual animal or product sampling methods are neither economical nor easy to implement. Due to the high labour costs in Switzerland, sampling costs represent a major proportion of the diagnostic costs for the analysis of a sample from an individual animal or product. To reduce these cost, two strategies can be followed: reducing the number of herds to be sampled, for example through risk-based approaches for sample size calculations (Hadorn et al., 2002; Stark et al., 2006; Schwermer et al., 2009) or reducing the

[1] Government report on agriculture 2010, Federal Office of Agriculture, FOAG

cost of sampling by using pooled herd samples or less expensive sampling procedures. In the last few years, the main focus in Switzerland was in reducing the amount of herds to be tested. However, as the potential for reduction of sample size by risk-based approaches is not infinite, the second strategy is also highly valuable and needs further investigation.

In Switzerland, dairies selling commercialised milk undergo each month at least two milk quality controls. Bulk-tank milk (BTM) samples are collected routinely twice a month and tested in the milk-testing scheme (somatic cell counts, bacterial counts and antibiotic-residue testing). Since 2011, the milk-testing diagnostics are conducted in one single laboratory. Hence, BTM samples represent a fast, easily-available, convenient, inexpensive and non-invasive medium that could be screened for several purposes, such as for epizootics and zoonoses surveillance, eradication programmes, mastitis surveillance or diagnosis and screening of substances or residues such as antibiotics or also monitoring of antibiotic resistances. Testing of BTM samples is a scientifically based and accepted method in several European countries and also worldwide: examples are the United States, Canada or Australia (Innes and Lynch, 1990; Van Wuijckhuise et al., 1998; Nylin et al., 2000; Jayarao and Henning, 2001; Paisley et al., 2001; Nuotio et al., 2003; Houe et al., 2006; Nuotio et al., 2007). In Switzerland, BTM samples were used for Bluetongue surveillance in the recent past (Kluiters et al.,2008).

Diagnostic tests

A literature review published by Jayarao in 2004, described the usefulness of BTM tests for the measurement and detection of: 1) somatic cells, 2) bacterial pathogens (foodborne, animal and zoonotic), 3) milk quality deteriorating bacteria, 4) cattle viruses, 5) antibodies in milk against bacterial, viral and gastrointestinal parasites, and 6) substances such as antibiotics, metabolites, drugs, toxins and trace minerals.

Worldwide, the applications of BTM testing for the diagnosis of cattle diseases are numerous. A large number of diagnostic tests for certain diseases have been described in the literature.

As a result of an international enquiry comprising 13 European laboratories conducted in this study, many already commercialised BTM diagnostic tests were identified on the market.

Objectives

The main aim of this research was a scientific, practical and economic evaluation of the potential use of BTM samples for surveillance of animal health, zoonoses and antibiotic resistance in Switzerland. In order to achieve this, specific objectives were defined as follows:

1. Identify candidate epizootic and zoonotic pathogens that could be surveyed or eradicated utilising BTM testing. This was achieved by conducting a literature review and a laboratory survey on surveillance systems using BTM and on currently available diagnostic tools (Chapter 2).

2. Evaluate the use of BTM samples for surveillance programmes to demonstrate freedom from animal diseases. A cost-effectiveness analysis of BTM testing for surveys for infectious bovine rhinotracheitis and bovine enzootic leucosis in Switzerland was used as an example, because these are the major cattle diseases where Switzerland needs to demonstrate disease freedom (Chapter 3).

3. Prioritize the candidate zoonotic pathogens identified in chapter 2 according to their public health importance in BTM. For this, a questionnaire study was performed with a panel of Swiss experts (Chapter 4).

4. Evaluate the use of BTM samples for surveillance of zoonotic pathogens identified as high priority in chapter 4. A cost-effectiveness analysis of surveillance programmes for *Listeria monocytogenes* and *Salmonella spp* in dairy cattle utilising BTM samples was conducted (Chapter 5).

5. A literature review on the feasibility of antibiotic resistance monitoring with BTM were carried out and is described in Chapter 6.

References

B.Jayarao: Past, present and future applications of bulk tank milk analysis to assess milk quality and herd health statusB.Jayarao. 2004.

Hadorn, D.C., Rufenacht, J., Hauser, R., Stark, K.D.C.: Risk-based design of repeated surveys for the documentation of freedom from non-highly contagious diseases. Preventive Veterinary Medicine 2002, 56: 179-192.

Houe, H., Lindberg, A., Moennig, V.: Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. J Vet Diagn Invest 2006, 18: 427-436.

Innes, P., Lynch, J.A.: Bulk Tank Survey of Dairy Farms in Ontario. Canadian Veterinary Journal-Revue Veterinaire Canadienne 1990, 31: 41-41.

Kluiters, G., Chaignat V., Schwermer H.: Spatial distribution of bluetongue surveillance and cases in Switzerland. Schweiz Arch Tierheilkd. 2008 Nov;150(11):543-52.

Jayarao, B.M., Henning, D.R.: Prevalence of foodborne pathogens in bulk tank milk. Journal of Dairy Science 2001, 84: 2157-2162.

Nuotio, L., Neuvonen, E., Hyytiainen, M.: Epidemiology and eradication of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) virus in Finland. Acta Veterinaria Scandinavica 2007, 49.

Nuotio, L., Rusanen, H., Sihvonen, L., Neuvonen, E.: Eradication of enzootic bovine leukosis from Finland. Preventive Veterinary Medicine 2003, 59: 43-49.

Nylin, B., Stroger, U., Ronsholt, L.: A retrospective evaluation of a Bovine Herpesvirus-1 (BHV-1) antibody ELISA on bulk-tank milk samples for classification of the BHV-1 status of Danish dairy herds. Preventive Veterinary Medicine 2000, 47: 91-105.

Paisley, L.G., Tharaldsen, J., Jarp, J.: A retrospective analysis of the infectious bovine rhinotracheitis (bovine herpes virus-1) surveillance program in Norway using Monte Carlo simulation models. Preventive Veterinary Medicine 2001, 50: 109-125.

Schwermer, H., Reding, I., Hadorn, D.C.: Risk-based sample size calculation for consecutive surveys to document freedom from animal diseases. Preventive Veterinary Medicine 2009, 92: 366-372.

Stark, K., Regula, G., Hernandez, J., Knopf, L., Fuchs, K., Morris, R., Davies, P.: Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: Review of current approaches. BMC Health Services Research 2006, 6: 20.

Van Wuijckhuise, L., Bosch, J., Franken, P., Frankena, K., Elbers, A.R.W.: Epidemiological characteristics of bovine herpesvirus 1 infections determined by bulk milk testing of all Dutch dairy herds. Veterinary Record 1998, 142: 181-184.

Chapter 2

A review of the capacity for the monitoring of epizootics, zoonoses and other pathogens by bulk-tank milk samples

Introduction

Bulk-tank milk (BTM) analysis is now widely accepted as a useful tool to evaluate milk quality, but also to monitor udder-health status in herds (Jayarao and Wolfgang, 2003). BTM tests are primarily related to mastitis problems. Today, BTM somatic cell counts (BMCC) and BTM cultures are common and routine approaches to assess the udder-health of dairy cows. The demand of consumers for safe and high quality milk has placed a significant responsibility on dairy producers, retailers and manufacturers to produce and market safe milk and milk products. The first step in the production of quality milk begins at the dairy farm. The task of producing quality milk and maintaining cows with low incidence of mastitis is a management challenge for all dairy producers.

Projects carried out in the US could show a good evaluation of the dairy producers BTM analysis to monitor herd mastitis pathogens and udder-health.

It is more difficult to assess the significance of environmental agents, being potential pathogens (coli forms, yeasts, *Nocardia* spp., *Streptococcus* spp. or other than *Streptococcus agalactiae*), in the bulk tank. While capable of causing mastitis, they may also appear as a result of a systemic infection or direct environmental contamination. BTM screening may be an effective first step in enabling the producer and the veterinarian to pin point and remedy more quickly and less expensive at least some of the predominant contributing factors to high herd somatic cell count (SCC) and mastitis.

Healthy animals are also the baseline for a healthy milk and livestock population in general. Over the years, BTM screening has proved its efficiency not only in udder health problem, but also in the screening of several epizootic and zoonotic pathogens. The potential of BTM

for the emphasis of bovine epizootics and zoonoses was also developed under the growing demand of national surveillance programmes and under the pressure to maintain healthy livestock for trading issues between countries.

In this review the aim is on the description of diagnostic tests applied on BTM, with consideration of the respective pathogen. More specifically, diagnostic procedures that have been used for any kind of pathogen should be identified and their eventual application on epizootic pathogens and zoonotic agents should be identified.

Diagnostic protocols used in bulk-tank milk samples for mastitis pathogen screening

Staphylococcus aureus

Staphyloccocus aureus is a very common pathogen of contagious mastitis in cattle and it can cause serious economic losses in the dairy industry. To develop effective pathogen diagnostic tools is an important step to monitor and control mastitis pathogens such as *Staphylococcus aureus*. BTM culture has been used as screening test or to identify strains causing mastitis within a herd. Studies about modified culture media were done to improve the detection of the pathogen strains (Ollis et al., 1995). Highly sensitive and specific real-time quantitative Polymerase Chain Reaction (PCR) were developed and used on BTM (Graber et al., 2007; Studer et al., 2008). Multiplex PCR were also developed and showed to be an easy and rapid method to detect pathogens such as *Staphylococcus aureus* (Phuektes et al., 2003). PCR methods could also be used for the detection of genes encoding enterotoxins, exofoliative toxins, toxic shock syndrome toxin 1 or detect the genotype variation among strains (Stephan et al., 2002; Jorgensen et al., 2005a; Jorgensen et al., 2005b)

Streptococcus agalactiae and spp.

A nationwide surveillance and eradication programme for *Streptococcus agalactiae* was initiated in Denmark as early as 1954 based on bacteriological examination of milk can and BTM samples. The programme was compulsory until 1988 and since then has become voluntary. Beginning in 1995, BTM samples were analysed every year. An evaluation study

of this programme was conducted and ways for useful application of BTM-testing schemes for screening purpose were described for *Streptococcus agalactiae* (Andersen et al., 2003). In another study, an evaluation of five selective media for isolation of catalase negative gram-positive cocci from bulk-tank milk was conducted and showed that Edward modified medium supplemented with colistin sulphate and oxolinic acid can be easily used as a selective medium on bulk-tank milk for the isolation of streptococci and streptococci-like microorganisms. It showed a sensitivity of 100%, a specificity of 87.5% and a positive predictive value of 99.4% (Sawant et al., 2002).

Escherichia coli

BTM samples have been screened for the presence of several genes encoding virulence factors associated with enterohemorrhagic forms of *Escherichia coli (E.coli)* using real-time and conventional PCR assays. 859 bulk-tank milk samples across 21 states of the United States were analysed. The eaeA gene encoding intimin was found in 23 % of the samples (Karns et al., 2007).

A survey was conducted in northeast Switzerland to identify the prevalence of *E.coli* O157 and other Shigatoxin-producing *E.coli.* Three hundred and ten bulk-tank milk samples were analysed by immunomagnetic separation (IMS) and PCR. No positive samples were found. This does perhaps imply good milking hygiene. Faecal contamination is considered the most common route of BTM contamination (Stephan and Buehler, 2001).

A study on the prevalence of *E.coli* 0157:H7 was conducted on 30 dairy farms in east Tennessee. Faecal samples from culled dairy cows and BTM samples from dairy cows were collected. Overall, 1.46% of the samples tested positive for *E.coli* O157:H7. Culture procedure and PCR for the indication of virulence factors were used to identify the pathogen. This study showed that raw milk and slaughtered dairy cows could indeed represent a potential hazard for human health and the usefulness of a development of on-farm and

preharvest control programmes for such foodborne pathogens ought to be carried out in the future (Murinda et al., 2002).

Mycoplasma spp.

Several surveys on BTM were conducted to isolate *Mycoplasma bovis*. A prevalence study was conducted in a province of Thailand with a commercially nested PCR kit after culture (Kampa et al., 2009). The prevalence of *Mycoplasma bovis* was 1.8%. This result did not differ from another study conducted on cultured *Mycoplasma bovis* from BTM samples in Prince Edward Island (Olde Riekerink et al., 2006). A higher prevalence was found in a survey in Northern Greece with a prevalence of 5.4% of BTM samples. BTM samples were cultured and *Mycoplasma bovis* isolates were identified by PCR (Filioussis et al., 2007). BTM samples testing could also be a valuable tool for screening dairy herds to identify infection with *Mycoplasma bovis* and permit a rapid removal or culling of infected cattle. This represented a major component of a successful control programme for *Mycoplasma bovis* (Fox et al., 2003).

Nocardia spp

A study showed that culturing BTM samples for *Nocardia spp.* was an effective way to identify herds with nocardial mastitis although *Nocardia spp.* are environmental pathogens. When BTM samples were cultured for four consecutive weeks the sensitivity and specificity of the BTM screening test were 75% and 97% respectively (Schoonderwoerd et al., 1990)

Diagnostic protocols used in bulk-tank milk samples for epizootic pathogen screening

Infectious bovine rhinotracheitis (IBR)

A routine BTM surveillance is already implemented in several European countries such as Norway, Denmark, Austria, Sweden and Finland. Different schemes are used: yearly testing of all dairy herds in Austria and Finland (Nuotio et al., 2007); dairy herds with more than 50 cows twice yearly in Sweden; all dairy herds four times a year, with special guidelines for territory such as near the German border with once per month testing in Denmark; or 10% of

all dairy cattle in Norway. Between 1984 and 1991, BTM sampling was already implemented to monitor the bovine herpesvirus (BHV)-1 status of the dairy herds in the Danish BHV-1 eradication campaign. These programmes were retrospectively analysed and the good performance of such surveillance programmes was shown (Nylin et al., 2000; Paisley et al., 2001). In a Dutch study, by BTM testing of all Dutch dairy herds, epidemiological risk factors for IBR such as herd size, animal density, purchase of stock and livestock type of farms were analysed and their influence on the IBR herd status was determined (Van Wuijckhuise et al., 1998).

For IBR, several already commercialised antibody diagnostic tests are available on the market (see Chapter 3, Table 2).

Enzootic bovine leucosis (EBL)

As for IBR, surveillance programmes for EBL are already implemented in several European countries, such as Norway, Denmark, Austria, England, Sweden and Finland (Nuotio et al., 2003). In the majority of countries, the sampling is coupled with the sampling of IBR, although the sampling interval can be different. A yearly testing is in use in Norway, Sweden, Austria, Finland and England or every three years in Denmark. Some ELISAs were already evaluated in the literature (Sargeant et al., 1997; Ridge and Galvin, 2005). Several already commercialised antibody diagnostic tests were also identified on the market (see Chapter 3, Table 2).

Bovine viral diarrhoea virus (BVD)

The level of BVDV antibodies in BTM correlates with the prevalence of seropositive cows in a dairy herd (Niskanen, 1993; Lindberg and Alenius, 1999; Beaudeau et al., 2001). Herds with persistently infected (PI) animals can be identified in screening through BTM ELISA, as in such a herd most cows have seroconverted after a short time. However, after removing the PI animals from a herd, a high prevalence from seropositive animals can remain for a long period and can lead, if referred to the detection of PI animals, to low specificity of BTM

diagnostic, with many false-positive tests results. BTM will not be suitable for screening herds for some years after the removal of PI animals. The BTM antibody testing will be a useful method to identify almost all true positive herds, but could also detect a number of false-positive herds (Houe et al., 2006).

BVDV can also be detected in BTM. PI animals can occasionally reach the adult age and contribute to the bulk-tank. Then large quantities of virus are shed in the milk from PI or transiently infected cows. Thus, detection of virus in BTM through virus detection diagnostic tools is possible. A RT-PCR technique in BTM was described by Drew et al., 1999. However, the most PI animals are almost always found among the young stock. PCR methods in BTM are obviously not suitable for the screening of PI animals in dairy herds. Nevertheless, they can be used as an initial diagnostic test to show if any PI animal is present among the lactating cows of a dairy farm, or as a back up test to detect false negative cows after individual PCR testing (Houe et al., 2006).

Foot and mouth disease

Two ELISA, a liquid-phase blocking ELISA (LPBE) and a specific isotype assay (SIA) were modified to detect antibodies against foot and mouth disease in cattle milk. Both ELISAs could be used for surveillance purposes, but only SIA could distinguish between vaccinated and naive animals (Armstrong, 1997; Armstrong et al., 2000). Another study by Amstrong demonstrated that SIA would be a valuable tool for testing bulk-tank milk samples to determine the herd protection level of vaccinated dairy herds at minimal costs (Armstrong and Mathew, 2001).

Bluetongue

In 2007, due to Bluetongue virus 8 spreading through Europe, Switzerland decided to increase its active surveillance. It was decided to test bulk-tank milk samples from all regions with a commercial indirect ELISA. The test showed a good sensitivity and specificity, 87.8% and 99.1% respectively. By a milk protein precipitation procedure before testing, the specificity

was increased to 99.6% and the sensitivity to 100%. This study determined that surveillance of BT trough bulk-tank milk was very efficient (Chaignat et al., 2010). Similar results were obtained by Kramps et al., 2008. Switzerland was among the first European countries to implement BTM in its surveillance programme for Bluetongue. Several countries like England and Sweden followed this example.

Bovine corona virus, bovine respiratory syncytial virus

An indirect ELISA was applied to BTM samples in a nationwide survey in Sweden to detect antibodies against bovine coronavirus (BCV). The study showed a widespread distribution of BCV antibodies in Swedish dairy herds and a significantly higher prevalence among larger than average sized herds (Traven et al., 1999). In another study, Elvander et al., 1995 showed the application of an ELISA for the bovine syncytial virus. By testing BTM a distinction between antibodies levels of diseased and healthy herds was possible (Elvander et al., 1995).

Diagnostic protocols used in bulk-tank milk samples for zoonotic pathogen screening

Brucella abortus

Brucella abortus screening programmes were already implemented in the seventies, e.g. the milk test programme in Ontario. This programme was based on testing pooled samples of milk or cream from the bulk tank among farms by the *Brucella* milk ring test (Gray and Martin, 1980). Today, several European countries - such as England, Austria, Germany and Sweden - are officially using bulk-tank milk testing to screen brucellosis in dairy herds.

In the literature, many diagnostic tests on milk are described as alternative to the *Brucella* milk ring test developed by Fleischhauer in 1937, such as enzyme immunoassay, and indirect enzyme-linked immunosorbent assays (ELISA) (Thoen et al., 1979; Nielsen et al., 1996; Vanzini et al., 1998). Indirect ELISA or fluorescence polarization assay for the detection of *Brucella abortus* in BTM samples were also evaluated with high sensitivities and specificities (Thoen et al., 1995; Vanzini et al., 2001; Gall et al., 2002).

Coxiella burnetii

Surveillance programmes for *Coxiella burnetii* using BTM samples are implemented in Sweden. A survey in 2008 followed by an active surveillance in 2009 gave an overall estimate of prevalence for dairy cattle of 8.2%. In the 1990s, BTM surveys in England and Wales were conducted using an ELISA test and showed that 21 % of dairy herds were infected (Paiba et al., 1999). Today, no active routine surveillance is conducted in England.

In cows, *Coxiella burnetii* are regularly shed via milk and the animals can be asymptomatic (Rodolakis et al., 2007). Therefore, milk and BTM represent a good medium for the isolation of the pathogen in dairy cows.

Coxiella burnetii can also be detected by nested PCR or real-time PCR assays. A screening study in the United States reported a prevalence of 94.3% in samples of BTM from U.S dairy herds. A real-time PCR was developed during the study (Kim et al., 2005). By testing BTM and individual milk samples with a real-time PCR, Guatteo et al. could assess the relationship between the PCR test results, the within-herd prevalence of the pathogen-shedder cows and the proportion of heavy pathogen shedder cows in a herd. A commercial real-time PCR assay was used (Guatteo et al., 2007). In Switzerland, from January to June 2006, bovine BTM samples obtained from two dairies were screened for Coxiella with a nested PCR, resulting in a prevalence of 4.7% (Fretz et al. 2007). Another survey was performed from May to November 2007 with a more sensitive quantitative PCR, which resulted in an even higher prevalence of 49% of 872 tested bulk milk samples (Baumgartner et al. 2009). The results of these studies suggest that BTM can be a very useful tool to identify, control and prevent *Coxiella* shedding in the dairy population. Diagnostic tools are also available commercially.

Listeria monocytogenes

Several prevalence studies were conducted in different countries to detect *Listeria monocytogenes* in BTM. In a Swiss study, conducted in northeastern Switzerland, *Listeria monocytogenes* could not be isolated from any of the 310 BTM samples - a prevalence of 0%.

In the United States, 56 out of 861 BTM samples were positive for *Listeria monocytogenes*, equal to a prevalence of 6.5%. In addition, no relationship between somatic cell count and presence of *Listeria monocytogenes* in the BTM could be demonstrated (Van Kessel et al., 2004). In an English study, conducted in northeastern Scotland, 180 bulk milk samples were tested on three different occasions and an overall incidence of 3.8% was found (Fenlon and Wilson, 1989). In Sweden, a prevalence study was conducted on BTM from farm tanks and from dairy tanks. *Listeria monocytogenes* was found in 1% of the farm tanks and in 19.6% of the dairy tanks (Waak et al., 2002). A Japanese study was conducted in the region of Nagano and 943 BTM samples were screened for *Listeria spp.*, *Listeria spp.* were isolated from 29 BTM samples corresponding to a prevalence of 3.1% (Yoshida et al., 1998). In all these studies, the diagnosis of *Listeria* was made based on pathogen isolation through culture methods.

Another study on BTM from dairies in the United States was conducted to identify risk factors associated with the presence of *Listeria monocytognes* in BTM. The results revealed that the contamination depended on the geographical region and the herd size in the collecting area of the dairy (Antognoli et al., 2009).

Salmonella spp.

ELISA for indirect detection of Salmonella and real time-PCR have already been adapted and developed for BTM or milk samples (Hoorfar et al., 1995; Hoorfar and Wedderkopp, 1995; Veling et al., 2001; Van Kessel et al., 2004; Omiccioli et al., 2009). In Sweden, a BTM screening was performed for *Salmonella Dublin* antibodies during a surveillance programme in 2009. In a Swiss study, bulk-tank milk samples (n=310) in the Northwest region were used for a screening study. In this study, all samples were negative (Stephan and Buehler, 2002).

***Mycobacterium avium subspecies paratuberculosis* (MAP)**

Mycobacterium avium subspecies paratuberculosis causes granulomatous enteritis in cattle. Johne's disease (JD) is responsible for an important economic loss through production losses

and premature culling. MAP is shed intermittently by sub-clinically and clinically infected cows into manure and within macrophages in milk. MAP represents a very controversial pathogen, because of its possible zoonotic potential and its capability to survive milk pasteurisation. Milk and milk products represent the most probable transmission route to humans. A PCR-based assay based on the detection of the insertion sequence 900 (IS900) was developed and described by Pillai and Jayarao, 2002.

A study in BTM was carried out in Switzerland to assess the prevalence of the MAP specific insertion sequence IS900 and examine the eventual correlation between MAP and somatic cell counts, total colony count and the presence of *Enterobacteriacea*. The prevalence was 19.7% and no correlation was found with somatic cell counts, total colony count and the presence of *Enterobacteriacea* (Stephan, 2002).

Prevalence of MAP in milk from the whole of Cyprus was estimated based on qPCR,and was found to be 28.6%. The author described the qPCR for IS900 and F57 as a more sensitive method for MAP detection in milk than culture (Slana et al., 2009).

An ELISA described by Nielsen and al. with a commercially available antigen and adapted with Klausen from the Danish veterinary laboratory in Copenhagen can be used with some modification for detection of high prevalence herds but not herds with a low prevalence; individual cow testing will be needed (Nielsen et al., 2000).

Apparently unspecific reactions are more predominant in serum samples than in milk samples, allowing lower cut-offs and pre-dilutions for milk samples. A commercially available ELISA for the detection of antibodies against MAP was evaluated in the same study (van Weering et al., 2007)

ELISA technology has gained an important place in herd-based testing schemes because of its low cost and high-throughput potential. (van Schaik et al., 2003)

Faecal culturing is considered the Gold Standard for diagnosis of MAP, but due to the intermittent shedding of the pathogen in the faeces, or the low number of pathogens in the

material, together with its slow growth characteristics, the isolation of the organism is fastidious and time-consuming. On large herds more challenging strategic measures have to be implemented due to limitations of costs, time or laboratory space. Serum ELISA or pooling of samples can be seen as additional possibilities in the JD surveillance. National control programmes of JD are an immense challenge because of the particularities of the epidemiology of the disease and the in majority poor performance of the test available (McKenna et al., 2006).

Using a peptide in a peptide mediated, capture PCR approach with primers specific for the MAP insertion element ISMav2 (Strommenger et al., 2001), the detection of MAP in BTM samples from infected herds was possible (Stratmann et al., 2002).

A basic procedure for isolation of MAP from milk includes centrifugation to collect pellet fraction, chemical decontamination and culture on slants containing antibiotics and other supplements. This study showed that the freshness of the milk sample is an important factor for a successful decontamination by identification of MAP in milk. Decontamination and addition of antibiotics are also important. Higher temperature enhanced the effect of decontamination on MAP survival. The best recovery of MAP was obtained from the pooled cream and pellet fractions. A study also suggest a protocol for isolation of MAP from raw milk (Gao et al., 2005).

MAP can survive the pasteurisation process at required minimum temperature of 72ºC and minimum holding time of 15s. From a survey of 312 samples of retail pasteurized cow's milk purchased from supermarkets in southern England and Wales, Millar et al. found PCR positive samples, but could not differentiate between viable or non viable MAP cells (Millar et al., 1996).

Viable MAP was cultured from 1.6% of bulk raw milk and 1.8% of commercially pasteurized milk samples from approved dairy processing establishments throughout the United Kingdom (Grant et al., 2002).

An immunomagnetic separation (IMS) technique was developed for the selective isolation of MAP cells from milk and it can be used in combination with detection method such as PCR or ELISAs (Grant et al., 1998).

Diagnostic protocols used in bulk-tank milk samples for parasite screening

Neospora caninum, Fasciola hepatica, Ostertagia ostertagi and Dictyocaulus viviparous

Several studies have also shown the potential of BTM to screen for parasites in dairy cattle populations (Chanlun et al., 2002; Bartels et al., 2005; Chanlun et al., 2006; Frossling et al., 2006; Bartels et al., 2007; Bennema et al., 2009).

Conclusion

BTM testing is a valuable tool for monitoring mastitis, epizootics, zoonoses and other pathogens. Many different diagnostic tests have been described in the scientific literature for many different pathogens. Applications on the national level include surveillance for demonstrating freedom from disease, surveillance of endemic diseases and early detection of introduction of new or re-emerging diseases. On the farm level, BTM testing can be used to aid decisions on herd health management, and for prevention and control of disease in the herd. While many diagnostic tests were originally developed for use in serum or other matrices also deliver valuable results on BTM, it is crucial that tests are specifically evaluated for their sensitivity and specificity in BTM, and for their fitness for the purpose of the surveillance or control programme.

References

Andersen, H.J., Pedersen, L.H., Aarestrup, F.M., Chriel, M.: Evaluation of the surveillance program of Streptococcus agalactiae in Danish dairy herds. Journal of Dairy Science 2003, 86: 1233-1239.

Antognoli, M.C., Lombard, J.E., Wagner, B.A., McCluskey, B.J., Van Kessel, J.S., Karns, J.S.: Risk Factors Associated with the Presence of Viable Listeria monocytogenes in Bulk Tank Milk from US Dairies. Zoonoses and Public Health 2009, 56: 77-83.

Armstrong, R.M.: Development of tests for antibodies against foot-and-mouth disease virus in cattle milk. Journal of Virological Methods 1997, 63: 175-180.

Armstrong, R.M., Mathew, E.S.: Predicting herd protection against foot-and-mouth disease by testing individual and bulk tank milk samples. Journal of Virological Methods 2001, 97: 87-99.

Armstrong, R.M., Mathew, E.S., Mackey, D.K.J.: Validation of the specific isotype assay to detect antibodies against foot-and-mouth disease virus in bovine milk. Journal of Virological Methods 2000, 85: 193-201.

Bartels, C.J.M., van Maanen, C., van der Meulen, A.M., Dijkstra, T., Wouda, W.: Evaluation of three enzyme-linked immunosorbent assays for detection of antibodies to Neospora caninum in bulk milk. Veterinary Parasitology 2005, 131: 235-246.

Bartels, C.J.M., van Schaik, G., van Maanen, K., Wouda, W., Dijkstra, T.: Factors associated with variation in Neospora caninum bulk-milk S/P ratios in initially bulk-milk negative testing Dutch dairy herds. Preventive Veterinary Medicine 2007, 81: 265-273.

Baumgartner, A., Niederhauser, I., Schaeren, W.: Occurrence of Coxiella burnetii DNA in bulk tank milk samplees in Switzerland. Archiv für Lebensmittelhygiene 2011, 62: 200-204.

Beaudeau, F., Belloc, C., Seegers, H., Assie, S., Pourquier, P., Joly, A.: Informative value of an indirect enzyme-linked immunosorbent assay (ELISA) for the detection of bovine viral diarrhoea virus (BVDV) antibodies in milk. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 2001, 48: 705-712.

Bennema, S., Vercruysse, J., Claerebout, E., Schnieder, T., Strube, C., Ducheyne, E., Hendrickx, G., Charlier, J.: The use of bulk-tank milk ELISAs to assess the spatial distribution of Fasciola hepatica, Ostertagia ostertagi and Dictyocaulus viviparus in dairy cattle in Flanders (Belgium). Veterinary Parasitology 2009, 165: 51-57.

Chaignat, V., Nitzsche, S., Scharrer, S., Feyer, D., Schwermer, H., Thur, B.: Milk concentration improves Bluetongue antibody detection by use of an indirect ELISA. Veterinary Microbiology 2010, 143: 179-183.

Chanlun, A., Emanuelson, U., Chanlun, S., Alumlamai, S., Bjorkman, C.: Application of repeated bulk milk testing for identification of infection dynamics of Neospora caninum in Thai dairy herds. Veterinary Parasitology 2006, 136: 243-250.

Chanlun, A., Naslund, K., Aiumlamai, S., Bjorkman, C.: Use of bulk milk for detection of Neospora caninum infection in dairy herds in Thailand. Veterinary Parasitology 2002, 110: 35-44.

Drew, T.W., Yapp, F., Paton, D.J.: The detection of bovine viral diarrhoea virus in bulk milk samples by the use of a single-tube RT-PCR. Veterinary Microbiology 1999, 64: 145-154.

Elvander, M., Edwards, S., Naslund, K., Linde, N.: Evaluation and Application of an Indirect Elisa for the Detection of Antibodies to Bovine Respiratory Syncytial Virus in Milk, Bulk Milk, and Serum. Journal of Veterinary Diagnostic Investigation 1995, 7: 177-182.

Fenlon, D.R., Wilson, J.: The Incidence of Listeria-Monocytogenes in Raw-Milk from Farm Bulk Tanks in Northeast Scotland. Journal of Applied Bacteriology 1989, 66: 191-196.

Filioussis, G., Christodoulopoulos, G., Thatcher, A., Petridou, V., Bourtzi-Chatzopoulou, E.: Isolation of Mycoplasma bovis from bovine clinical mastitis cases in Northern Greece. Veterinary Journal 2007, 173: 215-218.

Fox, L.K., Hancock, D.D., Mickelson, A., Britten, A.: Bulk tank milk analysis: Factors associated with appearance of Mycoplasma sp in milk. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 2003, 50: 235-240.

Fretz R, Schaeren W., Tanner M., Baumgartner A.: Screening of various foodstuffs for occurrence of Coxiella burnetii in Switzerland. International Journal of Food Microbiology 2007, 116(3):414-8.

Frossling, J., Lindberg, A., Bjorkman, C.: Evaluation of an iscom EIASA used for detection of antibodies to Neospora caninum in bulk milk. Preventive Veterinary Medicine 2006, 74: 120-129.

Gall, D., Nielsen, K., Bermudez, M.R., Moreno, F., Smith, P.: Fluorescence polarization assay for detection of Brucella abortus antibodies in bulk tank bovine milk samples. Clinical and Diagnostic Laboratory Immunology 2002, 9: 1356-1360.

Gao, A.L., Odumeru, J., Raymond, M., Mutharia, L.: Development of improved method for isolation of Mycobacterium avium subsp paratuberculosis from bulk tank milk: Effect of age of milk, centrifugation, and decontamination. Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire 2005, 69: 81-87.

Graber, H.U., Casey, M.G., Naskova, J., Steiner, A., Schaeren, W.: Development of a highly sensitive and specific assay to detect Staphylococcus aureus in bovine mastitic milk. J Dairy Sci 2007, 90: 4661-4669.

Grant, I.R., Ball, H.J., Rowe, M.T.: Isolation of Mycobacterium paratuberculosis from milk by immunomagnetic separation. Applied and Environmental Microbiology 1998, 64: 3153-3158.

Grant, I.R., Ball, H.J., Rowe, M.T.: Incidence of Mycobacterium paratuberculosis in bulk raw and commercially pasteurized cows' milk from approved dairy processing establishments in the United Kingdom. Applied and Environmental Microbiology 2002, 68: 2428-2435.

Gray, M.D., Martin, S.W.: Evaluation of Screening Programs for the Detection of Brucellosis in Dairy Herds. Canadian Journal of Comparative Medicine-Revue Canadienne De Medecine Comparee 1980, 44: 52-60.

Guatteo, R., Beaudeau, F., Joly, A., Seegers, H.: Assessing the within-herd prevalence of Coxiella burnetii milk-shedder cows using a real-time PCR applied to bulk tank milk. Zoonoses and Public Health 2007, 54: 191-194.

Hoorfar, J., Lind, P., Bitsch, V.: Evaluation of an O-Antigen Enzyme-Linked-Immunosorbent-Assay for Screening of Milk Samples for Salmonella-Dublin Infection in Dairy Herds. Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire 1995, 59: 142-148.

Hoorfar, J., Wedderkopp, A.: Enzyme-Linked-Immunosorbent-Assay for Screening of Milk Samples for Salmonella-Typhimurium in Dairy Herds. American Journal of Veterinary Research 1995, 56: 1549-1554.

Houe, H., Lindberg, A., Moennig, V.: Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. J Vet Diagn Invest 2006, 18: 427-436.

Jayarao, B.M., Wolfgang, D.R.: Bulk-tank milk analysis - A useful tool for improving milk quality and herd udder health. Veterinary Clinics of North America-Food Animal Practice 2003, 19: 75-+.

Jorgensen, H.J., Mork, T., Caugant, D.A., Kearns, A., Rorvik, L.M.: Genetic variation among Staphylococcus aureus strains from Norwegian bulk milk. Applied and Environmental Microbiology 2005a, 71: 8352-8361.

Jorgensen, H.J., Mork, T., Hogasen, H.R., Rorvik, L.M.: Enterotoxigenic Staphylococcus aureus in bulk milk in Norway. Journal of Applied Microbiology 2005b, 99: 158-166.

Kampa, J., Sukolapong, V., Buttasri, A., Charoenchai, A.: Prevalence of Mycoplasma bovis and Other Contagious Bovine Mastitis Pathogens in Bulk Tank Milk of Dairy Cattle Herds in Khon Kaen Province, Thailand. Thai Journal of Veterinary Medicine 2009, 39: 275-280.

Karns, J.S., Van Kessel, J.S., McClusky, B.J., Perdue, M.L.: Incidence of Escherichia coli O157 : H7 and E-coli virulence factors in US bulk tank milk as determined by polymerase chain reaction. Journal of Dairy Science 2007, 90: 3212-3219.

Kim, S.G., Kim, E.H., Lafferty, C.J., Dubovi, E.: Coxiella burnetii in bulk tank milk samples, United States. Emerging Infectious Diseases 2005, 11: 619-621.

Kramps, J.A., van Maanen, K., Mars, M.H., Popma, J.K., van Rijn, P.A.: Validation of a commercial ELISA for the detection of bluetongue virus (BTV)-specific antibodies in individual milk samples of Dutch dairy cows. Veterinary Microbiology 2008, 130: 80-87.

Lindberg, A.L., Alenius, S.: Principles for eradication of bovine viral diarrhoea virus (BVDV) infections in cattle populations. Vet Microbiol 1999, 64: 197-222.

McKenna, S.L.B., Keefe, G.P., Twari, A., VanLeeuwen, J., Barkema, H.W.: Johne's disease in Canada Part II: Disease impacts, risk factors, and control programs for dairy producers. Canadian Veterinary Journal-Revue Veterinaire Canadienne 2006, 47: 1089-1099.

Millar, D., Ford, J., Sanderson, J., Withey, S., Tizard, M., Doran, T., HermonTaylor, J.: IS900 PCR to detect Mycobacterium paratuberculosis in retail supplies of whole pasteurized cows' milk in England and Wales. Applied and Environmental Microbiology 1996, 62: 3446-3452.

Murinda, S.E., Nguyen, L.T., Ivey, S.J., Gillespie, B.E., Almeida, R.A., Draughon, F.A., Oliver, S.P.: Prevalence and molecular characterization of Escherichia coli O157 : H7 in bulk tank milk and fecal samples from cull cows: A 12-month survey of dairy farms in east Tennessee. Journal of Food Protection 2002, 65: 752-759.

Nielsen, K., Smith, P., Gall, D., Perez, B., Cosma, C., Mueller, P., Trottier, J., Cote, G., Boag, L., Bosse, J.: Development and validation of an indirect enzyme immunoassay for detection of antibody to Brucella abortus in milk. Veterinary Microbiology 1996, 52: 165-173.

Nielsen, S.S., Thamsborg, S.M., Houe, H., Bitsch, V.: Bulk-tank milk ELISA antibodies for estimating the prevalence of paratuberculosis in Danish dairy herds (vol 44, pg 1, 2000). Preventive Veterinary Medicine 2000, 46: 297-297.

Niskanen, R.: Relationship between the Levels of Antibodies to Bovine Viral Diarrhea Virus in Bulk Tank Milk and the Prevalence of Cows Exposed to the Virus. Veterinary Record 1993, 133: 341-344.

Nuotio, L., Neuvonen, E., Hyytiainen, M.: Epidemiology and eradication of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) virus in Finland. Acta Veterinaria Scandinavica 2007, 49.

Nuotio, L., Rusanen, H., Sihvonen, L., Neuvonen, E.: Eradication of enzootic bovine leukosis from Finland. Preventive Veterinary Medicine 2003, 59: 43-49.

Nylin, B., Stroger, U., Ronsholt, L.: A retrospective evaluation of a Bovine Herpesvirus-1 (BHV-1) antibody ELISA on bulk-tank milk samples for classification of the BHV-1 status of Danish dairy herds. Preventive Veterinary Medicine 2000, 47: 91-105.

Olde Riekerink, R.G., Barkema, H.W., Veenstra, S., Poole, D.E., Dingwell, R.T., Keefe, G.P.: Prevalence of contagious mastitis pathogens in bulk tank milk in Prince Edward Island. Can Vet J 2006, 47: 567-572.

Ollis, G.W., Rawluk, S.A., Schoonderwoerd, M., Schipper, C.: Detection of Staphylococcus-Aureus in Bulk Tank Milk Using Modified Baird-Parker Culture Media. Canadian Veterinary Journal-Revue Veterinaire Canadienne 1995, 36: 619-623.

Omiccioli, E., Amagliani, G., Brandi, G., Magnani, M.: A new platform for Real-Time PCR detection of Salmonella spp., Listeria monocytogenes and Escherichia coli O157 in milk. Food Microbiology 2009, 26: 615-622.

Paiba, G.A., Green, L.E., Lloyd, G., Patel, D., Morgan, K.L.: Prevalence of antibodies to Coxiella burnetii (Q fever) in bulk tank milk in England and Wales. Veterinary Record 1999, 144: 519-522.

Paisley, L.G., Tharaldsen, J., Jarp, J.: A retrospective analysis of the infectious bovine rhinotracheitis (bovine herpes virus-1) surveillance program in Norway using Monte Carlo simulation models. Preventive Veterinary Medicine 2001, 50: 109-125.

Phuektes, P., Browning, C.F., Anderson, G., Mansell, P.D.: Multiplex polymerase chain reaction as a mastitis screening test for Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae and Streptococcus uberis in bulk milk samples. Journal of Dairy Research 2003, 70: 149-155.

Pillai, S.R., Jayarao, B.M.: Application of IS900PCR for detection of Mycobacterium avium subsp paratuberculosis directly from raw milk. Journal of Dairy Science 2002, 85: 1052-1057.

Ridge, S.E., Galvin, J.W.: A comparison of two ELISAs for the detection of antibodies to bovine leucosis virus in bulk-milk. Australian Veterinary Journal 2005, 83: 431-434.

Rodolakis, A., Berri, M., Hechard, C., Caudron, C., Souriau, A., Bodier, C.C., Blanchard, B., Camuset, P., Devillechaise, P., Natorp, J.C., Vadet, J.P., Arricau-Bouvery, N.: Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds. Journal of Dairy Science 2007, 90: 5352-5360.

Sargeant, J.M., Kelton, D.F., Martin, S.W., Mann, E.D.: Evaluation of a bulk-milk ELISA test for the classification of herd-level bovine leukemia virus status. Preventive Veterinary Medicine 1997, 31: 223-230.

Sawant, A.A., Pillai, S.R., Jayarao, B.M.: Evaluation of five selective media for isolation of catalase-negative gram-positive cocci from bulk tank milk. Journal of Dairy Science 2002, 85: 1127-1132.

Schoonderwoerd, M., Mcfadzen, L.L., Manninen, K.I., Ollis, G.W.: Culturing of Bulk Tank Milk for the Presence of Nocardia Spp. Canadian Veterinary Journal-Revue Veterinaire Canadienne 1990, 31: 453-454.

Slana, I., Liapi, M., Moravkova, M., Kralova, A., Pavlik, I.: Mycobacterium avium subsp paratuberculosis in cow bulk tank milk in Cyprus detected by culture and quantitative IS900 and F57 real-time PCR. Preventive Veterinary Medicine 2009, 89: 223-226.

Stephan, R., Buehler, K.: A survey of the prevalence of Escherichia coli O157 and other Shigatoxin-producing Escherichia coli in bulk-tank milk samples from north-east Switzerland. Archiv für Lebensmittelhygiene 2001, 52: 122-123.

Stephan, R., Buehler, K., Lutz, C.: Prevalence of genes encoding enterotoxins, exfoliative toxins and toxic shock syndrome toxin 1 in Staphylococcus aureus strains isolated from bulk-tank milk samples in Switzerland. Milchwissenschaft-Milk Science International 2002, 57: 502-504.

Stephan, S.C.a.R.: Detection of Mycobacterium avium subspecies paratuberculosis specific IS 900 insertion in bulk-tank milk samples obtained from different regions throughout Switzerland. Bio Med Central 2002.

Stephan, R., Buehler, K., Prävalenz von Campylobacter spp., Salmonella spp. und Listeria monocytogenes in Bestandesmilchproben aus der Nordostschweiz. Archiv Fur Lebensmittelhygiene 2002, vol.53,no3,pp.62-65

Stratmann, J., Strommenger, B., Stevenson, K., Gerlach, G.F.: Development of a peptide-mediated capture PCR for detection of Mycobacterium avium subsp paratuberculosis in milk. Journal of Clinical Microbiology 2002, 40: 4244-4250.

Strommenger, B., Stevenson, K., Gerlach, G.F.: Isolation and diagnostic potential of ISMav2, a novel insertion sequence-like element from Mycobacterium avium subspecies paratuberculosis. FEMS Microbiol Lett 2001, 196: 31-37.

Studer, E., Schaeren, W., Naskova, J., Pfaeffli, H., Kaufmann, T., Kirchhofer, M., Steiner, A., Graber, H.U.: A longitudinal field study to evaluate the diagnostic properties of a quantitative real-time polymerase chain reaction-based assay to detect Staphylococcus aureus in milk. Journal of Dairy Science 2008, 91: 1893-1902.

Surveillance of zoonotic and other animal agents in Sweden, The national veterinary Institute (SVA), Uppsala, Sweden, 2009

Thoen, C.O., Haas, C.A., Angus, R.D., Townsend, A.S.: Evaluation of a Potassium-Chloride Extract of Brucella-Abortus in an Elisa for Detecting Brucella Antibodies in Bulk Tank Milk Samples from Cows. Veterinary Microbiology 1995, 45: 185-189.

Thoen, C.O., Pietz, D.E., Armbrust, A.L., Harrington, R.: Enzyme Immunoassay for Detecting Brucella Antibodies in Cows Milk. Journal of Clinical Microbiology 1979, 10: 222-225.

Traven, M., Bjornerot, L., Larsson, B.: Nationwide survey of antibodies to bovine coronavirus in bulk milk from Swedish dairy herds. Veterinary Record 1999, 144: 527-529.

Van Kessel, J.S., Karns, J.S., Gorski, L., McCluskey, B.J., Perdue, M.L.: Prevalence of Salmonellae, Listeria monocytogenes, and Fecal Coliforms in Bulk Tank Milk on US Dairies. Journal of Dairy Science 2004, 87: 2822-2830.

van Schaik, G., Rossiter, C.R., Stehman, S.M., Shin, S.J., Schukken, Y.H.: Longitudinal study to investigate variation in results of repeated ELISA and culture of fecal samples for Mycobacterium avium subsp paratuberculosis in commercial dairy herds. Am J Vet Res 2003, 64: 479-484.

van Weering, H., van Schaik, G., van der Meulen, A., Waal, M., Franken, P., van Maanen, K.: Diagnostic performance of the Pourquier ELISA for detection of antibodies against Mycobacterium avium subspecies paratuberculosis in individual milk and bulk milk samples of dairy herds. Vet Microbiol 2007, 125: 49-58.

Van Wuijckhuise, L., Bosch, J., Franken, P., Frankena, K., Elbers, A.R.W.: Epidemiological characteristics of bovine herpesvirus 1 infections determined by bulk milk testing of all Dutch dairy herds. Veterinary Record 1998, 142: 181-184.

Vanzini, V.R., Aguirre, N., Lugaresi, C.I., de Echaide, S.T., de Canavesio, V.G., Guglielmone, A.A., Marchesino, M.D., Nielsen, K.: Evaluation of an indirect ELISA for the diagnosis of bovine brucellosis in milk and serum samples in dairy cattle in Argentina. Preventive Veterinary Medicine 1998, 36: 211-217.

Vanzini, V.R., Aguirre, N.R., Valentini, B.S., de Echaide, S.T., Lugaresi, C.I., Marchesino, M.D., Nielsen, K.: Comparison of an indirect ELISA with the Brucella milk ring test for detection of antibodies to Brucella abortus in bulk milk samples. Veterinary Microbiology 2001, 82: 55-60.

Veling, J., van Zijderveld, F.G., Bemmel, A.M.V.Z.V., Schukken, Y.H., Barkema, H.W.: Evaluation of two enzyme-linked immunosorbent assays for detecting Salmonella enterica subsp enterica serovar Dublin antibodies in bulk milk. Clinical and Diagnostic Laboratory Immunology 2001, 8: 1049-1055.

Waak, E., Tham, W., Danielsson-Tham, M.L.: Prevalence and fingerprinting of Listeria monocytogenes strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks. Applied and Environmental Microbiology 2002, 68: 3366-3370.

Yoshida, T., Sato, M., Hirai, K.: Prevalence of Listeria species in raw milk from farm bulk tanks in Nagano Prefecture. Journal of Veterinary Medical Science 1998, 60: 311-314.

Chapter 3

Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhinotracheitis and bovine enzootic leucosis in Switzerland

Submitted to the Swiss Archive for Veterinary Science (Schweizer Archive der Tierheilkunde)

Received: 28 March 2011

Accepted: 20 January 2012

Schweiz. Arch. Tierheilk.
A. Reber, M. Reist, H. Schwermer, Band 154, Heft 5, Mai 2012, xxx–xxx
DOI 10.1024/0036-7281/a00000x

Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhinotracheitis and bovine enzootic leucosis in Switzerland

A. Reber[1], M. Reist[1], H. Schwermer[2]

[1]Institute of Veterinary Public Health, University of Bern and [2]Federal Veterinary Office, Bern, Switzerland

Summary

In Switzerland, annual surveys to substantiate freedom from infectious bovine rhinotracheitis (IBR) and enzootic bovine leucosis (EBL) are implemented by a random allocation of farms to the respective survey as well as blood sampling of individual animals at farm level. Contrary to many other European countries, bulk-tank milk (BTM) samples have not been used for active cattle disease surveillance for several years in Switzerland. The aim of this project was to provide a financial comparison between the current surveillance programme consisting of blood sampling only and a modified surveillance programme including BTM sampling. A financial spreadsheet model was used for cost comparison. Various surveillance scenarios were tested with different sample sizes and sampling frequencies for BTM samples. The costs could be halved without compromising the power to substantiate the freedom from IBR and EBL through the surveillance programme. Alternatively, the sensitivity could be markedly increased when keeping the costs at the actual level and doubling the sample size. The risk-based sample size of the actual programme results in a confidence of 94,18 % that the farm level prevalence is below 0,2 %. Which the doubled sample size, the confidence is 99,69 % respectively.

Keywords: bulk-tank milk, infectious bovine rhinotracheitis, enzootic bovine leucosis, financial comparison

Kosteneffizienz von Überwachungsprogrammen unter Nutzung von Tankmilchproben zum Nachweis der Freiheit von infektiöser boviner Rhinotracheitis und enzootischer boviner Leukose in der Schweiz

In der Schweiz wird der Nachweis der Freiheit von infektiöser boviner Rhinotracheitis (IBR) und enzootischer boviner Leukose (EBL) mittels *jährlicher* Untersuchung von Blutproben von Einzeltieren aus einer Zufallsstichprobe erbracht. Im Gegensatz zu verschiedenen europäischen *Ländern* wurden Tankmilchproben seit mehreren Jahren nicht mehr in der aktiven Überwachung von Tierseuchen genutzt. Das Ziel dieses Projektes war ein finanzieller Vergleich zwischen dem heutigen Überwachungsprogramm mittels Blutproben und einem modifizierten Überwachungsprogramm unter Einbezug von Tankmilchproben um die Kostenersparnisse einschätzen zu können. Ein finanzielles Spreadsheet-Model wurde für den Kostenvergleich benutzt. Szenarien mit verschiedenen Stichprobenumfängen und -frequenzen wurden entwickelt. Die Kosten könnten halbiert werden ohne eine Verschlechterung der Qualität des Überwachungsprogramms für den Freiheitsnachweis von IBR und EBL. Ebenso könnte die Sensitivität des Programms deutlich erhöht werden unter Beibehaltung der gegenwärtigen Kosten und einer Verdoppelung der Stichprobengrösse. Die risikobasiert berechnete Stichprobe des gegenwärtigen Programms erreicht ein Vertrauensniveau von 94,18 % bei einer Designprävalenz von 0,2 % infizierter Herden. Mit der doppelten Stichprobengrösse beträgt das Vertrauensniveau 99,69 %.

Schlüsselwörter: Tankmilch, infektiöse bovine Rhinotracheitis, enzootische bovine Leukose, finanzieller Vergleich

Introduction

Substantiating freedom from disease is the basis for international free trade of animals and animal products (OIE, Terrestrial animal health code, Vol.1, Section 1, Chapter 1.4, Article 1.4.6., 2010). In Switzerland, annual serological surveys are conducted in order to demonstrate freedom from infectious bovine rhinotracheitis (IBR), enzootic bovine leucosis (EBL), brucella melitensis, Aujeszky's disease and porcine reproductive and respiratory syndrome (PRRS). As Switzerland is free of these diseases, the low design prevalence at herd level assumed in sample size calculations necessitates the sampling of a large number of herds. The sampling procedure is a two-stage process. Firstly, the requested number of herds is randomly selected from the national flock and secondly, a predefined number of animals is tested within each selected herd. The tested animals can thus be seen as a representation of the whole herd. To reduce costs, two strategies can be followed: reducing the number of herds to be sampled, for example through risk-based approaches for sample size calculations (Hadorn et al., 2002; Stark et al., 2006; Schwermer et al., 2009) or reducing the cost of sampling by getting similar herd level sensitivity with fewer tests or less expensive sampling procedures. In the last few years, the main focus in Switzerland was reducing the number of herds that were tested. However, as the development of cost-effective tools for animal disease surveillance is of high importance to scientists and decision-makers in the field of veterinary public health, the second strategy is also highly valuable.

Bulk-tank milk (BTM) sampling represents a fast, easily available, inexpensive and non-invasive sampling method to investigate herds. In Northern European countries, BTM has been in use in epizootics eradication or surveillance programmes since the eighties and its implementation has since been expanded to many other European countries as well as Australia (Hutchison and Martin, 2005). The eradication or surveillance programmes already implemented for IBR and EBL in Austria, Sweden, Norway, Denmark, England, Holland and Finland were a strong motivation for this project (Van Wuijckhuise et al., 1998; Nylin et al., 2000; Paisley et al., 2001; Nuotio et al., 2003; Nuotio et al., 2007; Brun et al., 2007). In Switzerland, pooled individual milk samples were used in the control of IBR in the eighties (Ackermann et al., 1990)

In the milk-testing scheme in Switzerland, BTM samples are routinely collected from all dairy farms, and subsequently tested (somatic cell counts, bacterial counts and antibiotic-residue testing). All samples are tested in a single laboratory since the start of 2011. At least two BTM samples per month are collected from each Swiss dairy farm. The BTM samples are kept between 1–5°C without using preservatives. These samples represent a readily available sample matrix for screening. The BTM sampling procedure is also non-invasive for the animal, avoiding stress and negative effects that an invasive blood sampling can cause. Lower costs regarding farm visits, blood sampling procedures and materials render it a valuable and inexpensive sampling method.

This study aimed to compare the costs between the current surveillance programme for IBR and EBL with blood samples from individual animals, and a surveillance approach using BTM samples from dairy farms included in the milk-testing scheme in combination with the collection of blood samples from all other farms. Several surveillance scenarios were tested with two main goals: to either substantially reduce the costs or to increase the sensitivity of the surveillance programme maintaining the same costs. To reach these goals, a literature review of IBR and EBL BTM diagnostic tests was conducted and an overview of all available commercial tests was obtained to allow a better evaluation of the practicability of BTM testing. Data of the surveillance programme conducted in 2009 was used for the comparison. The results of this study should help to give guidance regarding the decision whether BTM testing should be used in the future for IBR and EBL surveillance in Switzerland.

Material and Methods

Bulk-tank milk sampling in Switzerland

BTM represents the entire milk production delivered by a dairy farm daily or every two days. The BTM samples are automatically collected on each farm by milk-collection tankers along the milk collecting routes. In Switzerland, a large number of farms are also sampled manually at milk collection locations, at dairies and at milk collecting or centrifugation plants. Specially-trained professionals take the samples in accordance with the international standards of the International Dairy Federation (IDF) and the Swiss law (Ordinance of 23 November 2005 on milk quality (MQV) and FVO, Technical directive for the execution of milk quality control of 30 Mai 2005: Version of 9 February 2009). BTM samples are refrigerated at 1–5°C and sent to a single laboratory for the milk inspection analyses.

Swiss livestock population 2009

The number of cattle holdings in Switzerland in 2009 was 44'589 (Animal Movement Database, TVD, 2009). The number of dairy farms participating in the milk-testing scheme was 27'131 (61% of all cattle farms) with a total of 578'689 dairy cows. The mean number of cows in these dairy herds in 2009 was 21.3 animals (Federal Office of Agriculture, FOAG, 2009). The majority of cows (81,6%) are kept on the farms participating in the milk-testing scheme. The other farms represent the cattle holdings where only beef cattle are reared, farms where cattle are reared non-commercially or dairy farms from which

BTM samples are not collected because they do not sell their milk to a dairy. In 2009, this represented a total of 17'458 farms and a percentage of 39 % of all Swiss cattle farms. We obtained the number of farms from which BTM samples could not be collected and used by calculating the difference between the total number of Swiss cattle farms and the total number of farms participating in the milk-testing scheme. Currently, all these farms have to be surveyed with individual animal blood samples. There has been a strong decline in the number of Swiss milk producers in the last decade. The numbers of producers has fallen by 28,2 % with a simultaneous increase in the number of dairy cows per farm.

Framework of the financial model for the surveillance of IBR and EBL

The model framework was created in an Excel spreadsheet (Excel 2007, Microsoft, Seattle, WA). The spreadsheet model developed by Menéndez (2008) for a financial evaluation project of animal disease surveillance programmes in Switzerland was used as a basis. In the model, the different steps of the surveillance programme are described in detail and their costs are calculated. These steps include planning, implementation, sampling, laboratory testing, data management, controlling, data analysis and communication. Each step contains sub-steps with a description of the procedure and associated costs. For the costs calculation of the IBR and EBL surveillance using BTM samples the model required further refinements, so several sub-steps were added. In the planning procedure, we added the labour expenses for allocating farms to dairy or beef farms as labour. In the implementation procedure, we added the communication of the assignment of farms to the milk testing scheme laboratory as labour. In the sampling procedure, we added the triage, sorting and the delivery of the milk samples to the laboratories through the milk testing scheme laboratory as operations and expenses. In the laboratory testing procedure, we added the testing of BTM IBR Enzyme-linked Immunosorbent assay (ELISA) and EBL ELISA as operations and expenses. To ensure that dry cows or cows on medication (e.g. cows with mastitis, being treated with antibiotics) would be included, the model foresaw that all farms with BTM samples were sampled and tested twice in an interval of three months.

Surveillance costs: input data

Costs had to be estimated in order to calculate the cost-effectiveness of the surveillance programme. The cost estimations were collected from the FVO, the Institute of Virology and Immunoprophylaxis (IVI), the milk-testing laboratory (Suisselab AG) and the Swiss Post. The labour costs for the different collaborators at the Federal and Cantonal Veterinary Offices had to be quantified as wage rates. The time each collaborator spent on the surveillance programme was also estimated. The labour costs were estimated at an hourly rate in Swiss Francs. The costs for a farm visit were estimated at 28 CHF and blood sampling per individual animal at 8.50 CHF. The material used for the blood sampling (e.g. tubes and needles) was estimated at 0.30 CHF per blood sample. The handling costs of BTM samples were estimated at 5 CHF per sample. The laboratory costs for blood serum ELISA and BTM ELISA were estimated in CHF per tested sample. The unit price per tested sample included labour, materials and general laboratory charges. The costs of a blood serum ELISA were estimated at 21.70 CHF and BTM ELISA at 25 CHF.

Annual Swiss IBR and EBL surveillance programme with individual animal blood samples

Currently, the sample size of this surveillance programme is calculated according to the risk-based approach first suggested by Hadorn et al., (2002), modified by Knopf et al., (2007) and Schwermer et al., (2009). A herd sensitivity of 99 % and a herd specificity of 100 % for IBR and EBL are used for the calculation of the sample size of the actual IBR and EBL surveillance programme. The sensitivity and specificity for the IBR blood serum ELISA are 99,3 % and 98,3 % (CHEKIT® Trachitest Serum, IDEXX Laboratories) and for the EBL blood serum ELISA 99,9 % and 99,8 % (CHEKIT® Leucose Serum, IDEXX Laboratories). These values were obtained from the Swiss reference laboratories for the named diseases. Blood samples of all cattle older than 24 months are collected on cattle farms. If there are fewer than 7 animals older than 24 months on a farm, younger cattle are also sampled to reach a number of 7 blood samples and thus ensure a sufficient level of herd sensitivity. For the calculation of the sample size, a herd sensitivity of 99 % and a herd specificity of 100 % are assumed. These parameters resulted in a sample size of 1'410 cattle farms for the survey in 2009.

Bulk-tank milk surveillance programme scenarios

The scenarios were built based on a sample size that allowed to declare with 99 % reliability that less than 0,2 % of herds are infected with IBR or EBL, as agreed in the bilateral treaty with the European Community (2002: Agreement between the European Community and the Swiss Confederation on trade in agricultural products. Official Journal of the European Communities L 114, 132–349). Five surveillance scenarios based on two objectives were compared with the current surveillance programme. The definition of scenarios followed the assumptions that either the performance of the current surveillance programme was sufficient for the regulators or that the costs were acceptable. Consequently, the first outline was a surveillance programme that should cost less, while achieving the same sensitivity as the current

surveillance programme, e.g. use the same sample size as the actual programme. For the second outline, the target was to increase sensitivity while using not more than the current costs, e.g. the costs were fixed. To achieve this, either the sample size or the sampling frequency was increased. From these two baseline outlines, five scenarios for the BTM surveillance programme were evaluated, representing different sample sizes, different sampling recurrence and different fluctuating costs. The first scenario «Milk 1» was based on the same sample size as the sampling programme from 2009. In this scenario, a total of 1'410 cattle farms had to be sampled once a year, 550 through blood samples with a total of 10'815 examined blood serum samples and 860 dairy farms through BTM samples. In the second scenario «Milk 2», an estimation of the costs of 100 additionally sampled dairy farms was made in order to evaluate the mean costs and sensitivity of additional BTM sampling. Thus the cost-effectiveness of an increase in the sample size could be evaluated. This approach for the estimation of extra costs for sampling of additional farms offers higher accuracy compared to just stating the cost inputs for an additional farm, as the general costs for the programme are here split among all farms. General costs include all the costs for planning, implementation and administration of the surveillance programme. For the «fixed costs» outline we observed that this would correspond to doubling the sample size. As it is easier to communicate, we choose this approach rather than keeping the costs exactly the same as in the actual programme. The third scenario «Milk 3» was based on the doubled sample size compared with the survey from 2009. The fourth scenario «Milk 4» based again on the same sample size of the Swiss disease surveillance from 2009, but samples were taken twice a year. This scenario would thus be able to detect a disease event earlier than annual surveys. The fifth scenario «Milk 5» contained the sampling of all dairy farms included in the milk-testing scheme once a year and actual sampling of blood samples. 27'131 BTM samples and 550 beef farms with a total of 10'815 blood serum samples had to be tested.

Bulk-tank milk diagnostic tests IBR and EBL

Diagnostic use of ELISAs on BTM samples is common for IBR and EBL (Klintevall et al., 1991; Hartman et al., 1997; Sargeant et al., 1997; Nylin et al., 2000; Stahl et al., 2002; Ridge and Galvin, 2005). After an enquiry with 13 laboratories in Europe, commercially IBR and EBL BTM ELISAs were identified on the market (Tab. 1).

Additional costs: Follow-up testing for non-negative bulk-tank milk samples

In the case of non-negative BTM test results, it was assumed that blood samples of every single animal from the farms would have to be collected and tested. To assess the potential proportion of non-negative BTM results per sampling round, the data from the BTM surveillance programme of 2008 for IBR, EBL and brucellosis from the Austrian Agency for Health and Food Safety (AGES) were used. In this programme, BTM from all Austrian dairy farms were tested once a year. As Switzerland, Austria is also free of IBR and EBL, and therefore, the epidemiological situation is similar. In the Austrian surveillance programme, 0,25 % of the BTM samples were not negative for IBR and 0,15 % for EBL. Therefore, if the scenario «Milk 1» was implemented in Switzerland, approximately 3 BTM samples could be not negative for IBR and approximately 2 BTM samples for EBL per sampling round. For the scenario «Milk 5», a total of approximately 68 BTM samples could be not negative for IBR and approximately 41 BTM samples for EBL. For the additional costs of the follow-up testing for non-negative BTM samples, the steps «sampling» and «laboratory testing» were taken into consideration because these represent fix costs for the re-testing in the model and do not depend on each Cantonal Veterinary Office. In the Swiss disease surveillance programme of 2009, the average of the tested blood samples per farm was 20.2. In the calculation, it was assumed that at least 20 animals per non-negative farm were retested individually.

Calculation of programme's sensitivity

To compare the detection power of the different scenarios, the probability to detect the farm-level design prevalence of 0,2 % was calculated using the freeware «freecalc» (AusVet Animal Health Services, Toowoomba, Australia). The sensitivity and specificity of testing an individual farm was set at 99 % and 100 % irrespectively whether the farm was tested using individual blood samples or BTM. The calculation was done with a population size of 41'100 cattle farms (Swiss Federal Statistical Office).

Results

Costs, the number of samples and the archived confidence of freedom for the current surveillance programme as well as for the different BTM scenarios are summarised in the Tables 2 and 3. The mean total surveillance costs for the current surveillance programme for IBR and EBL with blood samples is 1'662'468 CHF for the year 2009. The majority of the expenditures was for the laboratory testing procedures – some 73,9 % of the total costs. For the scenario «Milk 1», the total costs of this surveillance programme for IBR and EBL including BTM samples were 853'445 CHF. With this scenario the costs were reduced to approximately half of the current costs. For the scenario «Milk 2», the total costs were 864'445 CHF. Thus, for 100 additional BTM samples, the additional costs were only 11'000 CHF. Scenario «Milk 3» remained less expensive than the current risk-based programme, despite doubling the sample size. For the scenario «Milk 4» the extra costs in

Table 1: Commercially available BHV-1 and EBLV ELISAs in 2009 - (ND = no data available).

Producer	Disease	Testskits		Sensitivity	Specificity
Bio-X Diagnostics, Jemmel, Belgium	IBR	Bio K 238	Blood serum, milk and bulk milk	ND	ND
Hipra , Amer, Spain	IBR	CIVTEST BOV IBR	Blood serum, milk and bulk milk	ND	ND
Ingenesa, Madrid, Spain	EBL	Ingezim BLV	Blood serum and milk	ND	ND
Idexx, Maine, United States	IBR	BHV-1 Tank milk	Milk and bulk milk up to 50 animals	100; 98	100; 94
		Idexx Leukosis Milk Screening Ab	Milk and bulk milk	> 99	99
	EBL	Pourquier ELISA Leucose lait	Milk and bulk milk	ND	ND
		Pourquier ELISA IBR-IPV sérum et lait	Blood serum, milk and bulk milk	ND	ND
LSI, Laboratoire Service International, Lissieu, France	IBR	LSIVET MILK IBR Screening	Milk and bulk milk up to 50 animals	ND	ND
Svanova, Uppsala, Sweden	IBR	Svanovir IBR -ab	Blood serum, milk and bulk milk	97.4; Milk vs. Serum: 92.8	92.4; Milk vs. Serum:100
	EBL	Svanovir BLV-gp51-Ab	Blood serum, milk and bulk milk	*Ridge and Galvin, 2005: 50.4; Nuotio et al., 2003: 100 %	Ridge and Galvin, 2005: 99.9; Nuotio et al., 2003: 93.4 %
Synbiotics, Lyon, France	EBL	Lactelisa BLV Ab Mono Indirect	Milk and bulk milk up to 50 animals	ND	ND
		Lactelisa BLV Ab Bi Indirect	Milk and bulk milk up to 50 animals	ND	ND
		Lactelisa BLV Ab Tank 250 Bi indirect	Milk and bulk milk up to 250 animals	Ridge and Galvin, 2005: 100	Ridge and Galvin, 2005: 99.6

*In the study of Ridge and Galvin, 2005, comparing two BTM ELISAs, the sensitivity of one of the BTM test showed a very low sensitivity of 50.4 %. This obviously does not represent the true evaluation of the sensitivity. The tests used in absence of a gold standard could not estimate the true sensitivity and specificity of both EBL ELISAs, due to the fact that the two assays being compared were not independent.

comparison to the blood serum surveillance programme were 44'422 CHF. For the scenario «Milk 5», the total costs were 3'743'255 CHF. The laboratory costs for the BTM represented the major cost factor in this programme. The total costs of a re-sampling and re-testing were at least 3'300 CHF for the less expensive scenario «Milk 1». The total costs of re-sampling and re-testing were 27'300 CHF for the most comprehensive scenario «Milk 5».
In the scenarios «Milk 3» and «Milk 5» the achieved confidence levels were higher than required 99 %. In the actual programme and the scenarios «Milk 1» and «Milk 4» the sample size are equal in each sampling round and consequently the achieved confidence is the same. The testing of 100 more samples increased the confidence by 1 % in scenario «Milk 2».

Discussion

This financial comparison made it possible to assess the savings generated by including BTM samples in the surveillance programmes. Either the costs were reduced by 50 %, without any major impact on the quality of the surveillance programme. Or when the costs were maintained, the sample size or sample recurrence were increased, resulting in a higher sensitivity of the surveillance programme. For the sample size calculation, the herd sensitivity in the current blood sampling surveillance programme was estimated to be 99 %. Given the data on sensitivity of BTM tests there is no indication of a substantial decrease in the herd sensitivity as a result of the implementation of these tests (Tab. 1). The sample size is therefore not affected by this change, allowing us to use the same sample size calculation for the milk scenarios as for the actual blood sampling. The actual programme fulfils the required confidence level of 99 % through the utilization of a risk-based sample size calculation (Hadorn et al., 2002; Schwermer et al., 2009). In this approach the actual achieved confidence in an annual survey is combined with the results of prior surveys and can thus be lower than the required confidence level. In contrast, in scenario «Milk 3» the required confidence

Table 2: Summary of scenario designs for IBR/EBL-surveillance with different sample sizes and combinations of dairy and non-dairy farms.

Scenario	No. of bulk milk samples	No. of blood samples	Description
«Blood»	-	22,732	Actual programme; blood samples from dairy and non-dairy farms
«Milk 1»	860	10,815	Same sample size as actual programme; blood samples are only from non-dairy farms
«Milk 2»	860 + 100	10,815	«Milk 1» and 100 additional BTMS; blood samples are only from non-dairy farms
«Milk 3»	1,720	21,630	Double sample size as «Milk 1» for dairy and non-dairy farms
«Milk 4»	2 x 860	2 x 10,815	«Milk 1» twice a year for dairy and non-dairy farms
«Milk 5»	27,131	10,815	BTMS from all available dairy farms and blood samples from non-dairy farms as in «Milk 1»

Table 3: Summary of the surveillance scenario's sensitivity and costs: Detection probability refers to the probability to detect a farm level design prevalence of 0.2 % in the Swiss cattle population; General costs are all costs for planning, implementation and administration of the surveillance programme. The costs are in CHF. Percentages refer to the total costs of the surveillance programme. The costs per average farm are calculated by dividing the total costs by the number of farms sampled. * This detection probability refers to a interval of 6 months in contrast ta an interval of 12 months for the other scenarios.

Scenario	Detection probability	Total costs	Bulk-tank milk sampling & testing costs (%)	Blood sampling costs (%)	Blood testing costs (%)	General costs (%)	Costs per average farm
«Blood»	94.18 %	1'662'468		295'021(17.8)	1'229'036 (73.9)	183'411 (8.3)	1179
«Milk 1»	94.18 %	853'445	95'520 (11.2)	122'072 (14.3)	494'838 (58)	141'015 (16.5)	605
«Milk 2»	95.26 %	864'445	106'520 (12.3)	122'072 (14.1)	494'838 (57.3)	141'015 (16.3)	572
«Milk 3»	99.69 %	1'528'040	190'120 (12.5)	232'652 (15.2)	964'252 (63.1)	141'015 (9.2)	541
«Milk 4»	94.18 % *	1'706'890	191'040 (11.2)	244'144 (14.3)	989'676 (58)	282'030 (16.5)	1211
«Milk 5»	> 99.99 %	3'743'255	2'985'330 (79.7)	122'072 (3.3)	494'838 (13.2)	141'015 (3.8)	134

is achieved in each sampling round. The added value in scenario «Milk 4» is not a higher confidence per sampling round, but the shorter time interval between the sampling rounds. By this, the programme is better suited for early detection purposes. In the scenario «Milk 5», the confidence as close to 100 %, that it is out of the calculation power of the software used. In consequence, this scenario would provide the highest probability of freedom of all scenarios. However, as in this scenario a large part of the cattle farms are out of the sampling frame, the sample is not representative for the Swiss cattle population.

The factors representing the major costs in the scenarios were identified. These costs were related to herds not participating in the milk inspection scheme due to the costs of blood sampling of individual animals and testing of individual blood samples. Several possibilities are conceivable to reduce these costs, such as the pooling of blood samples, the sampling in slaughterhouses or the risk-based selection of farms for the sampling. Minor extra labour could be expected in the step «planning» of the surveillance programme for separating dairy farms, which can be tested through BTM samples, from beef farms, which still have to be tested through blood samples. In Switzerland, the TVD and the Information Database of the Swiss Veterinarian Authorities (ISVet) provide an excellent basis for conducting this classification more easily. In contrast, utilising additional BTM samples is rather inexpensive, as scenario «Milk 2» shows. Thus BTM samples also provide a quick and inexpensive means to modify the surveillance programme if requirements change, for example if testing of all dairy farms in a certain region in the case of a disease outbreak is required. By the sampling of all Swiss dairy farms – scenario «Milk 5» – the costs of the laboratory testing for the BTM samples represented the major cost factor of the surveillance programme. However, a reduction of the costs per BTM ELISA in the laboratories can be expected if the test is introduced on a routine basis.

The limitations of BTM samples also need to be considered. BTM samples only represent the cows delivering milk to the bulk-tank on the day of sampling, excluding dry cows, diseased cows, cows in the colostral period and other non-milking cattle. In each scenario, this problem was addressed by collecting and testing two BTM samples in a minimal interval of three months. It was expected, that a second round of sampling would be sufficient to compen-

sate this effect, as the dry period is on average 2 months and diseased cows should have recovered or have been culled in that time. The BTM diagnostic tools are sensitive to the number of pooled milk samples contained in the bulk-tank, the number of shedding cows and to the concentration of antibodies. The average acceptable maximal dilution for an ELISA for BTM seems to be < 50 animals in one bulk-tank sample, depending on the ELISA, as well as on the disease and the manufacturer. In an Australian study of a comparison of two ELISAs for detecting EBL, one of the ELISAs failed to detect EBL antibodies by a dilution of 1 in 40, whereas the comparable value for the other ELISA was 1 in 200 (Ridge and Galvin, 2005). By means of a dilution trial from a Danish study, a BTM BHV-1 blocking ELISA detected 75 % of the herds as BHV-1 seropositive with one out of ten cows being seropositive, but only up to 25 % of the herds with one cow being seropositive out of > 60 cows (Nylin et al., 2000). Switzerland has favourable conditions because of its small cattle herd size. The average number of cows in dairy farms in 2009 was 21.3. Additionally, only 2,9 % of dairy herds in Switzerland consist of more than 50, which means that 97,1 % of all Swiss dairy herds could be included in the BTM sampling surveillance programme and could be tested under optimal testing conditions. If the herd prevalence is low and the dilution is high, there will be a possible risk that a positive herd can remain undiscovered while the disease has already spread (Frankena et al., 1997; Nylin et al., 2000). A second round of sampling is also a valuable tool to detect this spread more quickly and also to detect false-negative herds with a recent infection history and which had tested negative in the first round of sampling (Houe et al., 2006).

In Switzerland, a trend towards a decrease of dairy farm numbers with a simultaneous increase of respective herd size is currently observed. These general conditions therefore need to be monitored, and the surveillance programme should be adjusted when needed.

Conclusion

BTM sampling is a cost-effective method for cattle disease surveillance. The FVO is therefore organising a BTM pilot survey for the surveillance of IBR and EBL and foresees the implementation of BTM sampling in future surveillance programmes. The utilization of BTM samples also increases the flexibility of the surveillance programmes to changing needs, for example increased surveillance intensity in case of disease events or increase in the early detection capabilities of the survey design.

Acknowledgements

The authors would like to thank all colleagues at the Federal Veterinary Office, the Institute for Virology and Immunoprophylaxis, Suisselab AG and the Institute of Veterinary Public Health, Vetsuisse Faculty, University of Bern, for their help and for providing valuable data. We would also like to thank Sonia Menéndez for the provision of her model and Barbara Häsler for her help providing data and valuable advice. Special thanks go to Michelle Schorer for corrections of the manuscript. The project was funded by the Federal Veterinary Office.

Efficacité économique des programmes de surveillance utilisant des échantillons de lait de citerne pour démontrer l'absence de rhino trachéite infectieuse bovine et de leucose enzootique bovine en Suisse

En Suisse, l'absence de rhinotrachéite bovine (IBR) et de leucose enzootique bovine (EBL) est démontrée par l'examen annuel d'échantillon sanguin d'animaux individuels choisi au hasard. Contrairement à plusieurs pays européens, les échantillons de lait de citerne ne sont plus utilisés depuis de nombreuses années dans la surveillance actives des épizooties. Le but de ce projet était une comparaison financière entre les programmes de surveillance actuels au moyen d'échantillons sanguins et un programme de surveillance modifié comprenant des échantillons de lait de citerne, ceci afin d'estimer les économies possibles. Un modèle de tableur financier a été utilisé pour comparer les coûts. On a développé des scénarios avec diverses quantités et fréquences d'échantillonnages. Les coûts

Ottimizzazione dei costi dei programmi di sorveglianza con l'uso di campioni di latte da cisterna per dimostrare l'assenza di rinotracheite infettiva bovina e leucosi bovina enzootica in Svizzera

In Svizzera, l'assenza di rinotracheite infettiva bovina (IBR) e di leucosi bovina enzootica (EBL) viene dimostrata tramite un esame annuale di campioni di sangue di singoli animali provenienti da un campione casualizzato. A differenza di molti paesi europei, l'uso di campioni di latte da cisterna non si utilizza più da diversi anni per la sorveglianza attiva delle malattie negli animali. L'obiettivo di questo progetto è di fare un confronto finanziario tra l'odierno programma di monitoraggio che utilizza campioni di sangue e un programma di monitoraggio modificato che include campioni di latte da cisterna al fine di stimare i risparmi sui costi. Un modello di calcolo finanziario è stato utilizzato per il confronto dei costi. Sono stati sviluppati scenari con differenti dimensioni e frequenze del

pourraient être diminués de moitié sans diminution de la qualité de programme de surveillance. De même, la sensibilité du programme pourrait être nettement augmentée en maintenant les cout actuels et en doublant le nombre d'échantillons. Les échantillons du programme actuels, basé sur le risque, atteignent un niveau de confiance de 94.18 % avec une prévalence désignée de 0.2 % de troupeaux affectés. En doublant le nombre d'échantillon ce niveau de confiance atteint 99.69 %.

campione. I costi potrebbero essere dimezzati senza deterioramento della qualità del programma di sorveglianza per dimostrare l'assenza IBR e EBL. Allo stesso modo, la sensibilità del programma potrebbe essere aumentata in modo evidente, pur mantenendo i costi attuali ma raddoppiando la dimensione del campione. Il campione, calcolato in base al rischio, del programma corrente ha raggiunto un livello di affidabilità del 94.18 % con una prevalenza dello 0.2 % degli allevamenti infettati. Raddoppiando la dimensione del campione, il livello di affidabilità è del 99.69 %.

References

Ackermann, M., Müller, H.K., Bruckner, L., Kihm, U.: Eradication of infectious bovine rhinotracheitis in Switzerland: review and prospects. Vet. Microbiol. 1990, 23:365–370.

Brun, E., Jordsmyr, H.M., Hellber, H., Mork, T. (editors): Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2007. Oslo: National veterinary Institute 2008: 49–55.

Frankena, K., Franken, P., Vandehoek, J., Koskamp, G., Kramps, J.A.: Probability of detecting antibodies to bovine herpesvirus 1 in bulk milk after the introduction of a positive animal on to a negative farm. Vet. Rec. 1997, 140: 90–92.

Hadorn, D.C., Rufenacht, J., Hauser, R., Stark, K.D.C.: Risk-based design of repeated surveys for the documentation of freedom from non-highly contagious diseases. Prev. Vet. Med. 2002, 56: 179–192.

Hartman, A., vanWuijckhuise, L., Frankena, K., Franken, P., Wever, P., deWit, J., Kramps, J.: Within-herd BHV-1 prevalence prediction from an ELISA on bulk milk. Vet. Rec. 1997, 140: 484–485.

Houe, H., Lindberg, A., Moennig, V.: Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. J. Vet. Diagn. Invest. 2006, 18: 427–436.

Hutchison, J., Martin, T.: An evaluation of surveillance for enzootic bovine leucosis in the australian dairy industry. Australian Government Department of Agriculture, Fisheries and Forestry Report 2005.

Klintevall, K., Näslund, K., Svedlund, G., Hajdu, L., Linde, N., Klingeborn, B.: Evaluation of an indirect ELISA for the detection of antibodies to bovine leukaemia virus in milk and serum. J. of Virol. Methods 1991, 33: 319–333.

Knopf, L., Schwermer, H., Stark, K.D.: A stochastic simulation model to determine the sample size of repeated national surveys to document freedom from bovine herpesvirus 1 (BoHV-1) infection. BMC Vet. Res. 2007, 3: 10.

Menéndez, S.: Costs of surveillance systems. Federal Veterinary Office Report 2008.

Nuotio, L., Rusanen, H., Sihvonen, L., Neuvonen, E.: Eradication of enzootic bovine leukosis from Finland. Prev. Vet. Med. 2003, 59: 43–49.

Nuotio, L., Neuvonen, E., Hyytiainen, M.: Epidemiology and eradication of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) virus in Finland. Acta Vet. Scand. 2007, 49.

Nylin, B., Stroger, U., Ronsholt, L.: A retrospective evaluation of a Bovine Herpesvirus-1 (BHV-1) antibody ELISA on bulk-tank milk samples for classification of the BHV-1 status of Danish dairy herds. Prev. Vet. Med. 2000, 47: 91–105.

Paisley, L.G., Tharaldsen, J., Jarp, J.: A retrospective analysis of the infectious bovine rhinotracheitis (bovine herpes virus-1) surveillance program in Norway using Monte Carlo simulation models. Prev. Vet. Med. 2001, 50: 109–125.

Ridge, S.E., Galvin, J.W.: A comparison of two ELISAs for the detection of antibodies to bovine leucosis virus in bulk-milk. Aust. Vet. J. 2005, 83: 431–434.

Sargeant, J.M., Kelton, D.F., Martin, S.W., Mann, E.D.: Evaluation of a bulk-milk ELISA test for the classification of herd-level bovine leukemia virus status. Prev. Vet. Med. 1997, 31: 223–230.

Schwermer, H., Reding, I., Hadorn, D.C.: Risk-based sample size calculation for consecutive surveys to document freedom from animal diseases. Prev. Vet. Med.2009, 92: 366–372.

Stahl, K., Rivera, H., Vagsholm, I., Moreno-Lopez, J.: Bulk milk testing for antibody seroprevalences to BVDV and BHV-1 in a rural region of Peru. Prev. Vet. Med. 2002, 56: 193–202.

Stark, K., Regula, G., Hernandez, J., Knopf, L., Fuchs, K., Morris, R., Davies, P.: Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: Review of current approaches. BMC Health Services Research 2006, 6: 20.

Van Wuijckhuise, L., Bosch, J., Franken, P., Frankena, K., Elbers, A.R.W.: Epidemiological characteristics of bovine herpesvirus 1 infections determined by bulk milk testing of all Dutch dairy herds. Vet. Rec. 1998, 142: 181–184.

Corresponding author

Heinzpeter Schwermer
Monitoring Department
Federal Veterinary Office
Schwarzenburgstr. 155
CH-3097 Liebefeld
Tel.: + 41 (0)31 323 30 53
Fax: + 41 (0)31 323 95 43
heinzpeter.schwermer@bvet.admin.ch

Received: 28 March 2011
Accepted: 20 January 2012

Chapter 4

Public health prioritisation of zoonotic pathogens that can be transmitted to humans by raw milk or milk

Introduction

Background

To be able to allocate resources for research and surveillance of infectious diseases efficiently, prioritising pathogens has become increasingly necessary in the field of public health. Several studies adopting different methods have been published for prioritizing foodborne pathogens. However, the focus of these publications was either alone on foodborne zoonotic pathogens in general or on pathogens relevant for health issues in dairy cattle. None of these studies was about zoonotic pathogens that can be transmitted to humans via milk and thus, to the best of our knowledge, no study about the prioritisation of zoonotic pathogens transmitted by raw or heat-treated milk was ever published in Switzerland (Mangen et al., 2009; Wells et al., 1998; Doherty, 2000; Mangen et al., 2006; Havelaar et al., 2008; Cardoen et al., 2009; Ruzante et al., 2010). In Switzerland, both trading of milk and cheese production are economically very important. The trade with milk is well controlled by official and private institutions and thus an exceptional high level of security is provided. By law, only heat-treated milk can be sold as foodstuff, and raw milk is not considered ready for consumption. However, raw milk can be sold to consumers leaving the task of heat treatment to them.

As we were interested in the economic evaluation of surveillance systems using bulk-tank milk sampling, the identification of the major zoonotic pathogens transmitted by milk or raw milk in Switzerland was necessary as a first step. Therefore, a literature review about zoonotic pathogens - their epidemiology and especially probability of surviving the heat treatment (thermisation or pasteurisation) in the milk - was carried out. To gain an overview of the Swiss situation, an evaluation of the exhaustive selection of zoonotic pathogens that could be

transmitted by raw milk was organized with a panel of Swiss experts. The aim was to gain a conclusive priority setting or selection to decide which pathogens are the most significant to be surveyed through bulk-tank milk (BTM) in Switzerland. The experts mainly valued raw milk as a source of infection for humans. The pathogen's survival of thermisation and pasteurisation was also considered as a potential risk of infection, but of much less importance.

A questionnaire was developed using the methodology based on the method for prioritisation of infectious diseases by Krause and the working group on prioritisation at the Robert Koch Institute, Germany (Krause, 2008) as a general model. This methodology provided a standardised, systematic, reproducible and plain tool. The aim of this study was to identify zoonotic pathogens which pose a risk for human health through the consumption of milk and which could be surveyed suitably in BTM.

Materials and Methods

First, a literature review revealed the zoonotic pathogens for prioritisation based on their presence and potential to be transmitted to humans through the consumption of raw milk. Afterwards, experts of the working group curtailed the list of pathogens. The final list included 16 pathogens that were integrated in an expert survey (Table 1).

Questionnaire

For each pathogen, the 6 categories (1) "burden of disease towards humans", (2) "epidemiologic dynamics in humans", (3) "incidence in milk", (4)"available scientific information", (5) "economic significance" and (6) "public attention or perception" were to be rated. Questions of the first category included the ranking of the severity of disease, the incidence and the mortality in humans caused by the pathogen in Switzerland. In the second category questions included the ranking of the outbreak potential, the trend, the transmission and the potential of the pathogen to spread. In the third category we questioned the ranking of the potential of transmission through raw milk, the viability and the potential of transmission

in heat treated milk, the viability and the potential of transmission in pasteurised milk and the potential of prevention of cases through BTM sampling. Questions of the fourth category included an appraisal of the current knowledge on epidemiologic information, evidence of risk factors or groups at risk, evidence of pathogenesis and transmission pathways for the pathogen. Questions of the fifth category included ranking of the production losses caused by the pathogen. Questions of the last category included the presence of national and international regulations on trade of animals and animal products and an appraisal of the current public attention or perception of the pathogen.

The pathogens were rated according to 14 criteria. For each criterion a numerical score of +1, 0 or -1 was available. The score of +1 indicated a high importance and a score of - 1 a low importance with respect to the criterion. A score of 0 represented an average importance. The numerical scores of each criterion were added giving a total numerical score for all 14 criteria (Krause, 2008). Additionally, a weight between 1 and 5 was assigned to each criterion by which the numerical score was multiplied. This weight was attributed to the criterion before the beginning of the survey by our working group and was not communicated to the elicited experts.

The experts were also asked to rate their expertise in a self-assessment for each pathogen. This individual rating was between 1 and 3 (1= low knowledge of the pathogen, 2= moderate knowledge of the pathogen, 3=high knowledge of the pathogen) and the total numerical score was multiplied by this value. For an unbiased comprehension by all participants the questionnaire was translated into French, German and Italian (German version is shown in graphic 1).

Expert elicitation

Experts from Swiss governmental, industrial and research institutions with different background were invited to participate in this study. The institutions enquired were the Federal Public Health Office (BAG), the Institute for Food Safety and Hygiene of the

Vetsuisse Faculty of Zurich, the Centre for Zoonoses, Bacteriological Animal Diseases and Antibiotic Resistance (ZOBA), Bern, the University Hospitals of Bern, Lausanne and Geneva, the Institute of Food Knowledge from the Swiss Federal Institute of Technology (ETH), Zurich, the Agroscope Liebefeld-Posieux (ALP), the Federal Food Chain Unit, the Veterinary Public Health Institute (VPHI) from the Vetsuisse Faculty of Bern, Cantonal Veterinary Offices, the Cantonal Institute of Microbiology of the Canton Ticino, milk industries and cantonal laboratories. Twenty-seven experts were identified and invited. Nineteen of the twenty-seven agreed to participate and to fill in the questionnaire. The experts were asked to fill in all questions for each pathogen. If they felt unable to answer a question, they were asked to answer it to the best of their knowledge. After the questionnaires were sent, seven experts declined to participate, three never returned the questionnaire and one questionnaire was incomplete. Reminder emails were sent 3 times to all non-respondents during a period of 1 month. As a result, a total of 8 experts participated in the expert survey. They could complete the questionnaire entirely or at least entirely for some pathogens.

Scoring

We conducted four different calculations for the final scoring of the pathogens: weighted score with expert's self-assessment, score with expert's self-assessment, weighted score without expert's self-assessment and score without expert's self-assessment. As it was likely that the rankings were different for each of this approach, we decided prior to the ranking, that the most relevant ranking should be the one with weighted scoring and expert's self-assessment. As it contained the most detailed information, this ranking should provide the most specific assessment for the Swiss situation. However, the results of the three other rankings should be used to assess the robustness of the whole process. As it got obvious that the influence of the expert's self—assessment was negligible, only two rankings were assessed in detail. The first ranking was with the means of weighted scores and the second one with the means of scores multiplied by the rate of the experts' self-assessment.

Results

Ranked mean scores of the pathogens for all four rankings and from all experts are shown in table 3. Most pathogens received a negative score, which indicates that the experts rated the majority of the criteria as of low importance for almost all pathogens. The prioritisation was determined by ranking the pathogens by the mean of the individual expert score for each pathogen. The resulting lists are summarized in tables 4 and 5.

According to this prioritisation, the currently most challenging zoonotic pathogen, which can be transmitted to humans by milk or raw milk in Switzerland, is *Listeria monocytogenes* with a weighted score of 2.5. However, in the list of scores, *Mycobacterium tuberculosis bovis* came on the top of the ranking followed by *Listeria monocytogenes* and *Brucella abortus*. Taking into account that Switzerland is free from tuberculosis and brucellosis in cattle, sheep and goats, *Listeria monocytogenes* remains to be the most relevant pathogen to be monitored in BTM with respect to a potential transmission to humans by milk. Interestingly, the rankings showed only little variation for most of the pathogens. The highest influence of the weights was on "*Mycotoxins*" and "*Campylobacter jejuni/coli*". Whereas "*Mycotoxins*" was ranked fourth with weights, it was ninth in the unweighted list. "*Campylobacter jejuni/coli*" was ranked tenth in the weighted list and sixth in the unweighted list.

Discussion

The methodology applied for the prioritisation was straight forward to implement, transparent and introduced the desired objectivity in the expert survey. We framed the questionnaire to be complete, precise and consisting of a reasonable number of questions and of an exhaustive and representative number of pathogens. In spite of this endeavour, the amount of work for each expert to fill in the questionnaire was time-consuming and the topics in the panel of questions wide-ranging. This was communicated to us in many of the expert feedbacks. Most of the "non-responders" did not complete the questionnaire because of a lack of time. This explains the low response rate of 30%. In expert opinion surveys, subjectivity and uncertainty

are strong components and multiple sources for bias cannot be avoided (Vose, 2009). We chose experts from several fields of work, thus the panel of experts invited to this survey was as representative as possible for zoonoses experts in Switzerland. Unfortunately, no completed questionnaires from experts from the industry could be integrated in the survey. Food safety experts and veterinarians were well represented. Of course, this decreases the representativeness of the prioritisation results. The integration of the experts' self-assessment for each pathogen was a promising approach to provide more objective evidence for the final score. Obviously, not all experts could have the same expertise in all 16 pathogens. This is also reflected in a large variation of the scores assigned to the pathogens by the different expert. Furthermore, the results of this study reveal the complexity and the multidisciplinary of tasks in the field of zoonoses. The low response rate as well as the critical self-assessments might indicate a lack of sound information and recent knowledge concerning pathogen transmission risks through raw milk and milk consumption. Many of the works concerning the risks of pathogen transmission through raw milk and milk consumption are aged, and thus little evidence from recent scientific studies is available. New studies with new diagnostics will, in some cases, deliver important results for the knowledge of pathogens in milk, either if they confirm or disprove the results of the aged studies.

Acknowledgement

The author would like to thank all experts who participated to the prioritisation for their valuable support.

References

Cardoen, S., Van Huffel, X., Berkvens, D., Quoilin, S., Ducoffre, G., Saegerman, C., Speybroeck, N., Imberechts, H., Herman, L., Ducatelle, R., Dierick, K.: Evidence-based semiquantitative methodology for prioritization of foodborne zoonoses. Foodborne Pathog 2009, Dis 6: 1083-1096.

Doherty, J.A., Establishing priorities for national communicable disease surveillance. Can J Infect 2000, Dis 11: 21-24.

Havelaar, A.H., Galindo, A.V., Kurowicka, D., Cooke, R.M.: Attribution of Foodborne Pathogens Using Structured Expert Elicitation. Foodborne Pathogens and Disease 2008, 5: 649-659.

Krause, G.: Prioritisation of infectious diseases in public health--call for comments. Euro Surveill 2008, 13.

Mangen, M.J., Batz, M.B., Kasbohrer, A., Hald, T., Morris, J.G., Jr., Taylor, M., Havelaar, A.H.: Integrated approaches for the public health prioritization of foodborne and zoonotic pathogens. Risk Anal 2009, 30: 782-797.

Mangen, M.J.J., Van Duynhoven, Y.T.H.P., Van Pelt, W., Kemmeren, J., Havelaar, A.H.: Priority setting of (foodborne) pathogens. European Journal of Public Health 2006, 16: 11-11.

Ruzante, J.M., Davidson, V.J., Caswell, J., Fazil, A., Cranfield, J.A.L., Henson, S.J., Anders, S.M., Schmidt, C., Farber, J.M.: A Multifactorial Risk Prioritization Framework for Foodborne Pathogens. Risk Analysis 2010, 30: 724-742.

Vose, D.: Risk analysis, a quantitative guide. 2009, Third edition: Chapter 14.

Wells, S.J., Ott, S.L., Seitzinger, A.H.: Key health issues for dairy cattle - New and old. Journal of Dairy Science 1998, 81: 3029-3035.

Table 1: Pathogens selected for prioritisation; listed in alphabetical order

Bacillus cereus
Brucella abortus
Campylobacter jejuni/coli
Central European tick-borne virus
Clostridium botulinum
Clostridium perfingens
Coxiella burnetii
Enterotoxic *E. coli (STEC)*
Listeria monocytogenes
Mycobacterium avium* subsp *paratuberculosis
Mycobacterium tuberculosis/bovis
Mycotoxins
Salmonellae non-typhi, non-parathyphi
Staphylococcus aureus
Streptococcus pyogenes
Yersinia enterolitica

Graphic 1: Criteria and definition of the respective scores for the prioritisation of pathogens (German questionnaire)

Zoonose-Priorisierung			
→modifiziert nach der Arbeit von der Arbeitsgruppe für Priorisierung von Robert Koch Institut (Krause et al., 2008)			
Selbst-Einschätzung als Expert/in*	**1**	**2**	**3**
* 1=geringe Expertise, 2=mittlere Expertise und 3=hohe Expertise			
Kriterien:		**Werte und Definition**	
Krankheitsbelastung (Mensch):	**-1**	**0**	**1**
Inzidenz in CH/Jahr	<1/100'000	1/100'000-20/100'000	>20/100'000
Schweregrad	Hospitalisation sehr selten, Arbeitsausfall weniger als 2 Tage, ohne Folgen	Hospitalisation selten, Arbeitausfall mehr als 5 Tage ist selten, selten Folgen	Hospitalisation ist häufig, Arbeitausfall mehr als 5 Tage ist häufig, Folgen sind möglich
Letalität	<0,01%	0,01%-1%	>1%
Epidemiologie der Krankheit (Mensch):			
Ausbruchspotenzial	Ausbrüche sind sehr selten	Ausbrüche mit 5 oder mehr Fällen sind selten	Ausbrüche mit 5 oder mehr Fällen sind häufig
Trend	Inzidenz verringert sich	Stabile Inzidenz	Inzidenz nimmt zu
Übertragung und Verbreitungspotenzial	niedrig	mittel	hoch
Bedeutung in Milch:			
Übertragungspotenzial in Rohmilch[1]	kein	wahrscheinlich	sicher vorhanden
Überleben und Übertragungspotenzial in thermisierter[1] Milch	kein	wahrscheinlich	sicher vorhanden
Überleben und Übertragungspotenzial in pasteurisierter[1] Milch	kein	wahrscheinlich	sicher vorhanden
Effiktivität einer Tankmilchuntersuchung im Hinblick auf Prävention	nicht effektiv, Kontamination primär während der Fabrikationsprozess	nicht unbedingt effektiv, Kontamination während der Fabrikationsprozess möglich	effektiv
Wissenschaftliches Wissen:			
Epidemiologische Information/Risikofaktoren und Risikogruppen/Pathogenese und Übertragungwege (Tier-Mensch)	bekannt mit wissenschaftlichem validiertem Wissen	grundsätzlich bekannt, aber wissenschaftlich nicht unbedingt validiert	nicht bekannt , ungenügendes Wissen
Wirtschaft :			
Wirtschaftliche Bedeutung und Produktionsverluste	niedrig	mittel	hoch
Allgemein (in CH):			
Nationale und Internationale Regelung (im Handel mit Produkten und lebenden Tieren)	keine Regelung	keine Internationale Regelung, aber nationale Vepflichtungen	Internationale und nationale Regelung
Öffentliches Interesse und Wahrnehmung:	niedrig	mittel	hoch

Table 3: Mean scores derived from an expert survey on prioritisation of zoonotic pathogens by 8 different experts. For unweighted scores, all criteria evaluated by the experts were scored equally, while a weighting factor was introduced for the weighted scores. For scores with expert self-assessment, an additional weighting is introduced according to how confident experts were about their knowledge on the respective pathogen

Experts	Description of the score	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
Expert 1	Weighted score without expert self-assessment		0	-19		-5	-14	-19	-29	4		-3		-11	-7	-26	-19
	Weighted score with expert self-assessment		0	-57		-10	-28	-57	-58	8		-9		-33	-21	-78	-57
	Unweighted score without expert self-assessment		-1	-4		-2	-5	-5	-8	-1		0		-3	-4	-6	-7
	Unweighted score with expert self-assessment		-3	-12		-4	-10	-15	-16	-2		0		-9	-12	-18	-21
Expert 2	Weighted score without expert self-assessment	-14	-18	-13	-34	4	-14	-33	-4	-1	-19	-13	-9	-19	-2	-38	-34
	Weighted score with expert self-assessment	-42	-18	-39	-34	8	-42	-33	-8	-3	-19	-13	-27	-57	-6	-38	-68
	Unweighted score without expert self-assessment	-5	-5	-4	-10	-1	-5	-5	-1	0	-7	-3	-3	-7	1	-11	-10
	Unweighted score with expert self-assessment	-15	-5	-12	-10	-2	-15	-5	-2	0	-7	-3	-9	-21	3	-11	-20
Expert 3	Weighted score without expert self-assessment	-5	-1	-3	-10	-10	-13	1	-5	8	-7	0		4	-12	-16	-13
	Weighted score with expert self-assessment	-10	-1	-6	-10	-20	-26	1	-5	16	-7	0		8	-24	-16	-26
	Unweighted score without expert self-assessment	-1	0	1	-2	-3	-3	2	-1	2	-1	0		2	-3	-2	-2
	Unweighted score with expert self-assessment	-2	0	2	-2	-6	-6	2	-1	4	-1	0		4	-6	-2	-4
Expert 4	Weighted score without expert self-assessment	-23		-14	-12	-10	-25		-19	-11			-12	-17	-18	-15	-27
	Weighted score with expert self-assessment	-69		-42	-12	-20	-50		-57	-22			-36	-34	-36	-30	-54
	Unweighted score without expert self-assessment	-8		-4	-4	-6	-8		-6	-4			-5	-4	-5	-5	-9
	Unweighted score with expert self-assessment	-24		-12	-4	-12	-16		-18	-8			-15	-8	-10	-10	-18
Expert 5	Weighted score without expert self-assessment	-19	-21	-10	-20	-14	-36	-11	-15	-5	-14	-7	-14	-14	-23	-27	-26
	Weighted score with expert self-assessment	-38	-63	-30	-20	-28	-108	-22	-45	-15	-28	-14	-14	-42	-69	-54	-78
	Unweighted score without expert self-assessment	-7	-7	-1	-5	-7	-12	-5	-6	-2	-4	-4	-6	-5	-9	-10	-8
	Unweighted score with expert self-assessment	-14	-21	-3	-5	-14	-36	-10	-18	-6	-8	-8	-6	-15	-27	-20	-24
Expert 6	Weighted score without expert self-assessment	-13		5		-20	-32		-7	11			4	-16			-23
	Weighted score with expert self-assessment	-26		10		-40	-64		-14	22			8	-32			-46
	Unweighted score without expert self-assessment	-5		2		-9	-10		-2	3			1	-4			-6
	Unweighted score with expert self-assessment	-10		4		-18	-20		-4	6			2	-8			-12
Expert 7	Weighted score without expert self-assessment		0	-13		-1	-32	10	-9	9	2	1	1	-4	-7		-31
	Weighted score with expert self-assessment		0	-13		-1	-32	10	-9	9	2	1	1	-4	-7		-31
	Unweighted score without expert self-assessment		0	-2		-2	-11	5	-2	2	1	2	0	0	0		-9
	Unweighted score with expert self-assessment		0	-2		-2	-11	5	-2	2	1	2	0	0	0		-9
Expert 8	Weighted score without expert self-assessment	-37		-8		-1	-22		-7	-16			-11	-8	-31		
	Weighted score with expert self-assessment	-74		-16		-2	-44		-14	-32			-11	-16	-62		
	Unweighted score without expert self-assessment	-11		-3		-2	-8		-2	-6			-4	-2	-8		
	Unweighted score with expert self-assessment	-22		-6		-4	-16		-4	-12			-4	-4	-16		

P1= Salmonellae non-typhi, non-parathyphi, P2= Enterotoxic E. coli (STEC), P3=Campylobacter jejuni/coli, P4= Listeria monocytogenes, P5= Streptococcus pyogenes, P6= Staphylococcus aureus , P7=Coxiella burnetii, P8= Brucella abortus, P9= Mycobacterium avium subsp paratuberculosis, P10= Mycobacterium tuberculosis/bovis, P11= Clostridium perfingens, P12= Clostridium botulinum, P13= Yersinia enterolitica, P14= Bacillus cereus, P15= Mycotoxins and P16= Central European tick-borne virus

Table 4: Prioritisation by weighted score for selected pathogens with expert self-assessment, i.e. criteria with a greater relevance received more weight towards the final score

Pathogens	**Median**
Listeria monocytogenes	2.5
Brucella abortus	-1
Mycobacterium tuberculosis/bovis	-9
Mycotoxins	-12.5
Mycobacterium avium subsp *paratuberculosis*	-13
Enterotoxische *E. coli (STEC)*	-14
Clostridium botulinum	-15
Central European tick-borne virus	-16
Coxiella burnetii	-22
Campylobacter jejuni/coli	-23
Staphylococcus aureus	-24
Salmonellae non-typhi, non-parathyphi	-32.5
Streptococcus pyogenes	-38
Bacillus cereus	-40
Clostridium perfingens	-43
Yersinia enterolitica	-54

Table 5: Prioritisation by unweighted score for selected pathogens, i.e. all criteria evaluated by the experts were scored equally

Pathogens	**Median**
Mycobacterium tuberculosis/bovis	0
Listeria monocytogenes	-1
Brucella abortus	-3
Enterotoxische *E. coli (STEC)*	-4
Mycobacterium avium subsp *paratuberculosis*	-4
Campylobacter jejuni/coli	-4.5
Central European tick-borne virus	-4.5
Coxiella burnetii	-5
Mycotoxins	-5
Clostridium botulinum	-5
Salmonellae non-typhi, non-parathyphi	-8
Staphylococcus aureus	-10
Streptococcus pyogenes	-11
Bacillus cereus	-14.5
Clostridium perfingens	-15.5
Yersinia enterolitica	-18

Chapter 5

Calculation of costs and benefits of surveillance systems for listeriosis and salmonellosis utilizing bulk-tank milk samples

Introduction

Salmonella spp. and *Listeria monocytogenes* are two pathogens that can cause diseases – salmonellosis and listeriosis - in many species including humans. Foodstuffs are recognized as the major route of human infection for these two zoonoses. Therefore, livestock represents an important reservoir for these pathogens.

Salmonella (S.) are rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria. They have a wide range of hosts, including humans. Three species have been described in the genus *Salmonella*: *S. enterica*, *S.bongori* and *S. subterranea*. In each species, several subspecies are recognized, that are further subdivided into several serovars. One of the most common subspecies is *Salmonella enterica subsp. enterica*. The serovars Dublin and Typhimurium appear to be the most common that have been isolated from cattle.

In the genus Listeria (L.), seven species have been described; namely *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. grayii*, and *L. murrayi*. Only two, *L. monocytogenes* and *L. ivanoii* are pathogenic. The first causes disease in both humans and animals, whilst the second causes disease predominantly in sheep. *Listeria* species are Gram-positive, non-spore-forming rods, are ubiquitous in the environment and can grow in a wide range of pH-values, temperatures and salt concentrations. *Listeria monocytogenes* represents a problematic pathogen for the food industry, because of its ability to survive and multiply under extreme conditions.

Salmonellosis is the second most reported zoonosis in the European Union (EU) - and also in Switzerland - with a total of 109,844 and 1,325 confirmed cases in humans in 2009[2] [3]. Many

[2] European Food Safety Agency (EFSA) Reports, 2009

foodstuffs from different livestock species have been identified as sources of human salmonellosis; most important are eggs, pork and poultry. In comparison to these foodstuffs, beef represents a rare source of human disease with a low proportion (0.1-0.3%) of human disease cases attributed to it. In 2009, Denmark reported in its Annual Report on Zoonoses that 0.1-0.3% Salmonella cases were attributed to beef. In their reports for the same year, Sweden reported a proportion of 0.1% and the EU (EFSA) of 0.2%. In addition, a low (<1.8%) proportion of Salmonella positive beef carcass samples have been reported in several European countries (Wahlstrom et al., 2010). Higher prevalence has been reported from the testing of faecal samples from dairy herds from some EU countries, the United States and Canada (Huston et al., 2002; Blau et al., 2005; Callaway et al., 2005; Lailler et al., 2005). A large proportion of human cases of salmonellosis have an unknown source and obviously some of these cases could originate from cattle. In two Swiss studies involving bulk-tank milk (BTM) samples (n=310) in the Northwest region and faecal samples (n= 1,000) from slaughtered healthy cattle, the proportion of positive samples was zero (Al-Saigh et al., 2004, Stephan and Buelher, 2002.). The transmission of Salmonella from cattle to humans can occur through several routes, including consumption of milk, beef or transmission by direct contact with faeces. In 2009, 22 cases of salmonellosis in cattle[4] and 1,325 cases in humans[5] were reported in Switzerland.

Listeria monocytogenes is another major foodborne pathogen for humans. This pathogen is widespread in the environment, in processing plants and is known to affect several animal species, especially cattle and other ruminants, which can excrete the pathogen. The excretion can be continuous through faeces from asymptomatic, healthy cattle carriers (Roberts and Wiedmann, 2003; Nightingale et al., 2004). *Listeria* is rarely reported as a cause of mastitis,

[3] Zoonosis Report, Federal Veterinary Office (FVO), 2009

[4] Zoonosis report 2009, FVO

[5] Federal Office of Public Health (FOPH) report, 2009

but the importance of this pathogen for this pathology is likely to be underestimated. In the case of mastitis, direct contamination of the milk occurs (Fedio et al., 1990; Bourry et al., 1995; Jensen et al., 1996). Raw milk, and in few cases pasteurised milk, are a known source of infection for humans (Doyle et al., 1987). In humans, there is great public health concern towards listeriosis, because it can cause severe meningitis, encephalitis, septicaemia or abortion. In those persons infected, it has a high lethality rate of 20 to 30 % and a high hospitalisation rate. Nevertheless, the number of human cases is low in comparison with other foodborne pathogens, such as *Salmonella spp.* or *Campylobacter*. During the last decade, the trend in several European countries showed an increasing number of human cases and an increase in the incidence rate (Denny and McLauchlin, 2008; Goulet et al., 2008). In 2009, the number of reported human cases in the Swiss population was 43 and the number of cases in cattle was 2. The later number is likely a massive underestimation, as several studies in the EU and in the United States showed prevalences from 11.7 to 16.1% of *Listeria monocytogenes* on dairy farms (Husu, 1990; Hassan et al., 2000; Erdogan et al., 2001). Studies carried out on BTM samples in several countries also showed prevalence between 1 to 12.6% (Rohrbach et al., 1992; Steele et al., 1997; Jayarao and Henning, 2001; Waak et al., 2002; Muraoka et al., 2003; Van Kessel et al., 2004; Jayarao et al., 2006). Farmers are known to consume raw milk and raw milk seems to be seen by an increasing part of the population as a healthy foodstuff (Headrick et al., 1997; Hegarty et al., 2002; Jayarao et al., 2006; Oliver et al., 2009). Its consumption could increase considerably in the future. Changes in food consumption mentalities could bring new challenges for the surveillance of zoonotic pathogens along the food chain. This will be the case for serious zoonotic pathogens such as *Listeria monocytogenes*.

To distinguish between infected BTM and uninfected BTM, Enzyme like immunosorbent assays (ELISA) for indirect detection of *Salmonella* and Polymerase chain reaction (PCR) for the direct detection of Listeria monocytogenes have already been adapted and developed for

BTM or milk samples (Thomas et al., 1991; Hoorfar et al., 1995; Hoorfar and Wedderkopp, 1995; Nogva et al., 2000; Veling et al., 2001; Van Kessel et al., 2004; Omiccioli et al., 2009). The aim of this study was to evaluate two approaches for the surveillance of zoonotic pathogens using BTM. The candidate pathogens were selected after a prioritisation process with a panel of Swiss experts and for their major importance as foodborne pathogens and zoonotic significance. The first approach was to monitor the pathogen in BTM samples to avoid that contaminated milk enters the food chain and consequently infects humans. The aim of this surveillance programme will be to decrease the number of human cases. This approach is appropriate for *Listeria monocytogenes* that may be present in the BTM and pose a threat by contaminating foodstuffs. The second approach was the screening of antibodies in BTM samples to identify infected herds, in order to find shedding animals that would then be treated or culled before they could reach the food chain. The aim of this surveillance programme was the sanitation of dairy cattle herds. The chosen pathogen for this approach was *Salmonella spp*.. In Sweden, such an approach was performed with a BTM screening for *Salmonella Dublin* antibodies in 2009[6].

To see if these zoonosis surveillance programmes would be economically feasible, a cost-effectiveness analysis was carried out. Two scenario tree models and economic models were implemented. The costs of the surveillance programmes and direct medical costs associated with human disease cases were estimated. A surveillance programme was deemed to be feasible, if the benefits, e.g. saved costs due to the prevention of human disease cases, exceeded the costs of surveillance and control.

Materials and methods

Bulk-tank milk samples in Switzerland

[6] Surveillance of zoonotic and other animal agents in Sweden, The national veterinary Institute (SVA), Uppsala, Sweden, 2009

BTM represents the entire milk production delivered by a dairy farm. The BTM samples are either collected automatically during milk collection from the farm's bulk tank into the transporter, or farms are sampled manually from milk-cans at collecting location, such as dairies, milk collecting or centrifugation plants. Official professionals with special training take the samples as laid down by the Swiss milk quality ordinance (MQV) and the technical directive for the execution of milk quality control of the Federal Veterinary Office (FVO). BTM samples are refrigerated at 1-5ºC and sent to the laboratory for the milk inspection analyses. In 2009, the number of dairy farms participating in the milk inspection scheme was 27,131 (61% of all cattle farms) with a total of 578,689 dairy cows. This represents the reference population of the surveillance system.

Adaptation and development of the scenario tree models

The methodology based on scenarios trees to demonstrate freedom from disease according to (Martin et al., 2007) was adapted to develop a scenario tree model for a zoonoses surveillances programme with BTM testing. The two candidate zoonoses were *Listeria monocytogenes* and *Salmonellae spp.*. The two pathogens were chosen as examples for zoonoses that have either a low morbidity but a high fatality as it is the case for listeriosis, or a high morbidity but a low case fatality as for salmonellosis.

Two stochastic scenario tree models were developed in a spreadsheet using Excel 2007 (Microsoft, Seattle, WA) and @ Risk 5.0 (Palisade, Corporation) to calculate the probability that the surveillance process will detect a (antigen or antibody) positive BTM sample in the population tested, given the predetermined level of infection in the tested population, called the design prevalence.

We developed two scenario tree models for *Listeria monocytogenes* and *Salmonella* with identical structure, but with a different diagnostic test used as the node for detection of positive herd. For *Listeria,* the detection node was an antigen diagnostic test and for *Salmonellae* spp. an antibody diagnostic test.

Input parameters for the surveillance model

In the scenario tree models, the design prevalence for *Listeria monocytogenes* and *Salmonella* was modelled as pert distributions in @Risk 5.0 (Palisade Corporation) and run with 1000 iterations.

For Listeria, the test sensitivity of the PCR based on the fluorogenic 5'nuclease assay was used. The sensitivity of this test has been described to be 95.2% (Cox et al., 1998).

For Salmonella, the test sensitivity of the ELISA diagnostic test was modelled as pert distributions in @Risk 5.0 (Palisade Corporation) and run with 1000 iterations. In the literature, the sensitivity of lipopolysacharide ELISA for detecting *Salmonella enterica* subsp.*enterica serovar Dublin* were found to be 54 %, 88% and 100% respectively (Hoorfar et al., 1995; Veling et al., 2001; Wedderkopp et al., 2001).

Framework of the financial model and input data

The surveillance scenario was to test once a year all Swiss dairy farms participating in the milk testing scheme. For *Listeria monocytogenes*, the presence of the pathogen will be screened in the BTM and for *Salmonella* the presence of antibodies.

The model framework was created in an Excel spreadsheet (Excel 2007, Microsoft, Seattle, WA). In the model the different steps of the surveillance programmes were detailed. These steps included: planning, implementation, sampling, laboratory testing, data management, data analysis and communication. For each step, the labour time or the operations and expenses of each procedure were identified and their costs were calculated. All data for the costs estimation were collected from the Federal Veterinary Office (FVO) and the Centrum for Zoonoses, Bacterial animal diseases and Antibiotic resistance (ZOBA). The only identified cost payer in this surveillance programme was the FVO. The labour costs for the different collaborators at the FVO had to be quantified in wage rate. The time each collaborator spent on the surveillance programme was estimated on the basis of the data of the organisation of the national testing programme on milk products. The labour costs were

estimated at an hourly rate in Swiss Francs. The one step with different costs between the models was laboratory testing. For *Listeria monocytogenes*, a PCR test was conducted and for Salmonella an ELISA. The costs for a BTM PCR for *Listeria monocytogenes* were estimated for individual sample at 75 CHF and for ≥10 samples at 24.50 CHF. The costs for a BTM ELISA for Salmonella were estimated for an individual sample at 17 CHF and for ≥ 10 samples at 7 CHF per sample. A contribution of the Federal Public Health Office (BAG), of the Agroscope Liebefeld-Posieux (ALP), of the Cantonal Veterinary Offices or of the cantonal laboratories in the planning of the surveillance programme was not included in this calculation.

Benefits: costs of illness estimates of listeriosis

For the costs of illness estimates, the total direct medical expenses of the disease were calculated. If these losses could be avoided the society would benefit. The productivity losses from illness or premature death and the indirect non-medical expenses (e.g. lifelong chronic disability of new born) were not included in the calculation. The incidence rate of listeriosis cases per year from 2005 to 2010 was collected at the BAG. The median of the number of cases for these 5 years was calculated and used as model input.

Hospitalisation rate for listeriosis is almost 92% (Mead et al., 1999). Because the symptoms of listeriosis for healthy people are usually mild - such as gastroenteritis or influenza type symptoms - an underreporting of cases is realistically probable. Therefore, it was assumed that all the cases reported to the BAG had visited a physician for severe symptoms and were hospitalised cases. Hospitalisation costs were collected and published by the Federal Office of Statistics in the statistics on diagnosed cases and costs by AP-DRG Code in 2008. Several codes describing severe pathologies for listeriosis were identified. These were meningitis or encephalomeningitis, septicaemia, pneumonia, endocarditis and peritonitis by adults and meningitis, septicaemia, granulomatosis infantiseptica, pneumonia, abortion, amniotic infection and endometritis by newborns and mothers. An AP-DRG code represents a specific

pathology and the related costs can be identified. However, it was not possible to relate pathologies to all human cases. Thus, the costs range of the direct medical costs was estimated by multiplying all the cases by the lowest and the highest total costs per case.

99% of human listeriosis cases have a foodborne source (Mead et al., 1999). In a 10-year study from the Swiss National Reference Centrum for listeriosis in Lausanne, 41% of cases were attributed to milk products (Bille, 2004). It was assumed that a maximum of 41% of direct medical costs could be attributed to infections acquired through contaminated milk.

Costs of illness estimates of salmonellosis

For salmonellosis, the direct medical costs were also estimated. The productivity losses from illness or premature death were not included in the calculation. The incidence rates of human salmonellosis cases per year in Switzerland were collected from the BAG. As for listeriosis, the incidence rates for the years between 2005 and 2010 were known. The median of the number of cases for these 5 years was calculated and used in the model.

Hospitalisation rates for salmonellosis were estimated to be 2% (Mead et al., 1999). 5% visit a physician and recover fully. 93 % do not visit a physician and recover fully (Buzby et al., 1996). Among hospitalisation for salmonellosis, gastroenteritis (61%) and septicaemia (23%) were the most common diagnoses (Trevejo et al., 2003). As for listeriosis, all gastroenteritis and septicaemia hospitalised cases were multiplied by the lower and the higher pathologies and thus costs. The proportion of human cases attributed to beef (assuming that cases from milk products were included in this estimation) was estimated to be between 0.1 and 0.3% of all cases.

Results of the financial model

BTM surveillance costs were estimated for *Listeria monocytogenes* screening to range between 910,000 and 2,280,000 CHF annually. For *Salmonella* antibodies screening the costs ranged between 435,000 and 706,000 CHF.

The total direct medical costs for human listeriosis per year in Switzerland were estimated to be between 274,000 and 1,439,000 CHF. The milk products are responsible for only 41 % of the cases. Therefore, the real direct costs for listeriosis induced by milk products are estimated to be between 113,000 and 590,000 CHF at maximum.

The total direct medical costs for human salmonellosis per year in Switzerland were estimated to be between 12,477,000 and 40,000,000 CHF. Beef is responsible for 0.3%, at most, of the cases. Therefore, the real direct costs for salmonellosis induced by beef are estimated between 37,500 and 120,000 CHF.

Discussion

In both diseases, the costs exceed the benefits. We tried to include conservative estimates for the benefits, as we did not want to overestimate it. However, even with the highest values in our estimation the costs are always lower than the benefits. For listeriosis, one might question the approach to quantify the live of humans monetarily. But this is usual in any estimation of costs (Buzby et al., 1996). If a surveillance programme aims on the prevention of fatalities in humans, listeriosis can still be a candidate disease for a systematic surveillance in BTM. Interestingly, while salmonellosis accounts for massive losses, the contribution of beef-born infections is very low. As salmonellosis accounts also for losses in the agricultural sector, the benefits would increase if these losses would be included in the calculation. With the results of this study, surveillance for zoonotic pathogens in BTM seems to be not an economical valuable option.

The economical estimation of the costs and benefits of surveillance and control programmes is often very complicated and provides estimates with wide margins as often a lot of the costs are only roughly estimated. Additionally, such work is often aiming to evaluate one special programme and not a general approach. Indeed, the benefits of surveillance only are often hard to find, therefore, in general, surveillance costs are often only a (small) proportion of the costs of a control or intervention programme.

The aim of this study was to investigate the surveillance of two zoonoses in BTM for the economical benefits that might be achieved. For this purpose we conducted a "light" calculation of the costs and benefits, with only the surveillance costs weighted against the benefits. With this approach we wanted to simplify the calculation of costs compared to a full model. Additionally, we assumed to get narrower limits of the costs, if we only include costs we have good estimates of.

The presented methodology was exact enough to be used as a screening tool to test surveillance of zoonoses that might be economical. Indeed, we did no even include the intervention costs and assumed 100% sensitivity and we neglected the costs of false positive results. As the surveillance was not economical without these additional costs, further refinement of the calculation is not necessary. On the other hand, the existing models could be extended to full models without much additional work.

References

Al-Saigh, H., Zweifel, C., Blanco, J., Blanco, J.E., Blanco, M., Usera, M.A., Stephan, R.,: Fecal shedding of Escherichia coli O157, Salmonella, and Campylobacter in Swiss cattle at slaughter. Journal of Food Protection 2004, 67 : 679-684.

Bille, J.: Listeria et denrées alimentaires. Federal Public Health Office Bulletin 2004, 5: 60-63

Blau, D.M., McCluskey, B.J., Ladely, S.R., Dargatz, D.A., Fedorka-Cray, P.J., Ferris, K.E., Headrick, M.L.: Salmonella in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. J Food Prot 2005, 68: 696-702.

Bourry, A., Poutrel, B., Rocourt, J.: Bovine Mastitis Caused by Listeria-Monocytogenes - Characteristics of Natural and Experimental Infections. Journal of Medical Microbiology 1995, 43: 125-132.

Buzby, J.C., Roberts, T., Lin, C.-T.J., MacDonald, J.M.: Bacterial Foodborne Disease: Medical Costs and Productivity Losses 1996.

Callaway, T.R., Keen, J.E., Edrington, T.S., Baumgard, L.H., Spicer, L., Fonda, E.S., Griswold, K.E., Overton, T.R., VanAmburgh, M.E., Anderson, R.C., Genovese, K.J., Poole, T.L., Harvey, R.B., Nisbet, D.J.: Fecal prevalence and diversity of Salmonella species in lactating dairy cattle in four states. J Dairy Sci 2005, 88: 3603-3608.

Cox, T., Frazier, C., Tuttle, J., Flood, S., Yagi, L., Yamashiro, C.T., Behari, R., Paszko, C., Cano, R.J.: Rapid detection of Listeria monocytogenes in dairy samples utilizing a PCR-based fluorogenic 5 ' nuclease assay. Journal of Industrial Microbiology & Biotechnology 1998, 21: 167-174.

Denny, J., McLauchlin, J.: Human Listeria monocytogenes infections in Europe--an opportunity for improved European surveillance. Euro Surveill 2008, 13.

Doyle, M. P.,Glass, K.A., Beery, J. T., Garcia, G. A., Pollard, D. J., Schultz, R.D.: Survival of Listeria monocytogenes in milk during high-temperature, short-time pasteurization. Applied and Environnemental Microbiology 1987, Vol. 53 No. 7: 1433-1438.

Erdogan, H.M., Cetinkaya, B., Green, L.E., Cripps, P.J., Morgan, K.L.: Prevalence, incidence, signs and treatment of clinical listeriosis in dairy cattle in England. Vet Rec 2001, 149: 289-293.

Fedio, W.M., Schoonderwoerd, M., Shute, R.H., Jackson, H.: A Case of Bovine Mastitis Caused by Listeria-Monocytogenes. Canadian Veterinary Journal-Revue Veterinaire Canadienne 1990, 31: 773-775.

Goulet, V., Hedberg, C., Le Monnier, A., de Valk, H.: Increasing incidence of listeriosis in France and other European countries. Emerging Infectious Diseases 2008, 14: 734-740.

Hassan, L., Mohammad, H.O., McDonough, P.L., Gonzalez, R.N.: A cross-sectional study on the prevalence of Listeria monocytogenes and Salmonella in New York dairy herds. Journal of Dairy Science 2000, 83: 2441-2447.

Hoorfar, J., Lind, P., Bitsch, V.: Evaluation of an O-Antigen Enzyme-Linked-Immunosorbent-Assay for Screening of Milk Samples for Salmonella-Dublin Infection in Dairy Herds. Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire 1995, 59: 142-148.

Huston, C.L., Wittum, T.E., Love, B.C., Keen, J.E.: Prevalence of fecal shedding of Salmonella spp in dairy herds. Journal of the American Veterinary Medical Association 2002, 220: 645-649.

Husu, J.R.: Epidemiological studies on the occurrence of Listeria monocytogenes in the feces of dairy cattle. Zentralbl Veterinarmed 1990,B 37: 276-282.

Headrick, M.L., Timbo, B., Klontz, K.C., Werner, S.B.: Profile of raw milk consumers in California. Public Health Rep 1997, 112: 418-422.

Hegarty, H., O'Sullivan, M.B., Buckley, J., Foley-Nolan, C.: Continued raw milk consumption on farms: why? Commun Dis Public Health 2002, 5: 151-156.

Hoorfar, J., Lind, P., Bitsch, V.: Evaluation of an O-Antigen Enzyme-Linked-Immunosorbent-Assay for Screening of Milk Samples for Salmonella-Dublin Infection in Dairy Herds. Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire 1995, 59: 142-148.

Hoorfar, J., Wedderkopp, A.: Enzyme-Linked-Immunosorbent-Assay for Screening of Milk Samples for Salmonella-Typhimurium in Dairy Herds. American Journal of Veterinary Research 1995, 56: 1549-1554.

Jayarao, B.M., Donaldson, S.C., Straley, B.A., Sawant, A.A., Hegde, N.V., Brown, J.L.: A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. Journal of Dairy Science 2006, 89: 2451-2458.

Jayarao, B.M., Henning, D.R.: Prevalence of foodborne pathogens in bulk tank milk. Journal of Dairy Science 2001, 84: 2157-2162.

Jensen, N.E., Aarestrup, F.M., Jensen, J., Wegener, H.C.: Listeria monocytogenes in bovine mastitis. Possible implication for human health. International Journal of Food Microbiology 1996, 32: 209-216.

Lailler, R., Sanaa, M., Chadoeuf, J., Fontez, B., Brisabois, A., Colmin, C., Millemann, Y.: Prevalence of multidrug resistant (MDR) Salmonella in bovine dairy herds in western France. Preventive Veterinary Medicine 2005, 70: 177-189.

Martin, P.A.J., Cameron, A.R., Greiner, M.: Demonstrating freedom from disease using multiple complex data sources: 1: A new methodology based on scenario trees. Preventive Veterinary Medicine 2007, 79: 71-97.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V.: Food-related illness and death in the United States. Emerging Infectious Diseases 1999, 5: 607-625.

Muraoka, W., Gay, C., Knowles, D., Borucki, M.: Prevalence of Listeria monocytogenes subtypes in bulk milk of the Pacific Northwest. J Food Prot 2003, 66: 1413-1419.

Nightingale, K.K., Schukken, Y.H., Nightingale, C.R., Fortes, E.D., Ho, A.J., Her, Z., Grohn, Y.T., McDonough, P.L., Wiedmann, M.: Ecology and transmission of Listeria monocytogenes infecting ruminants and in the farm environment. Applied and Environmental Microbiology 2004, 70: 4458-4467.

Nogva, H.K., Rudi, K., Naterstad, K., Holck, A., Lillehaug, D.: Application of 5 '-nuclease PCR for quantitative detection of Listeria monocytogenes in pure cultures, water, skim

milk, and unpasteurized whole milk. Applied and Environmental Microbiology 2000, 66: 4266-4271.

Oliver, S.P., Boor, K.J., Murphy, S.C., Murinda, S.E.: Food safety hazards associated with consumption of raw milk. Foodborne Pathog Dis 2009, 6: 793-806.

Omiccioli, E., Amagliani, G., Brandi, G., Magnani, M.: A new platform for Real-Time PCR detection of Salmonella spp., Listeria monocytogenes and Escherichia coli O157 in milk. Food Microbiology 2009, 26: 615-622.

Roberts, A.J., Wiedmann, M.: Pathogen, host and environmental factors contributing to the pathogenesis of listeriosis. Cellular and Molecular Life Sciences 2003, 60: 904-918.

Rohrbach, B.W., Draughon, F.A., Davidson, P.M., Oliver, S.P.: Prevalence of Listeria-Monocytogenes, Campylobacter-Jejuni, Yersinia-Enterocolitica, and Salmonella in Bulk Tank Milk - Risk-Factors and Risk of Human Exposure. Journal of Food Protection 1992, 55: 93-97.

Stephan, R., Buehler, K.,. Prävalenz von Campylobacter spp., Salmonella spp. und Listeria monocytogenes in Bestandesmilchproben aus der Nordostschweiz. Archiv Fur Lebensmittelhygiene 2002, vol.53,no3: pp.62-65

Steele, M.L., McNab, W.B., Poppe, C., Griffiths, M.W., Chen, S., Degrandis, S.A., Fruhner, L.C., Larkin, C.A., Lynch, J.A., Odumeru, J.A.: Survey of Ontario bulk tank raw milk for food-borne pathogens. Journal of Food Protection 1997, 60: 1341-1346.

Thomas, E.J.G., King, R.K., Burchak, J., Gannon, V.P.J.: Sensitive and Specific Detection of Listeria-Monocytogenes in Milk and Ground-Beef with the Polymerase Chain-Reaction. Applied and Environmental Microbiology 1991, 57: 2576-2580.

Waak, E., Tham, W., Danielsson-Tham, M.L.: Prevalence and fingerprinting of Listeria monocytogenes strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks. Applied and Environmental Microbiology 2002, 68: 3366-3370.

Wahlstrom, H., Andersson, Y., Plym-Forshell, L., Pires, S.M.: Source attribution of human Salmonella cases in Sweden. Epidemiol Infect 2010, 1-8.

Van Kessel, J.S., Karns, J.S., Gorski, L., McCluskey, B.J., Perdue, M.L.: Prevalence of Salmonellae, Listeria monocytogenes, and Fecal Coliforms in Bulk Tank Milk on US Dairies. Journal of Dairy Science 2004, 87: 2822-2830.

Veling, J., van Zijderveld, F.G., Bemmel, A.M.V.Z.V., Schukken, Y.H., Barkema, H.W.: Evaluation of two enzyme-linked immunosorbent assays for detecting Salmonella enterica subsp enterica serovar Dublin antibodies in bulk milk. Clinical and Diagnostic Laboratory Immunology 2001, 8: 1049-1055.

Wedderkopp, A., Stroger, U., Bitsch, V., Lind, P.: Testing of bulk tank milk for Salmonella Dublin infection in Danish dairy herds. Can J Vet Res 2001, 65: 15-21.

Chapter 6

Potential of bulk-tank milk samples for antibiotic resistance monitoring

Introduction

Practitioners, public health and veterinary public health officials and researchers are concerned about transferable drug resistance in microorganisms as it has potential public health consequences and it complicates the treatment of infectious diseases in both humans and animals. The transmission of resistant bacteria from animals to humans can occur via food consumption or direct contact. Dairy cows are a potential source for both transmission pathways. By increasing the selection pressure, the use of antibiotics for the prophylaxis and treatment of udder infections in dairy cattle can reasonably result in a selection of resistant strains of bacteria and the potential transmission of its resistance genes to humans.

Since 2006, Switzerland is carrying out a continuous monitoring of antibiotic resistance in several livestock species, meat and milk products [7]. In 2008, cattle faeces samples taken at slaughterhouses were analysed for *Campylobacter* spp. and *Escherichia coli* [8]. In 2006, the indicator bacteria *Enterococcus* spp was also analysed in milk products [9].

In addition to the current monitoring programme based on sampling at slaughter and at retail, approaches for sampling at farm-level could provide valuable additional information. Through a literature review, this paper evaluates the potential and the value of bulk-tank milk (BTM) samples as a tool for antibiotic resistance monitoring.

Bulk-tank milk samples to monitor antibiotic resistance

BTM can be a medium to transfer antibiotic resistant bacteria to humans. The consumption of raw milk containing antibiotic resistant bacteria can result in a bacterial colonization of the

[7] in accordance with Article 291d from the Ordinance on epizootic disease from the 27th of June 1995

[8] Annual report 2008, Monitoring of antibiotic resistance, Federal veterinary office FVO

[9] Annual report 2006, Monitoring of antibiotic resistance, FVO

gut and can pose a potential public health risk (van den Bogaard and Stobberingh, 1999). Consumption of raw milk is very common on dairy farms as it is less expensive than pasteurised retail milk. Additionally, consumption of raw milk is appreciated for its numerous health qualities (Jayarao et al., 2006a). An increased interest from the public was also observed for raw milk consumption in the latest years. This consumption poses a risk for consumers not only to get bacterial diseases (Oliver et al., 2009), but also for the transmission of antibiotic resistance to humans. Several studies have shown the presence of pathogenic bacteria in BTM and these microorganisms can obviously carry resistance genes (Jayarao and Wang, 1999; Jayarao and Henning, 2001; Stephan et al., 2002; Holm et al., 2004; Karns et al., 2005; Oliver et al., 2005; Karns et al., 2007). The risk of milk-borne transmission of antibiotic resistant bacteria to humans by consuming milk and milk products is noticeably minimized by pasteurisation. However the presence of bacteria in raw milk, which can survive pasteurisation and can carry antibiotic resistance such as *Listeria monocytogenes*, is also of great concern. Several studies have shown the presence of *Listeria* in BTM (Rohrbach et al., 1992; Steele et al., 1997; Yoshida et al., 1998; Jayarao and Henning, 2001; Waak et al., 2002; Van Kessel et al., 2004; Oliver et al., 2005). In addition, through feeding calves with wasted raw milk, antibiotic resistant pathogens can also be transmitted to young stock, spread to the adult stage, persist and disseminate in the dairy environment (Selim and Cullor, 1997).

In the Czech Republic, a BTM study on the prevalence and the resistance of antimicrobial drugs from selected zoonotic and indicator pathogens such as *Staphylococcus aureus, coagulase-negative Staphylococci, Listeria monocytogenes, Escherichia coli, Enteroccoccus faecalis, Enterococcus faecium and Bacillus cereus* has already been carried out (Schlegelova et al., 2002). Hundred and eleven BTM samples were investigated. Resistance against one or more antimicrobial drugs was found in 93% of the S. aureus, 40% of the *CN Staphylococci*, 73% of the *E. coli*, 88% of the *E.faecalis* and 55% of the *E.faecium. Listeria monocytogenes* was isolated in 1.8% of the BTM and one strain showed multidrug resistance. In another

study, indicator bacteria (*Enterococcus and E. coli*) isolated from 799 BTM samples in Austria were analysed against 30 different antibiotics for resistance. Fifteen percent of the isolates were resistant against one or more than one antibiotic (Fuchs et al., 2004). Another study from six farms in central Pennsylvania was performed on gram-negative bacteria (GNB) in BTM. GNB were isolated from 46 of 54 (85%) BTM samples. Eleven different species of antimicrobial resistant GNB were found. The study demonstrates that BTM can be a major source of antimicrobial-resistant GNB (Jayarao et al., 2006b).

From another approach, BTM can also be an important and valuable tool to get the farm-level trend in antibiotic resistance and help to a better management in the use of antibiotics on dairy farms. The choice of the target pathogens and the interpretation of the results have to be done carefully. In a study on *Salmonella enterica subsp enterica and E.coli,* antimicrobial susceptibility was described and compared (Berge et al., 2007). Twenty-three of all isolates were multidrug resistant (MDR). The MDR strains of *Salmonella and E.coli* had a different antimicrobial pattern and the antimicrobial pattern of Salmonella tended to be serovar dependent. Screening results in BTM samples can also be correlated to a spatial pattern analysis of specific antibiotic resistance to detect spatial clustering of resistant samples (Fuchs et al., 2004). Critical regions where farms have more antibiotic resistance can be identified and treated in an accurate manner e.g. through improved management measures the number of mastitis cases can be decreased and the use of antibiotics can be reduced. Decreasing the use of antibiotics in dairy cattle can obviously decrease the selection pressure of several bacteria and decrease the potential of selecting antibiotic resistant strains of bacteria.

Diagnostic tests for antibiotic resistance in bulk-tank milk

Most studies used a two-step approach consisting of isolation of relevant bacteria followed by phenotypic antimicrobial resistance testing. A micro-dilution technique for the determination of the minimum inhibitory concentration was the test performed in several BTM studies (Schlegelova et al., 2002; Fuchs et al., 2004; Jayarao et al., 2006b). Another test used on

bacteria from BTM was the antimicrobial disk susceptibility test (Jayarao et al., 2006b; Berge et al., 2007). Other methods such as PCR, hybridization or hybridization using microarray technology can be used for the direct detection of specific resistant genes without prior isolation of bacteria (Perreten et al., 2005; Virgin et al., 2009).

Usefulness of BTM for different purposes of antibiotic resistance monitoring programmes

BTM could potentially be used for antibiotic resistance monitoring programmes with different objectives:

1. Obtain an overview on the prevalence of resistant bacteria or resistance genes in the cattle population, and detect trends for changes in resistance over time

   Advantages of using BTM for this surveillance objective include the low cost of sampling and that obtaining an equal distribution of samples across the year for continuous monitoring is easily achieved. Indicator bacteria with a relatively high prevalence in BTM could be used for monitoring. In the current surveillance, *E. coli* and *Enterococci* are used as indicator bacteria. For BTM, indicator bacteria with a higher prevalence in milk would need to be used in order to make the surveillance efficient. The main disadvantage of BTM for this surveillance objective is that it only includes cows delivering milk to BTM. It would thus be difficult to compare prevalence of resistance in the cattle population with data from other monitoring programmes using faecal samples.

2. Provide feedback to farmers regarding the effectiveness of different antibiotics for the treatment of cows with mastitis.

   The advantages of BTM for this surveillance objective are the low cost compared to milk samples from individual cows with mastitis, and that continuous monitoring of the resistance situation within the herd is possible.

   Disadvantages are that in BTM, there is a very low prevalence of mastitis pathogens.

Resistance testing in BTM would therefore not represent the resistance situation in mastitis pathogens, which might differ substantially from the resistance situation in other bacteria found more frequently in BTM.

3. Monitor resistant bacteria with potential public health relevance.

   The advantage of using BTM for this surveillance objective is that detection of resistant bacteria has direct relevance for public health for milk consumed as raw milk. Also, there is a good potential for early detection of emerging resistant pathogens through continuous monitoring of BTM.

   However, only a relatively small percentage of milk is consumed as raw milk. For direct transmission of resistant bacteria from cows to humans, faecal contamination is likely to be more relevant than milk. In BTM there is also only a small prevalence of zoonotic bacteria, in which antimicrobial resistance has the highest public health relevance.

Compared to other sampling approaches, BTM is likely to be very well suited for many surveillance objectives. The current paper is based on published literature. Up to date, no study has been performed on comparing the efficiency of an antimicrobial resistance monitoring programme in BTM to the monitoring programmes which are currently used. Thus, it was not possible to provide a direct comparison between different sample types for monitoring antibiotic resistance. Also, data available in the scientific literature were not sufficient for an economic evaluation of different sampling methodologies.

Conclusion

The literature on BTM monitoring for antibiotic resistance is limited. The routine monitoring of BTM represents a possible method to measure the trend of antibiotic resistance in cattle and could be used in future monitoring programmes.

References

Annual report 2008, Monitoring of antibiotic resistance, Federal veterinary office (FVO)

Annual report 2006, Monitoring of antibiotic resistance, Federal veterinary office (FVO)

Berge, A.C.B., Champagne, S.C., Finger, R.M., Sischo, W.M.: The use of bulk tank milk samples to monitor trends in antimicrobial resistance on dairy farms. Foodborne Pathogens and Disease 2007, 4: 397-407.

Fuchs, K., Deutz, A., Wagner, P., Kofer, J.: Spatial point pattern analysis to study the resistance behaviour of enterococcus to tetracycline in bulk milk. Proceedings of the SVEPM: 24 - 26 March 2004 2004: 134 - 142.

Holm, C., Jepsen, J., Larsen, M., Jespersen, L.: Predominant microflora of downgraded Danish bulk tank milk. Journal of Dairy Science 2004, 87: 1151-1157.

Jayarao, B.M., Donaldson, S.C., Straley, B.A., Sawant, A.A., Hegde, N.V., Brown, J.L.: A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. Journal of Dairy Science 2006a, 89: 2451-2458.

Jayarao, B.M., Henning, D.R.: Prevalence of foodborne pathogens in bulk tank milk. Journal of Dairy Science 2001, 84: 2157-2162.

Jayarao, B.M., Straley, B.A., Donaldson, S.C., Hedge, N.V., Sawant, A.A., Srinivasan, V., Oliver, S.P.: Public Health Significance of Antimicrobial-Resistant Gram-Negative Bacteria in Raw Bulk Tank Milk. Foodborne Pathogens and Disease 2006b, 3: 222-233.

Jayarao, B.M., Wang, L.: A study on the prevalence of gram-negative bacteria in bulk tank milk. Journal of Dairy Science 1999, 82: 2620-2624.

Karns, J.S., Van Kessel, J.S., McCluskey, B.J., Perdue, M.L.: Prevalence of Salmonella enterica in bulk tank milk from US dairies as determined by polymerase chain reaction. Journal of Dairy Science 2005, 88: 3475-3479.

Karns, J.S., Van Kessel, J.S., McClusky, B.J., Perdue, M.L.: Incidence of Escherichia coli O157 : H7 and E-coli virulence factors in US bulk tank milk as determined by polymerase chain reaction. Journal of Dairy Science 2007, 90: 3212-3219.

Oliver, S.P., Boor, K.J., Murphy, S.C., Murinda, S.E.: Food Safety Hazards Associated with Consumption of Raw Milk. Foodborne Pathogens and Disease 2009, 6: 793-806.

Oliver, S.P., Jayarao, B.M., Almeida, R.A.: Foodborne pathogens in milk and the dairy farm environment: Food safety and public health implications. Foodborne Pathogens and Disease 2005, 2: 115-129.

Perreten, V., Vorlet-Fawer, L., Slickers, P., Ehricht, R., Kuhnert, P., Frey, J.: Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. Journal of Clinical Microbiology 2005, 43: 2291-2302.

Rohrbach, B.W., Draughon, F.A., Davidson, P.M., Oliver, S.P.: Prevalence of Listeria-Monocytogenes, Campylobacter-Jejuni, Yersinia-Enterocolitica, and Salmonella in Bulk Tank Milk - Risk-Factors and Risk of Human Exposure. Journal of Food Protection 1992, 55: 93-97.

Schlegelova, J., Babak, V., Klimova, E., Lukasova, J., Navratilova, P., Sustackova, A., Sediva, I., Rysanek, D.: Prevalence of and resistance to anti-microbial drugs in selected microbial species isolated from bulk milk samples. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 2002, 49: 216-225.

Selim, S.A., Cullor, J.S.: Number of viable bacteria and presumptive antibiotic residues in milk fed to calves on commercial dairies. Journal of the American Veterinary Medical Association 1997, 211: 1029-&.

Steele, M.L., McNab, W.B., Poppe, C., Griffiths, M.W., Chen, S., Degrandis, S.A., Fruhner, L.C., Larkin, C.A., Lynch, J.A., Odumeru, J.A.: Survey of Ontario bulk tank raw milk for food-borne pathogens. Journal of Food Protection 1997, 60: 1341-1346.

Stephan, R., Buehler, K., Lutz, C.: Prevalence of genes encoding enterotoxins, exfoliative toxins and toxic shock syndrome toxin 1 in Staphylococcus aureus strains isolated from bulk-tank milk samples in Switzerland. Milchwissenschaft-Milk Science International 2002, 57: 502-504.

van den Bogaard, A.E., Stobberingh, E.E.: Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs 1999, 58: 589-607.

Van Kessel, J.S., Karns, J.S., Gorski, L., McCluskey, B.J., Perdue, M.L.: Prevalence of Salmonellae, Listeria monocytogenes, and Fecal Coliforms in Bulk Tank Milk on US Dairies. Journal of Dairy Science 2004, 87: 2822-2830.

Virgin, J.E., Van Slyke, T.M., Lombard, J.E., Zadoks, R.N.: Short communication: methicillin-resistant Staphylococcus aureus detection in US bulk tank milk. J Dairy Sci 2009, 92: 4988-4991.

Waak, E., Tham, W., Danielsson-Tham, M.L.: Prevalence and fingerprinting of Listeria monocytogenes strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks. Applied and Environmental Microbiology 2002, 68: 3366-3370.

Yoshida, T., Sato, M., Hirai, K.: Prevalence of Listeria species in raw milk from farm bulk tanks in Nagano Prefecture. Journal of Veterinary Medical Science 1998, 60: 311-314.

Chapter 7

Discussion and Conclusion

This doctoral thesis aimed to describe the potential of bulk-tank milk (BTM) in the monitoring of antibiotic resistance, epizootics and zoonoses.

The literature review conducted in Chapter 2 showed the wide range of microorganisms that can be surveyed or isolated in BTM. Several studies showed the palette of available or investigated diagnostic tools for identification of antigen or antibodies.

The study conducted in Chapter 3 showed the great potential of BTM for the surveillance of epizootics in a financial analysis of the surveillance programmes of infectious bovine tracheitis (IBR) and Enzotic Bovine Leucosis (EBL). This reinforced the initiative of the Federal Veterinary Office to implement an active surveillance for IBR and EBL through BTM in the future. A pilot study started in 2011 and the testing of BTM was applied in the official surveillance in 2012. As showed in the review in Chapter 2 other epizootic candidates have a great potential to be surveyed with BTM. The successful surveillance programme of Bluetongue implemented in 2007 was the first example of a successful programme in the last years and showed additionally the advantage of BTM testing in the surveillance of emerging animal diseases in Switzerland (Chaignat et al., 2010).

The prioritisation conducted in Chapter 4 helped us to identify problematic zoonotic pathogens in BTM and sorted the important ones which could be surveyed in the future. Such a prioritisation was not published in Switzerland, or, as far as we know, in other countries. The prioritisation revealed that *Listeria monocytogenes* is currently the most important zoonotic pathogen in heat-treated and raw milk in Switzerland. The next important pathogens are *Mycobacterium tuberculosis* or *bovis* and *Brucella abortus*. However, both pathogens are absent from the Swiss cattle population and thus surveillance would only be useful for the early detection of the introduction of these pathogens into Switzerland.

The surveillance of zoonotic pathogens represents another challenge. Milking hygiene is a very important component of the quality of the milk and most of the Swiss farmers are trained and very careful during this procedure and know its importance for milk quality. This careful work decreases seriously the risk of contamination of the BTM with microorganisms from the environment. Additionally, BTM is obviously raw milk, which will normally not reach the food chain without having undergone a heat-treatment (e.g. thermisation, pasteurisation or ultrapasteurisation). In Switzerland, raw milk may be sold under specific conditions, but is not legally regarded as food ready for consumption. Raw milk has to be labelled that it is not a ready to drink product and that it has to be heated to a temperature of minimum 70°C, which will kill most of the zoonotic pathogens. However, there is an increasing demand for raw milk due to perceived health benefits. No statistics are available on the quantitative development of raw milk consumption in Switzerland.

Chapter 5 showed that even though zoonotic pathogens can be successfully detected in BTM, the potential benefit of new surveillance programmes must exceed the costs associated with surveillance. A substantiatial benefit in the theoretical surveillance programmes of Listeria monocytogenes and Salmonella spp. could not be shown in the current situation in Switzerland. Although for Listeria, given a lethality of 20% to 30%, the potential of saving a human life could be considered as a sufficient benefit independent of the cost-benefit ratio. But unfortunately in such calculation a human life has to be quantified and such cost-benefit calculations have to be done with quantifiable measures.

In Chapter 6, despite the limited literature, the possibilities of antibiotic resistance surveillance through BTM in cattle could be assessed and its use in continuous future monitoring programmes in Switzerland could be presumed. A pilot study on using BTM for antimicrobial resistance monitoring was conducted in 2011.

In conclusion, BTM is a valuable matrix for the surveillance of antimicrobial resistance, epizootics and zoonoses. However, to exploit its full potential, more research is needed on the

performance of available diagnostic tests, on the coverage of BTM for the total cattle population, and on the cost-efficiency of BTM surveillance.

References

Chaignat, V., Nitzsche, S., Scharrer, S., Feyer, D., Schwermer, H., Thur, B.: Milk concentration improves Bluetongue antibody detection by use of an indirect ELISA. Veterinary Microbiology 2010, 143: 179-183.

Acknowledgement:

Je tiens à remercier chaleureusement toutes les personnes qui ont participés de près ou de loin à cette thèse. Je tiens à remercier tout particulièrement les personnes suivantes :

Le Dr. Heinzpeter Schwermer pour la bonne collaboration, son temps, les discussions enrichissantes, sa confiance et son aide durant toute la durée du projet.

Mon chef, le Dr. Martin Reist pour ces précieux conseils, pour avoir toujours été à l'écoute et pour m'avoir toujours poussé vers l'avant.

Le Dr. Marcus Doherr pour sa bonne humeur et son aide dans l'écriture du manuscrit.

La directrice de notre institut, Gertraud Schüpbach pour son aide précieuse dans l'écriture du manuscrit et sa disponibilité, malgré ces grandes responsabilités et ces nombreux travaux.

Tous mes co-auteurs, Walter Schaerren, Hans Graber et Andrea Hänni pour le partage de connaissance, leur aide et leur disponibilité durant toute la durée du projet.

Un remerciement à tous mes collègues de l'Institut, Valérie Grangier, Franziska Wohlfender, Patrick Korff, Sara Schärrer, et tout particulièrement à:

Sarah Blickenstorfer pour m'avoir toujours soutenue et supportée même dans les moments difficiles, d'avoir toujours été là pour dialoguer et refaire le monde avec moi. Il y a des amitiés qui resteront pour toujours, tu es une d'entre elles… Merci pout tout.

Maribe Lefevre pour ta gentillesse, ta joie de vivre, ton aide et ton soutien. Les personnes comme toi sont rares et quand on les rencontre dans sa vie, c'est que l'on est chanceux… Merci.

Myriam Harisberger pour ton amitié, ton aide, ton soutien, d'avoir toujours été disponible pour discuter ou me conseiller et pour la très bonne collaboration pour l'organisation du Technical Corner… Merci.

Paz Gordon, tu es la personne la plus douce et disponible que je connaisse et ton amitié, ton soutien ont été très précieux pour moi… Merci.

Norikazu Isoda pour ton amitié, ton soutien et ton humour. J'ai toujours aimé le Japon, mais depuis que je te connais encore plus… Un grand merci.

Judith Hunninghaus, la petite dernière, mais pas des moindre, tu es le positivisme incarné, ton soutien et ta bonne humeur m'ont donné du courage… Merci

Anna Fahrion, une allemande avec une touche de France, merci pour ton amitié et de m'avoir donné la possibilité de parler un peu français…Merci

Patrick Presi pour ton amitié et ton soutien, on partage le français et l'Italie, l'on était fait pour s'entendre… Merci.

Bart van den Borne, tu m'as fait découvrir les Pays-Bas dans le bureau avec les Stroopwaffeln et cela vaut déjà un remerciement… Un grand merci encore pour ton amitié.

Salomé Dürr pour ton amitié et ta gentillesse. Une touche de Bâle dans les bureaux, ça fait toujours du bien… Merci.

Natacha Wu pour ton amitié indéfectible, ton soutien, pour m'avoir toujours redonnée confiance en moi, pour m'avoir toujours supportée, malgré les hauts et les bas et pour m'avoir tant apportée… tu es mon port d'attache dans la tempête…proverbe taïwanais…Merci pour tout.

Et enfin, Naomi Goy, sans toi, je ne serais pas là où je suis et qui je suis aujourd'hui... Sincèrement et de tout mon cœur, merci.

Un remerciement particulier aussi à tous mes anciens collègues de l'Office vétérinaire fédéral pour notre bonne collaboration.

Je tiens à remercier d'une manière vraiment particulière mes parents, ma sœur et ma famille pour leur soutien, leur confiance et de m'avoir donné la possibilité de faire ce que j'aime dans la vie, sans concession. De tout mon cœur merci.