Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science

Tick-borne encephalitis

A food safety risk for humans consuming unpasteurized milk and milk products from goat, sheep and cattle in Sweden

Alexander Hanberger

Uppsala
2020

Degree Project 30 credits within the Veterinary Medicine Programme

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Alexander Hanberger

***Supervisor**: Ann Albihn, Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences*

***Assistant Supervisor**: Anna Omazic & Elina Lahti, National Veterinary Institute, Sweden*

***Examiner**: Sofia Boqvist, Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences*

Degree Project in Veterinary Medicine

***Credits**: 30 HEC*
***Level**: Second cycle, A2E*
***Course code**: EX0869*
Course coordinating department: *Department of Clinical Sciences*

***Place of publication**: Uppsala*
***Year of publication**: 2020*
***Online publication**: https://stud.epsilon.slu.se*
Cover illustration:

***Key words**: TBEV, Tick borne encephalitis, milk, pasteurization*

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SUMMARY

Tick-borne encephalitis (TBE) is one of the most important vector-borne human infections affecting the central nervous system and is caused by tick-borne encephalitis virus (TBEV) which is transmitted to humans primarily by ticks, mainly *Ixodes* spp. The ticks are expanding their distribution in Sweden and the number of reported cases of human TBE in Sweden has been increasing considerably since the end of the 20th century. Ruminants do not show clinical signs when infected by TBEV but can secrete the virus via milk which can cause food-borne outbreaks of TBE. Several outbreaks have been reported in Europe but no such case has yet been reported in Sweden. In this study, the presence of TBEV antibodies in bulk milk from 108 dairy farms in Sweden were analyzed using an enzyme-linked immunosorbent assay (ELISA). Antibodies were detected in 3.7% (n=4) of the samples and 15.7% (n=17) had levels of antibodies on the border between positive and negative (borderline value). The results indicate that it could be a risk for humans to contract TBE if consuming unpasteurized milk in Sweden. A descriptive study based on a questionnaire to the farmers was also included in this study. Based on the questionnaire results, several factors may contribute to the risk of food-borne TBE on Swedish farms such as (lack of) pasteurization of milk and low human vaccination status. Further studies are needed to investigate if there is TBEV present in milk or ticks from seropositive and potentially seropositive (borderline) farms.

CONTENT

INTRODUCTION

Tick-borne encephalitis (TBE) is one of the most important vector-borne human infections affecting the central nervous system (Gritsun *et al.*, 2003b). The disease is caused by tick-borne encephalitis virus (TBEV) which is transmitted to humans primarily by ticks, mainly *Ixodes* spp. (Gritsun *et al.*, 2003a, 2003b). The number of human cases of TBE reported in Sweden has been increasing considerably the last decade (The Public Health Agency of Sweden, 2019) and the ticks are expanding their geographical distribution (Jaenson *et al.*, 2012b, 2016; Omazic *et al.*, in manuscript). The taiga tick, *I. persulcatus,* which can carry a new and potentially more pathogenic subtype of TBEV, has been reported in Sweden since 2015 (Jaenson *et al.*, 2016). Ruminants can become infected by TBEV but do not show clinical signs. They can, however, secrete the virus via milk which can be a source of TBE in humans when consuming unpasteurized milk or milk products (Balogh *et al.*, 2012). Several outbreaks of food-borne TBE have been reported in Europe (Balogh *et al.*, 2010; Brockmann *et al.*, 2018; Dorko *et al.*, 2018; Markovinović *et al.*, 2016). Even though artisan production of cheese and other milk products is popular in Sweden and some producers and consumers prefer unpasteurized milk, no case of TBE due to consumption of unpasteurized milk or milk products has yet been reported in Sweden. Animals can successfully serve as sentinels in finding new TBEV foci (Rieille *et a*l., 2017; Tonteri *et al.*, 2016) which can be important when the geographical distribution of the virus varies (Randolph *et al.*, 2000). There is limited knowledge of presence of TBEV in domestic ruminants in Sweden. The aim of this study was to estimate the occurrence of TBEV antibodies in bulk milk samples from goat, sheep and dairy cow herds in Sweden. Furthermore, the study aimed to review the literature regarding how TBEV risk areas can be detected and how food-borne TBE can be prevented. Specific questions that will be answered are:

- What is the occurrence of TBEV-specific antibodies in Swedish bulk milk samples?
- Can bulk milk be used for monitoring of TBEV risk areas?
- Which factors may increase the risk of food-borne TBE on Swedish farms?
- Which preventive methods can be used to reduce the risk of food-borne TBE?

This study was performed, and will also be continued, within the research project NordForsk CLINF (*Climate-change effects on the epidemiology of infectious diseases and the impacts on Northern Societies*). The CLINF project investigates the impact of climate change on the geographic epidemiology and distribution of human and animal infectious diseases in the Nordic region and Russia (CLINF, 2019). This study was performed with financial support by Ivar and Elsa Sandberg foundation.

LITERATURE REVIEW

Tick-borne encephalitis virus

TBE is one of the most important vector-borne human infections of the central nervous system (Gritsun *et al*., 2003b). The disease is caused by TBEV which belongs to the *Flaviviridae* family and the genus *Flavivirus*. It is a single stranded RNA virus with an envelope and therefore sensitive to heat and detergents. The virus has been found in Europe, Russia, Mongolia, northern parts of China and Japan *(Dumpis et al*., 1999; Gritsun *et al*., 2003a; Valarcher *et al*., 2015). Due to the complexity of the virus ecology, the distribution of TBEV foci varies between seasons (Randolph *et al*., 2000). The virus is divided into three subtypes; European, Siberian and Far Eastern. These subtypes differ in their endemic regions and even though all subtypes of TBEV can cause severe disease, the Siberian and Far Eastern are considered more pathogenic than the European subtype (Gritsun *et al*., 2003a, 2003b). TBE is transmitted by ticks, manly *Ixodes* spp. *Ixodes ricinus* (common tick) is the main transmitter of the European subtype of TBEV while *I. persulcatus* (Taiga tick) is the most important vector in spreading the Siberian and Far Eastern subtype (Gritsun *et al*., 2003a, 2003b). The ticks are expanding their geographical distribution in Sweden. During the last 30 years, the range of *I. ricinus* has spread northward (Jaenson *et al*., 2012b) and a study from 2016 showed that *I. persulcatus* has expanded its geographical range into northern Sweden (Jaenson *et al*., 2016). This has been confirmed by the findings in a nationwide tick collection study made by the National Veterinary Institute in Sweden. That study showed that *I. persulcatus* not only were found along the northern coastline areas but also in the inland regions, suggesting expansion of its geographical range (Omazic *et al*., in manuscript).

TBE in humans

Approximately 70-95% of all TBE infections in humans are estimated to be asymptomatic. When clinical signs are noted, they vary from mild flu-like symptoms to severe including fever, fatigue, anorexia, muscle and headaches, nausea, photophobia, confusion, paralysis, coma and even death (Gritsun *et a*l., 2003a; Kaiser, 2012; Růžek *et al.*, 2010). The number of reported human cases of TBE in Sweden has increased. The average number of reported cases in the late 1990's was 50 cases/year. In 2017, 391 cases were reported (the highest number so far), followed by 385 cases in 2018 and 365 cases in 2019. According to the Public Health Agency of Sweden the increase of incidence is 5% per year since 2005 (figure 1).

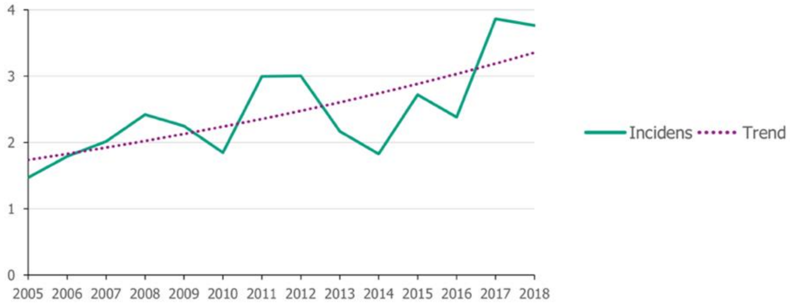

Figure 1. *Number of reported TBE cases/100 000 people in Sweden in the years 2005-2018 (The Public Health Agency of Sweden, 2019).*

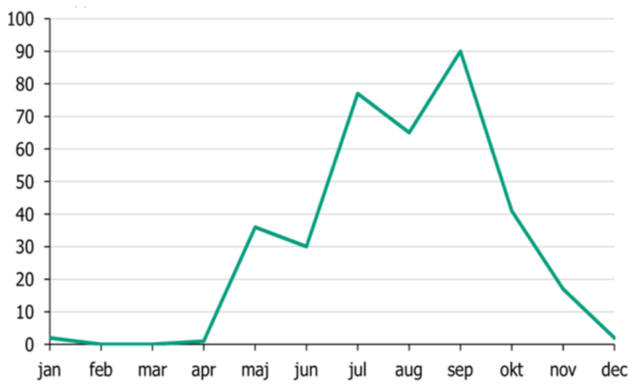

Figure 2. *Number of reported TBE cases each month in Sweden in 2018 (The Public Health Agency of Sweden, 2019).*

In 2018, unusually high numbers of cases were reported already in May (Figure 2). The peak of reported cases is usually in July to September. The disease is also spreading westwards; although the majority of cases in 2018 were reported from the eastern part of Sweden, in the regions around Stockholm, there were more cases reported in the south-western regions than previously (Figure 3). The increasing temperature due to the climate change is supposed to be a reason for the expanding distribution of TBEV in Sweden (Jaenson *et al.*, 2012b, 2016)

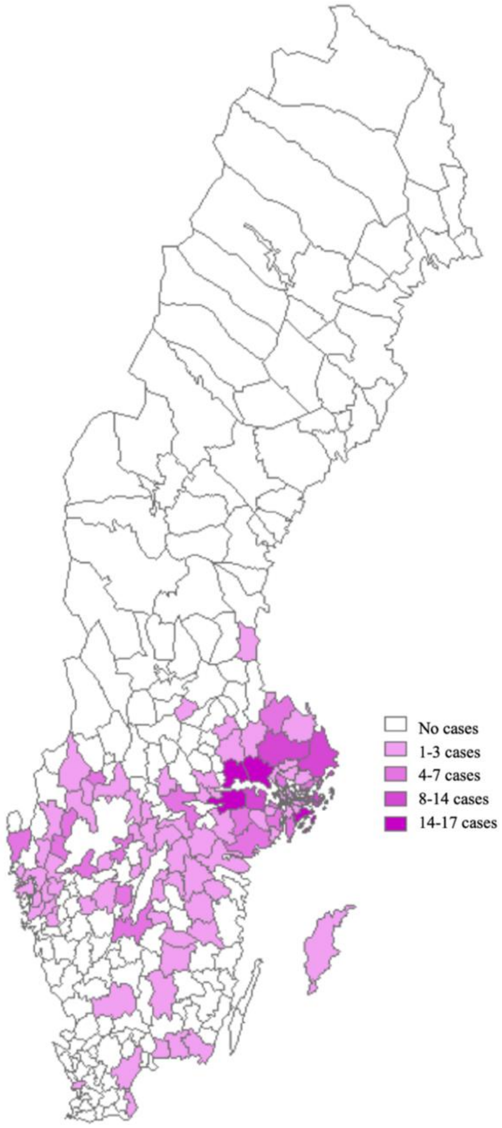

Figure 3. *Number of reported TBE cases in each municipality of Sweden in 2018 (The Public Health Agency of Sweden, Statistics Sweden, 2019).*

Outbreaks associated with milk and cheese consumption

Consumption of unpasteurized milk is associated with a risk of TBE infection (Bušová *et al.*, 2018). Alimentary outbreaks have been reported in central and eastern parts of Europe as shown in Table 1. To the authors knowledge, no case of TBE due to consumption of unpasteurized milk or milk products has yet been reported in Sweden.

Table 1. Reported outbreaks of *TBE in Europe caused by consumption of unpasteurized milk or milk products*

Year	Place	Reported cases	Source	Reference
2016	Germany	2	Goat cheese	(Brockmann *et al.*, 2018)
2016	Slovakia	44	Sheep cheese	(Dorko *et al.*, 2018)
2015	Croatia	7	Goat cheese Goat milk	(Markovinović *et al.*, 2016)
2014	Slovakia	11	Sheep cheese	(Dorko *et al.*, 2018)
2011	Hungary	11	Cow milk	(Caini *et al.*, 2012)
2007	Hungary	25	Goat milk	(Balogh *et al.*, 2010)

TBEV in ruminants

There is limited knowledge of presence of TBEV in domestic ruminants in Sweden. However, a recent study reported that TBEV specific antibodies were found in sera from Norwegian dairy cows (Paulsen *et al.,* 2018). TBEV RNA was also found in milk and the authors discuss the potential risk of consuming unpasteurized milk from Norway. The samples with TBEV RNA came from farms mainly in the southern parts of Norway except for one in Brønnøy municipality, located at the same latitude as southern Lapland, Sweden. (Paulsen *et al.*, 2018). So far, there is no evidence suggesting that ruminants develop clinical symptoms when infected by TBEV. During an experimental study, infected goats did not show any clinical signs or changes in body temperature comparing to uninfected animals (Balogh *et al*., 2012). They can however secrete the virus via milk, in some cases for more than three weeks post first exposure of TBEV (Balogh *et al*., 2012; Gritsun *et al*., 2003a).

Stability of TBEV in milk

In a study conducted in 2016 the stability of TBEV in fresh goat milk was investigated. The virus was stable for several days (>74 h) in untreated milk stored at refrigeration temperatures (4°C). Additionally, in this study, the thermal segments of a cheese making process were simulated to produce a similar cheese that caused an alimentary outbreak of TBE in Austria in 2008, where six humans developed TBE. The results revealed that using this method (slowly heating the milk to 30°C, allowing it to gradually cool to 22°C and thereafter incubate it for 16

h) reduced the quantity of virus but left infectious virus detectable. The study also included a pasteurization, where the milk was heated to 72°C for 15 seconds and then immediately cooled to 4°C. No virus was detected after pasteurization, indicating that it could be an effective method of inactivating TBEV (Offerdahl *et al*., 2016). Even though TBEV is sensitive to heat and detergents, the virus can sustain some time in normal gastric juice (pH 1.49–1.80). Because milk and milk products transfer from the human stomach quickly (milk can reach the duodenum within minutes after consumption), virus can reach the intestines without losing its infectivity (Balogh *et al*., 2012; Gritsun *et al*., 2003a).

Animals as sentinels

Several studies suggest that animals could serve as sentinels to discover new geographic TBEV foci. A sentinel is an organism, often an animal, that can provide information about occurrence of disease in a specific area. Tis information can be used for detection or evaluation of risks. In Finland, sera collected from moose after hunting was successfully used for detection of a possible new TBEV focal area (Tonteri *et al.*, 2016). In Switzerland, individual goat sera were analyzed for TBEV specific antibodies and the results were used to locate potentially new regional foci, which were later confirmed with TBEV RNA extraction from ticks collected in the areas (Rieille *et al.*, 2017). Paulsen *et al*. (2018) where able to locate TBEV RNA in Norwegian cow milk in an area with no reported cases of human TBE.

Milk production and consumption in Sweden

In 2016, 28.6 million ton milk was produced in Sweden. Four million ton where sold directly from the farm or consumed by the farmer themselves (The Swedish Board of Agriculture, 2019). The Swedish Food Agency discourages consumption of unpasteurized milk particularly for children, pregnant women, older or immunosuppressed individuals (The Swedish Food Agency, 2019). In Sweden, milk can be sold only if it is pasteurized or treated in other ways with equivalent effect. However, there is one exception. A small amount (specified to maximum of 70 liters per week) of unpasteurized milk can be sold, but only if it is done directly from the farm and if a written information to the consumer is provided. This information must include that the milk is unpasteurized and therefore may contain pathogenic bacteria and recommend-dation about storage and heat treatment prior to consumption. Furthermore, the information must include that it is particularly important to heat the milk if it is consumed by children or immunosuppressed individuals (LIVSFS 2016:5). Even though the national authorities discourage consumption of unpasteurized milk and historical data show improved public health due to pasteurization (Claeys *et al*., 2013), there are advocates for unpasteurized products. Arguments supporting unpasteurized products include better quality and taste, artisan traditions, profitability for farmers and reduced milk allergy due to consumption. A review article investigating several arguments supporting consumption of unpasteurized milk refutes almost all arguments put forward by unpasteurized milk proponents except the change of the organoleptic profile of milk when heat treated (Claeys *et al.*, 2013).

Prevention of TBEV transmission

There are several methods in which transmission of TBEV via milk can be prevented. A common and effective way is through pasteurization (Balogh *et al*., 2012; Gröner *et al.*, 2018) and heating the milk to 72°C for 15 seconds is enough to inactivate TBEV (Offerdahl *et al*., 2016). Other preventive actions can be made, focusing on different levels; humans, farm animals or ticks.

Vaccination

Human vaccination

Vaccination of people is an effective way of preventing the disease. In the 1980's, Austria launched a vaccine program and managed to bring down the number of human TBE cases dramatically. Between the year 1977 and 1982, the province of Carinthia had an average of 155 recorded cases per year compared to an average of under 5 recorded cases per year in the beginning of the 20th century, after the vaccine program (Kunz, 2003). In Sweden, the Public Health Agency recommends vaccination for residents or frequent visitors of TBE risk areas. The definition of a risk area changes over time and the Public Health Agency refer to local health care providers for information about each county (The Public Health Agency of Sweden, 2019). However, in most counties people must pay for the vaccination themselves, which may cause that people not get vaccinated as recommended. If vaccinated with a vaccine based on the European subtype of TBEV, the patient will also develop a sufficient protection against Far Eastern and Siberian subtypes (Kunz, 2003).

Animal vaccination

Studies have showed that vaccination of ruminants is an effective way to prevent spreading of TBEV to humans via unpasteurized milk and milk products. Immunized individuals do not develop viremia and therefore do not shed the virus (Balogh *et al*., 2012; Salát *et al*., 2018). TBEV vaccine developed for humans is effective also on goats (Balogh *et al*., 2012). However, to this day there is no TBEV vaccine available for veterinary use but there is a vaccine candidate designed and tested successfully (Salát *et al.,* 2018).

Tick prophylaxis

A way of controlling TBEV is by reducing the number of tick infestations. In Sweden, there are numbers of antiparasitic drugs registered for pour-on treatment of ruminants. For instance, eprinomectin, an insecticide which affects the nervous system of the parasites and can be used on lactating cows without milk withdrawal period (FASS vet, 2019). Insect repellent can also be used to bring down the number of tick bites. One study showed that Swedish plant extract diluted in acetone had a repellent effect on *I. ricinus* comparable with that of DEET (N,N-diethyl-m-tuluamide), a commonly used commercial repellent (Jaenson *et al.*, 2006). Other methods of avoiding tick bites is by keeping the animals at pastures with low number of ticks for example avoiding forest pasture and pastures with high grass and bushes.

MATERIAL AND METHODS

Recruitment of participating farms

An e-mail with information about the study was sent out to all farmers keeping cattle listed by the Swedish Board of Agriculture. In addition to this, a registration form enabling farmers to register to the study was published at the National Veterinary Institute's website and the link to this form was also sent to Swedish Goat Breeding Association and Swedish artisan dairy producers. The link was also shared on social media. All who replied and wished to participate were included in the study.

Sample material, instructions and questionnaire

Milk tubes for bulk milk (a combination of milk from several individual animals) samples and ice packs for transportation along with return envelope, sampling instructions and a written questionnaire were sent out to all participating farms. The questionnaire included questions in Swedish about production, handling of milk, tick-borne diseases in the area, vaccination status and more (appendix 1). The samples were taken between 5th and 14th of August 2019. After collection of the milk sample, the participants were asked to store the milk at -20°C (at least 1 h) before transportation with ice packs. After arriving to the National Veterinary Institute, Uppsala, with mail, the milk samples were coded and stored at -20°C until preparation. The questionnaires were also coded before analysis.

Milk analysis

Sample preparation

The samples were left to thaw at room temperature. When completely thawed and thoroughly mixed, approximately 1.8 ml of milk was transferred into 2 ml Eppendorf tubes and centrifuged at 16.000 rpm for 10 minutes to separate the skim milk (middle layer) from the cream (top layer) and the sediment (bottom layer). The skimmed milk was transferred into 2 ml micro tubes (polypropylene with screw cap) and stored at -80°C.

Serological analyses

Skimmed milk was analysed in a two-step ELISA (PROGEN IMMUNZYM FSME IgG ALL SPECIES) for detection of Ig-G antibodies against TBEV. Except for the milk samples, each plate also included 5 calibrator samples as well as a high concentration positive, a low concentration positive and a negative sample. The analysis was carried out according to the manufacturer's instructions; 10 μl skimmed milk was diluted with 500 μl working buffer (1 part 0.1 M Tris/HCl pH7.4 + 9 parts distilled water) and placed for incubation in a well of a 96 well plate coated with inactivated TBE virus. All samples were prepared in duplicates. The plates were covered by adhesive foil and left to incubate for 60 minutes at room temperature and then washed 3 times with 200 μl working buffer. In the next step, 200 μl of conjugate solution (blue colored protein G peroxidase) was added to each well and again covered with foil and left to incubate in room temperature for another 60 minutes. The samples were washed (200 μl working buffer x 3) and 200 μl of substrate (tetramethylbenzidine) was added to each well before the last incubation of 30 minutes. 50 μl of stop solution (0.5 M sulphuric acid) was added in each well and the plate was analyzed using an ELISA reader (Epoch 2 Microplate

Spectrophotometer). The optical density was measured at a wavelength of 450 nm within 10 minutes after adding the stop solution. To fit the optical density values to the antibody concentration a reference curve was plotted from the calibrator samples using a four-parametric logistics regression (calculated with software from MyAssays). Concentration unit is Vienna units (VIEU)/ml and samples with values above 126 VIEU/ml was considered positive and samples with values below 63 VIEU/ml was considered negative. Samples with values between 63 and 126 VIEU/ml were considered what PROGEN calls borderline.

Epidemiological analyses

The data from the completed questionnaires was organized using Microsoft Excel and analyzed descriptively. A map showing the participating farms was made using the free web application Map Customizer.

Literature review

A literature review was done to investigate methods of monitoring of TBEV risk areas and methods of prevention of food-borne TBE. Scientific publications were found using Pubmed and Google Scholar. Keywords included *TBE, outbreak, unpasteurized, milk.*

RESULTS

A total of 108 farms participated in this study; 85 dairy cattle farms, 21 goat farms, one sheep farm and one water buffalo farm. Most the farms were located in the southern parts of Sweden (Figure 5). The size of the dairy farms varied between 2 and 620 (average 76) lactating animals. The goat farms varied between 2 and 80 (average 33) lactating animals. The sheep farm had four lactating animals and the water buffalo farm had 13 lactating animals.

Serological results

A total of 108 bulk milk samples were analyzed. Antibodies were detected in 3.7% (n=4) of the samples and 15.7% (n=17) had levels of antibodies on the borderline between positive and negative (table 2, figure 4). The positive bulk milk samples were from cattle farms in the counties of Gotland, Örebro, Värmland and Jämtland (Figure 5).

Table 2. *Results from ELISA-analysis of TBEV-antibodies in bulk milk samples from cow, goat, sheep and water buffalo*

Animal	Total	Positive	Borderline	Negative
Dairy cattle	n=85	4.7% (n=4)	20% (n=17)	75.3% (n=64)
Goat	n=21	0	0	100% (n=21)
Sheep	n=1	0	0	100% (n=1)
Water buffalo	n=1	0	0	100% (n=1)
Total	n=108	3.7% (n=4)	15.7% (n=17)	80.6% (n=87)

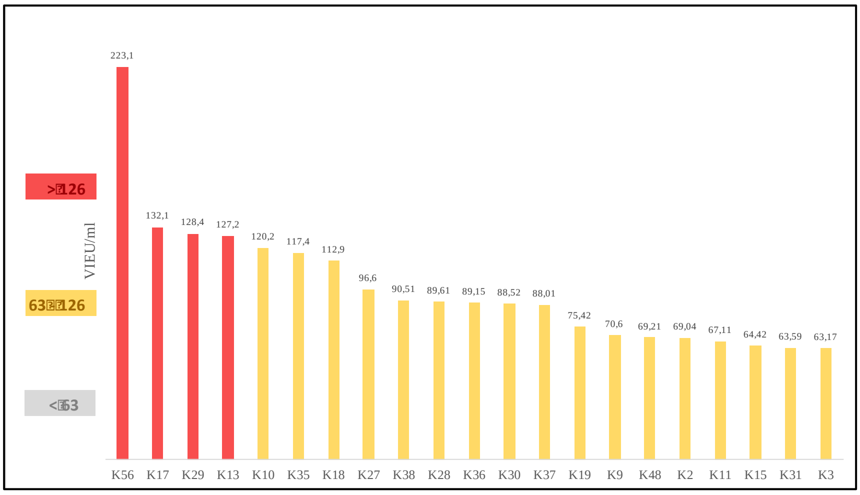

Figure 4. VIEU/ml of the positive and borderline bulk milk samples

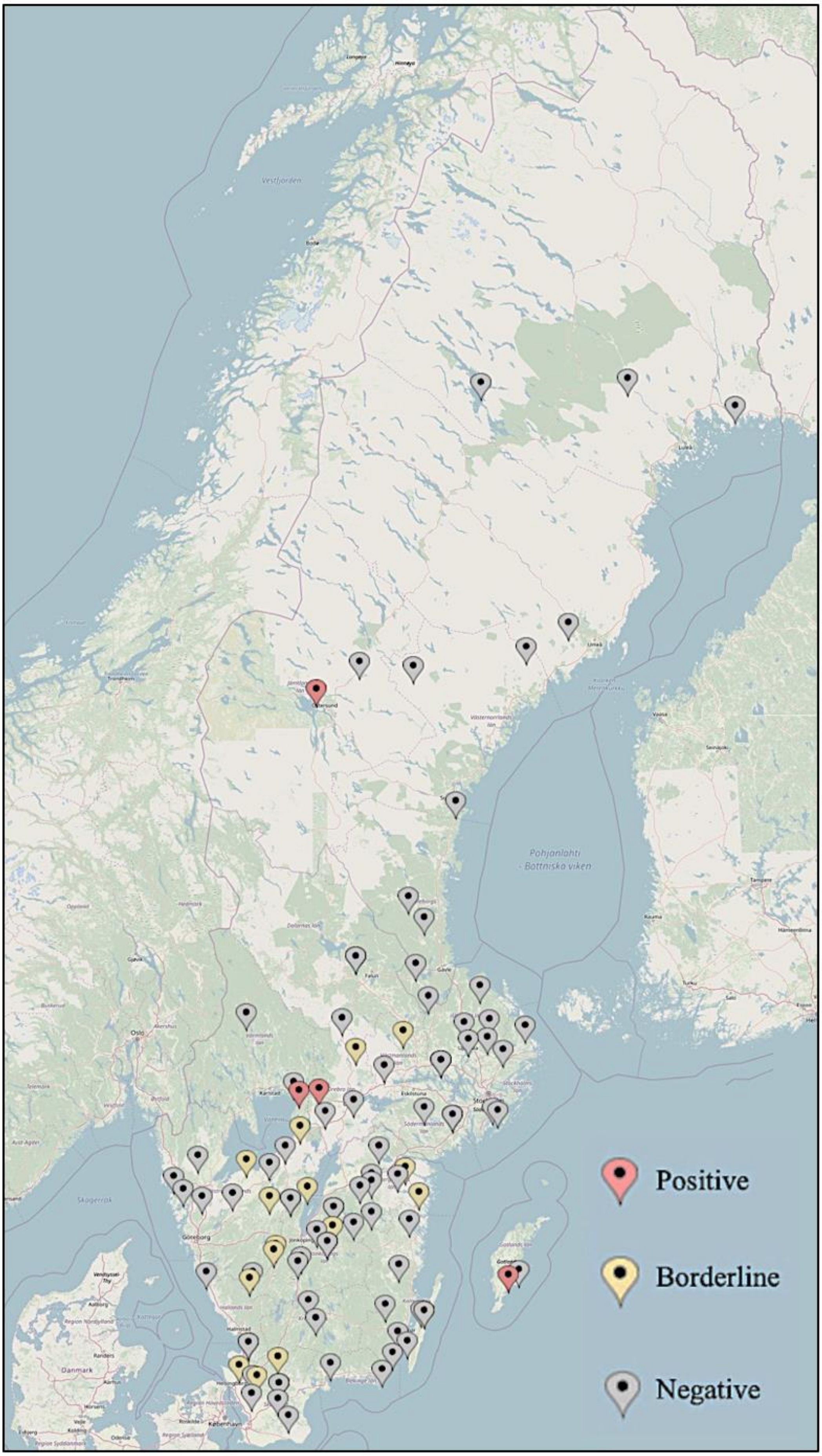

Figure 5. Geographical locations of the Swedish farms included in the study

Epidemiological results

A total of 108 questionnaires were completed. The distribution of the milk produced on the farms is shown in figure 6. The question "*If milk or milk products are sold on the farm, is it pasteurized?*" was answered in 73 questionnaires, including some who previously stated that the farm exclusively sent the milk to a dairy plant. Of these 73, 18% (n=13) pasteurized all milk, 9% (n=7) some of the milk and 73% (n=53) none of the milk. The extent of pasteurization on the 36 participating farms that did not send all their milk to a dairy plant (stating that they exclusively or partially sell from the farm or use the milk for own consumption) is shown in figure 7.

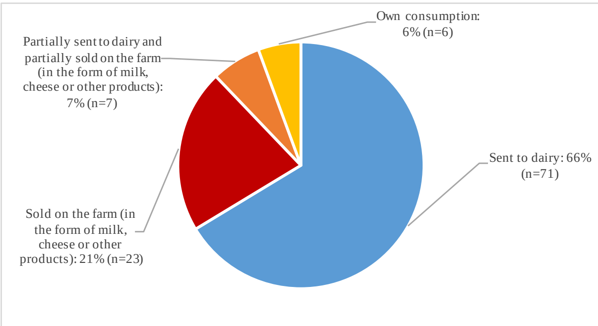

Figure 6. The distribution of the milk produced on the participating farms in Sweden.

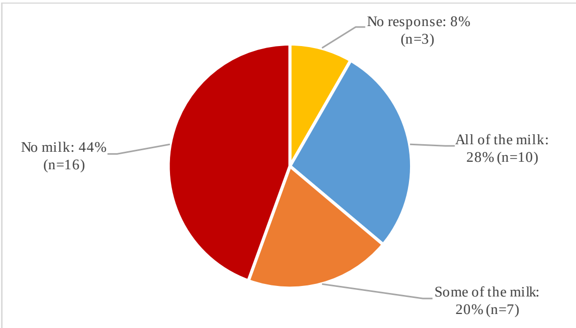

Figure 7. Pasteurization of milk produced on participating farms that did not send all their milk to dairy.

55% (n=59) did not use prophylactic drugs against ticks at all, 26% (n=29) used it only for pets (dogs or cats), 12% (n=13) used it only on farm animals and 8% (n=7) used it on both pets and farm animals. The experience of changes in the number of ticks is shown in figure 8.

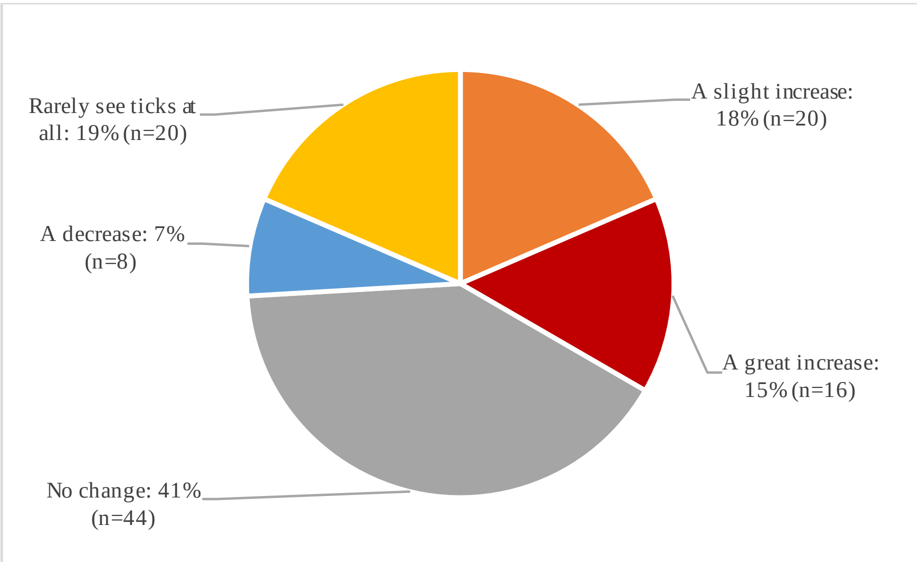

Figure 8. The experienced changes in the number of ticks the past five years in Sweden.

The vaccination status against TBEV of the employees on all the 108 participating farms and the 25 farms that sold, partially sold or consumed milk that was not at all or just partially pasteurized is shown in figure 9 and 10.

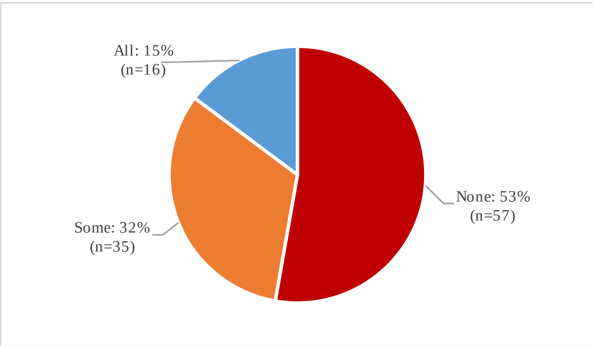

Figure 9. Vaccination status of the workers on all the participating farms.

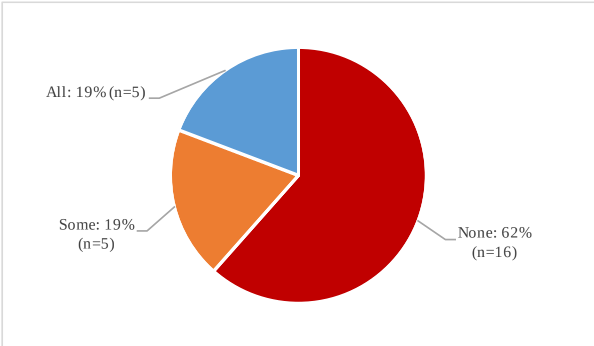

Figure 10. Vaccination status of the workers on the 25 farms that sold, partially sold or consumed milk that is not or just partially pasteurized.

Regarding cases of TBE, two of the farms (2%) mentioned that one person on the respective farm had suffered from TBE and one farm (1%) mentioned that more than one person on the farm had been ill in TBE. The last question of the questionnaire was an open question that let the participant add any information of their choice, which 36 of them did. Seven participants wrote that the number of ticks was unusually low in 2019. One participating farm who had both cows and goats stated that cows tend to have a lot more ticks than goats. One participant wrote that she suffered from TBE one year ago. She regularly drank unpasteurized milk from her cows and at that time, the cows had a lot of ticks and she herself had one tick that year.

DISCUSSION

This is the first study to detect TBEV specific antibodies in unpasteurized bulk milk in Sweden. The results indicate that there could be a risk for humans to contract TBE when consuming unpasteurized milk in Sweden. Further studies will be made to investigate if TBEV is present in milk or ticks from seropositive and potentially seropositive (borderline) farms. The occurrence of TBEV antibodies in cow bulk milk in Sweden in this study was 4.7%. The result is lower than the study in Norway made by Paulsen *et al*. (2018) (13.4% seropositivity) but comparison to other studies is complex because no other study used bulk milk samples (see further discussion about reference values below). The locations of the seropositive farms were not concentrated to the eastern part of Sweden, in the regions around Stockholm, where most human cases of TBE are reported (The Public Health Agency of Sweden, 2019). This was unexpected and could be due to TBE foci being small, multifocal and constantly changing location (Randolph *et al.*, 2000). One of the seropositive farms was located in Jämtland county, which is not known as a risk area for TBE and had no case of human TBE recorded in 2018 (The Public Health Agency of Sweden, 2019). According to the owner of this farm, no animals have been brought in from other regions since the production started more than 20 years ago (personal contact).

Today, ticks are expanding their geographical distribution in Sweden (Jaenson *et al.,* 2012b, 2016; Omazic *et al*., in manuscript) and the risk areas for TBE are increasing (The Public Health Agency of Sweden, 2019) and might be constantly changing (Randolph *et al*., 2000). The *I. ricinus* is moving in a northwest direction and *I. persulcatus* is advancing westwards into the northern parts of Sweden, especially along the coast, and could be carrying a possibly more pathogenic subtype of TBEV. These areas have not previously been known as risk areas for TBE and the human vaccination status may therefore be low (e.g. Jämtland county). Based on results obtained from this study, there might also be a potential risk for transmission of TBEV via unpasteurized milk in Sweden. If the increase of risk areas continue, the need for monitoring of the disease might become more important. TBE is a notifiable disease in Sweden, but monitoring risk areas based on human cases may be unspecific since people and their companion animals travel more these days. Using farm animals as sentinels for surveillance of a specific geographical area has proven to be a successful method. Samples from wildlife, such as blood samples collected in connection to hunting, can be used as a rather cheap surveillance method (Tonteri *et al*., 2016) but some wild animals can also move over wide areas, thus giving false information. Individual blood samples from farm animals can be an effective way of finding new risk areas (Rieille *et al.,* 2017), but this method may require trained personal to obtain the samples and several samples from each farm, thus increasing the cost and animal suffering, unless sampling is done at slaughter. Antibodies against TBEV in bulk milk can give important information about geographical distribution of infection. Bulk milk samples could be used as a guidance for detection of new TBE foci. Other advantages are that farmers easily can take the samples themselves, it is a non-invasive method and the cost is relatively low. Seropositive bulk milk can then be used for guidance where to look for infectious TBEV in ticks or milk instead of doing this at random and therefore at a much higher cost. One potential weakness of this method is that positive bulk milk results could be due to seropositive animals brought in from other regions.

Some other questions must be considered before using bulk milk sample for surveillance of TBEV occurrence. The reference values for the ELISA-kit used in this study were based on individual serum samples. Using these reference values for bulk milk samples samples may lead false negative results and it can thus be questionable if these cut-off values should be used to determine if a farm is seropositive or not. Combining several individual milk samples to one sample may dilute the antibodies if there are only a few seropositive animals on the farm at that time. Thus, bulk milk sample lower than 126 VIEU/ml (borderline), or even lower than 63 VIEU/ml (negative) could contain milk from seropositive individuals. Further studies to investigate the dilution effect of antibodies from seropositive milk when combined with milk from seronegative individuals into one bulk milk sample should be conducted to be able to interpret the result more exact.

Based on the results from the questionnaire, some factors on Swedish farms may contribute to the risk of food-borne TBE. The results show that unpasteurized milk or milk products are sold from or consumed directly on farms. Selling unpasteurized milk in small amounts is permitted in Sweden (LIVSFS 2016:5) and the result is therefore not surprising. Unfortunately the questionnaire did not reveal if proper written information about the product was provided to the persons buying the product. The lack of such information could be a potential risk factor for transmission of food-borne TBE. Also, the vaccination status of workers on the participating farms seems to be low, only 15% (n=16) of the participating farms stated that all the workers were vaccinated against TBEV. The usage of prophylactic drugs against ticks on farm animals were also low, 19% (n=20) of the farms used this.

The questions in the questionnaire could have been designed more adequately. For example, the participants who stated that they use the milk for own consumption added this information in text, since there was no such option in the questionnaire (appendix 1). The number might have been much higher if such a question was asked differently, for example including an *own consumption* answer option and letting the participants choose more than one answer to that question.

So far, no case of TBE due to consumption of unpasteurized milk or milk products has been reported in Sweden. Possible unrecorded cases of food-borne TBE could be due to lack of knowledge (both by the public and health professionals) of this potential way of infection or due to patients having mild symptoms and therefore not seeking medical attention. Also, patients could have tick bites in close connection to the onset of illness and therefore, alimentary reasons might not have been suspected. One of the respondents of the questionnaire, who was a regular consumer of unpasteurized milk, had suffered from TBE. The participant had one tick bite before becoming ill and assumed the disease was due to the tick.

Finally, there are successful methods of avoiding the risk of food-borne TBEV. Reducing the animal exposure of ticks will reduce the risk of TBEV transmission from animals to humans. It will also reduce the risk of other tick borne diseases such as babesiosis and anaplasmosis, which can cause significant health problems for the animals. The questionnaire results showed that only 18,5% (n=20) of the participating farms use tick prophylactic drugs on farm animals. However, this alone is no secure way of avoiding TBEV. Vaccination of farm animals against

TBEV could prevent viremia and thus prevent spreading of the virus via milk. However, there is currently no vaccine available for veterinary use and it is questionable if farmers would be willing to pay for vaccination against TBEV when it does not cause disease in the animals. Vaccination of humans is an effective way to prevent the disease (both food- and tick-borne) and should be done in risk areas. Vaccination of humans cannot completely reduce the risk of food-borne TBE since some people cannot be vaccinated for example due to immunodeficiency and some choose not to vaccinate as part of the anti-vaccination movement. Also, this will not protect people occasionally visiting TBE-hot spot areas, e.g. tourists. Pasteurization of milk is an effective way for inactivation of TBEV, thus avoiding the risk of food-borne TBE. Reviewing these options, pasteurization of milk is the most practical and inexpensive way of preventing food-borne TBE. Pasteurization also serves other purposes in preventing other food-borne pathogens, such as such as *E. coli*, *Salmonella*, *Listeria monocytogenes* etc. Knowing this, it is also important to understand why some producers and consumers choose unpasteurized milk and milk products. The review article by Clays et al. (2013) found no scientifically viable arguments for unpasteurized products except the change of taste. There could be other, more complex factors behind choosing unpasteurized products, such as culture and tradition.

This study will be continued within the NordForsk CLINF project. The ELISA results will be confirmed by Western-Blot analysis, another method of detecting antibodies using electrophoresis. Furthermore, total RNA will be extracted from individual milk samples collected from herds with a seropositive bulk milk sample (<63 VIEU/ml) and analysed for TBEV using q-PCR. TBEV positive milk samples will be applied to cell culture for virus cultivation and isolation. Ticks from seropositive farms will be analyzed for TBEV positivity using q-PCR.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Fästingburen hjärninflammation, TBE, är en allvarlig sjukdom som blir allt vanligare i Sverige. Det rapporteras fler fall och sjukdomen upptäcks över större områden än tidigare. Sjukdomen orsakas av ett TBE-virus (TBEV) som överförs till människor framför allt från den vanliga fästingen *Ixodes ricinus*. Fästingarna sprids nu över större områden och sedan 2015 har även den så kallade tajgafästingen, *I. persulcatus*, påvisats vid flera tillfällen och flera lokalisationer längs Norrlandskusten. Tajgafästingen kan bära på en typ av TBEV som kan ge allvarligare symtom än den typ som finns i Sverige idag men ännu har inget sådant sjukdomsfall rapporterats i Sverige. Våra lantbruks- och husdjur kan bli infekterade av TBEV men uppvisar i regel inga sjukdomstecken. Men idisslare, till exempel kor, får och getter, kan utsöndra virus i mjölk. Detta gör att människor kan smittas av TBEV genom att konsumera opastöriserad mjölk eller mjölkprodukter. Forskning visar att TBEV kan överleva flera dagar i mjölk som förvaras i kylskåpstemperatur men smittspridning kan förhindras om mjölken pastöriseras. Man kan även förhindra att viruset sprids via mjölk genom att vaccinera djuren mot TBEV. Det är för många okänt att TBEV kan spridas med opastöriserad mjölk och något sådant sjukdomsfall har ännu inte påvisats i Sverige. Det förekommer dock i andra delar av Europa där man i större utsträckning konsumerar opastöriserade mjölkprodukter. Då har människor drabbats av TBE efter konsumtion av bland annat mjölk och ost från ko, get och får.

Eftersom riskområdena för att drabbas av TBE ökar och ändras konstant finns ett behov av att övervaka både sjukdomen och potentiella riskområden. Genom att hitta spår av TBE-infektion i djur kan detta användas på olika sätt för att kartlägga dessa riskområden. Blodprov från älgar insamlade i samband med jakt har använts i Finland för att hitta nya riskområden och i Norge har man framgångsrikt undersökt mjölkprov från enstaka djur framgångsrikt.

Målet med denna studie var att undersöka förekomsten av antikroppar mot TBEV tankmjölk från ko-, get-, och fårbesättningar i Sverige. Tankmjölk är en blandning av alla gårdens mjölkande djur. Studien undersökte om det finns antikroppar och om det skulle kunna vara en metod för att hitta nya riskområden för TBE. Tankmjölk från 108 besättningar har analyserats med ELISA-teknik. Antikroppar mot TBEV hittades i tankmjölk från 4 gårdar och 17 gårdar hade nivåer av antikroppar som gränsade till att vara ett positivt prov. Samtliga dessa prover var från nötkreatursbesättningar. Dessa gårdar låg framför allt inte i de områden där flest fall av TBE hos människa rapporteras. En positiv gård upptäcktes i Jämtland. Resultatet visar att det kan föreligga en risk att drabbas av TBE genom att dricka opastöriserad mjölk från svenska gårdar. Det visar också att analys av tankmjölk för att kartlägga riskområden för TBE skulle kunna vara en effektiv metod. En enkätundersökning med frågor till lantbrukare om bland annat produktion, hantering av mjölk, fästingburna sjukdomar i området, vaccinationsstatus hos gårdens folk, med mera gjordes också i samband med studien. Enkäten visade bland annat att mjölk säljs eller konsumeras direkt på gårdar utan att pastöriseras och att vaccinationstäckningen i många fall är otillräcklig.

Studien har utförts med medel från Ivar och Elsa Sandbergs fond och inom forskningsprojektet NordForsk-CLINF som undersöker klimatförändringens påverkan på infektionssjukdomar i Norden och Ryssland. Projektet ska bland annat undersöka om det finns levande virus i mjölk från positiva gårdar.

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APPENDIX 1

STATENS VETERINÄRMEDICINSKA ANSTALT

Enkätfrågor till projektet TBE-virus i mjölk

När ni fyllt i svaren sparar ni enkäten på er dator och sedan skickar ni den till anna.omazic@sva.se. **Märk mejlet med ”TBE-virus i mjölk”**. Ni kan också välja att fylla i enkäten för hand och då skickar ni den tillsammans med proverna. Det finns plats för egna kommentarer sist i enkäten om ni tycker vi missat något eller vill lägga till information.

Stort tack för att ni fyller i vår enkät!

Vilken typ av mjölkproduktion har ni på gården?

- ☐ kor
- ☐ får
- ☐ get

Ange antalet mjölkande djur i besättningen.

☐

Hur har nivån på er gårds mjölkproduktion ändrats under de senaste 5 åren?

- ☐ ökat
- ☐ minskat
- ☐ Inga större förändringar i produktion

Hur avsätts mjölken som produceras på gården?

- ☐ Skickas till mejeri
- ☐ Säljs på gården (i form av mjölk, ost eller annan produkt)
- ☐ Skickas delvis till mejeri och säljs delvis på gården (i form av mjölk, ost eller annan produkt)

Om mjölk eller mjölkprodukter säljs på gården; pastöriseras mjölken eller mjölkprodukten innan försäljning?

- ☐ Ja, all mjölk
- ☐ Ja, en del av mjölken
- ☐ Nej

På vilken typ av betesmark går djuren huvudsakligen?

- ☐ Åkermark
- ☐ Naturbetesmark med buskar och träd
- ☐ Strandängar och/eller våtmark
- ☐ Blandat

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STATENS VETERINÄRMEDICINSKA ANSTALT

Finns det någon form av ytvatten på betena?

- ☐ Nej
- ☐ Rinnande bäckar/åar
- ☐ Stillastående dammar/sjöar
- ☐ Flera av ovanstående

Används något förebyggande medel mot fästningar hos sällskaps- och produktionsdjuren?

- ☐ Nej
- ☐ Endast för eventuella sällskapsdjur (hund eller katt)
- ☐ Endast för produktionsdjur
- ☐ Både för sällskapsdjur och produktionsdjur

Upplever du att antalet fästingar har ökat under de senaste 5 åren?

- ☐ Ökat något
- ☐ Ökat mycket
- ☐ Lika som tidigare (ingen ökning)
- ☐ Har minskat
- ☐ Vet ej, ser inga fästingar eller bara mycket sällan.

Har ni haft ökade problem med fästingburna sjukdomar i besättningen, t.ex. betesfeber (anaplasmos) eller sommarsjuka (babesios) under de senaste 5 åren?

- ☐ Ja, betesfeber (anaplasmos)
- ☐ Ja, sommarsjuka, blodpinkning (babesios)
- ☐ Ja, både fall av betesfeber (anaplasmos) och sommarsjuka (babesios)
- ☐ Nej, inga fall med fästingburna sjukdomar vad vi vet

Har ni haft ökade problem med kastningar/aborter i besättningen under de senaste 5 åren?

- ☐ Ja
- ☐ Nej

Är personer som lever och/eller arbetar på gården vaccinerade mot TBE?

- ☐ Nej
- ☐ Vissa personer
- ☐ Alla

Har någon person som bor/arbetar på gården drabbats av TBE under de senaste 20 åren?

- ☐ Nej
- ☐ Ja, en person
- ☐ Ja, flera personer

Har någon i närliggande område drabbats av TBE?

- ☐ Nej
- ☐ Ja, en person
- ☐ Ja, flera personer
- ☐ Vet ej

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STATENS VETERINÄRMEDICINSKA ANSTALT

Övriga kommentarer (frivilligt) – Fyll i övrig information som kan vara viktig för projektet. Texta gärna om du skriver för hand!

Läs mer om hantering av personuppgifter

Genom att skicka in dina uppgifter samtycker du till att vi behandlar personuppgifter i enlighet med dataskyddsförordningen (EU) 2016/679.

2019-06-28