

Toxoplasmosis and Border Disease in 54 Swedish Sheep Flocks

Seroprevalence and Incidence during one Gestation Period

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Lundén, A., U. Carlsson and K. Näslund: Toxoplasmosis and border disease in 54 Swedish sheep flocks: Seroprevalence and incidence during one gestation period. Acta vet. scand. 1992, 33, 175-184. – Serum samples from 704 animals from 54 Swedish sheep flocks were analysed by ELISA twice during 1 breeding season for antibodies to *Toxoplasma gondii* and border disease virus (BDV). An ELISA, originally developed for the detection of antibodies to bovine viral diarrhoea virus (BVDV) in cattle, was assessed on sheep sera and the results were compared with those obtained in a virus neutralization test. The correlation between the 2 assays proved good. Before breeding, 132 (19%) sheep in 42 flocks had antibodies to *T. gondii* and 7 (1%) sheep in 5 flocks were seropositive to BDV. During the observation period 4 sheep seroconverted to *T. gondii* and 13 to BDV, giving an incidence rate of 0.7% and 1.9% respectively. No clinical signs due to the infections were observed. In 5 flocks the frequency of barrenness, abortion or stillbirths exceeded 5%, 5% and 8%, respectively, but there was no evidence that this was attributable to the agents studied. The proportion of BDV-positive flocks was significantly higher among flocks that had been in contact with cattle than among those that had not.

***Toxoplasma gondii*; BDV; BVDV; ELISA; epidemiology; reproductive failure.**

Introduction

The protozoan parasite *Toxoplasma gondii* and the pestivirus border disease virus (BDV) can both cause reproductive disorders in sheep. Following infection of non-immune pregnant ewes, the microorganisms cross the placenta to the foetus, and can cause foetal death resulting in barrenness, abortion or stillbirth (Dubey & Towle 1986, Terpstra 1985).

In its lifecycle *T. gondii* exploits cats as its final host and many warmblooded animals as intermediate hosts. Strict herbivores such as sheep are infected by ingesting the oocysts excreted in cat faeces. Oocysts contaminating

feed, water and pastures can remain infective for several months and thus serve as a source of infection for prolonged periods (Dubey & Towle 1986).

Border disease virus is closely related to bovine virus diarrhoea virus (BVDV) (Done et al. 1980, Wensvoort et al. 1989) and ovine or bovine foetuses exposed to BDV or BVDV in early pregnancy may become immunotolerant to the virus, with the infection continuing postnatally with persistence (Terpstra 1985, Coria & McClurkin 1978). The transmission route of BDV is mainly by contact with sheep that are persistent excretors of the virus (Nettleton 1988), but it has

also been implied that pestivirus of sheep and cattle will infect the alternate species (Radostits & Littlejohns 1988). In fact, recent observations demonstrate that contact between pregnant ewes and persistently BVDV-infected cattle can result in border disease (BD) outbreaks (Carlsson 1991).

Both *T. gondii* and BDV have a worldwide distribution, and toxoplasmosis is in many countries regarded as one of the most important infectious causes of ovine abortion (Dubey & Towle 1986). In Sweden, *T. gondii* and BDV are the most frequently diagnosed causes of infectious ovine abortion, while other agents play a minor role (M. Elvander, personal communication). In previous Swedish investigations, antibodies to *T. gondii* have been found in 60-70% of the sheep examined (Uggla & Hjort 1984), while no serological survey regarding BDV has been conducted hitherto.

The aim of the present study was to serologically investigate the prevalence of *T. gondii* and BDV infections and the incidence of the 2 infections during 1 gestation period in healthy Swedish sheep. The intention was also to examine whether sheep farms that also keep cattle are more prone to BDV infections than are sheep flocks free from contact with cattle. The studies were performed by using enzyme-linked immunosorbent assays (ELISAs), and since this method has not previously been described for the diagnosis of BD, an ELISA, originally devised for the quantitation of bovine antibodies to BVDV, was assessed on sheep sera.

Material and methods

Animals

Altogether 704 sheep from 54 flocks in different parts of Sweden (Fig. 1) were examined serologically during the breeding season

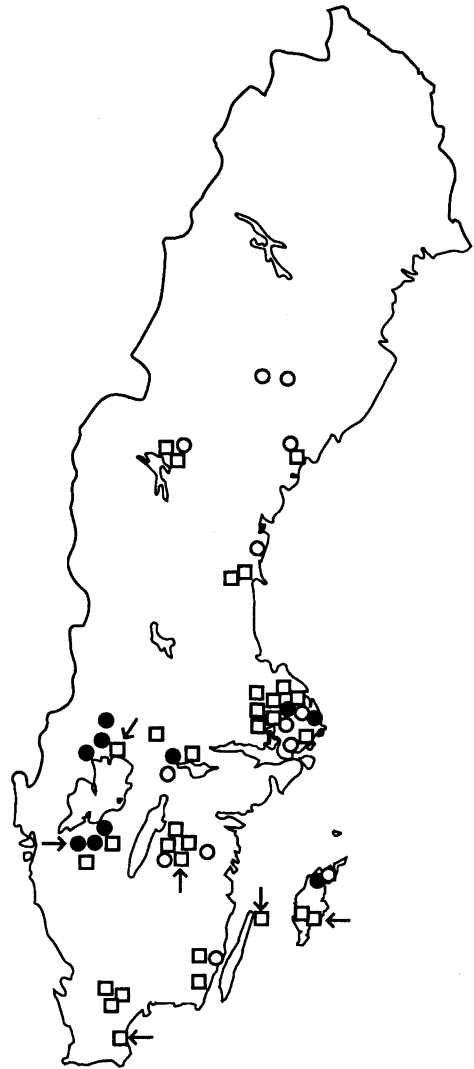


Figure 1. Map of Sweden showing localization of 54 sheep flocks examined serologically for infection with *Toxoplasma gondii* and border disease virus (BDV).

- : Flocks with >40% of sheep tested seropositive to *T. gondii*
- : Flocks with 1-40% of sheep tested seropositive to *T. gondii*;
- : Flocks with all sheep tested seronegative to *T. gondii*;
- : Flocks with some sheep seropositive to BVD.

of 1986-87. The flocks studied and animals sampled were selected from healthy sheep flocks by local veterinarians. Flock size ranged from 14 to 533 ewes, with a median of 54 ewes. In each flock, blood samples were collected from 7 to 30 sheep. The sheep sampled were 628 ewes and 76 rams; 413 (59%) were lambs born in the spring of 1986 (374 ewe lambs and 40 ram lambs), and 291 (41%) were older than 1 year. Most of the sheep were of the Swedish Landrace.

Of the 767 sheep initially sampled, 63 were not sampled a second time. Two of these were ewes found barren, 1 had aborted and 2 given birth to dead lambs and had therefore been culled before the second sampling occasion. Fortysix sheep had died, were sold, missing or culled for reasons other than barrenness, abortion or stillbirth. The cause of the remaining 12 withdrawals was not known.

Information on the frequency of barrenness, abortions and stillbirths in 51 of the flocks studied was obtained from the Swedish Sheep Recording Scheme (Anon. 1987), or was supplemented directly by the flock owners. No data were available from 3 flocks.

The flock owners were asked to complete a questionnaire as to whether cattle were kept on the farm, and if so, whether sheep and cattle were housed together and/or whether the 2 species grazed together.

Blood sampling

From the sheep in question, blood samples were collected twice, first at tugging in the autumn of 1986, and then 5 months later at lambing in the spring of 1987.

The samples were collected in Vacutainer tubes and transported to the laboratory at ambient temperature. The samples were centrifuged and sera stored at -20°C until analysed.

Serological examinations

All sera were analysed for antibodies to *T. gondii* as well as to BDV by ELISA as described below. The BDV-ELISA used had originally been developed for detection of antibodies to BVDV in serum and milk from cattle (SVANOVA Biotech AB, Uppsala, Sweden). To evaluate its suitability for detection of ovine antibodies to BDV, 137 sheep sera were analysed both by the ELISA and by a virus microneutralization test (VNT). After comparing the results, the ELISA was used throughout the study to detect antibodies to BDV.

For the detection of antibodies to *T. gondii*, the indirect fluorescent antibody test (IFAT) (Uggla & Hjort 1984) was used as a complement to the ELISA, as described below.

Toxoplasma serology

The Toxoplasma ELISA used was performed principally according to Voller *et al.* (1976), and has been described in detail by Uggla *et al.* (1990). As antigen, a soluble preparation of freeze-thawed and sonicated, purified *T. gondii* tachyzoites (National Bacteriological Laboratory, Stockholm, Sweden) was used. A horseradish peroxidase (HRP) conjugated rabbit anti-sheep immunoglobulin preparation (Dakopatts, Copenhagen, Denmark) was used in a dilution of 1:1000 in phosphate-buffered saline, pH 7.2, with 0.2% Tween-20 (PBS-T) and 2.5% horse serum. As substrate, 5-amino salicylic acid (5-AS, Merck, Darmstadt, Germany) or tetramethyl benzidine (TMB, Merck) was used. Sera with an absorbance value (A_{450}) exceeding 0.15 were regarded as positive. ($0.15 = \text{mean } A_{450} + 3 \text{ S.D. of } 46 \text{ sheep sera negative to } T. gondii$ according to IFAT.)

All sera were analysed in duplicate at 1:400 dilution, and those with an A_{450} close to the cut-off level were also tested by IFAT at 1:20

dilution, which allowed a final judgement to be made. Sera from animals that seroconverted during the observation period were analysed by IFAT at 2-fold serial dilutions, starting at 1:20 to their endpoint titres.

BDV serology

Virus neutralization test. Sera were inactivated at 56°C for 30 min before being tested in 2-fold dilutions (1:4-1:1024) for neutralizing antibodies to the cytopathic Oregon C24V strain, a strain which has been proved reliable for use in routine diagnostic tests for BD serodiagnosis (Brockman et al. 1988).

Each dilution was mixed with an equal volume of the virus suspension containing 100 tissue culture infective doses₅₀ (TCID₅₀) per 0.1 ml. Fifty µl of each serum-virus mixture was added into 3 wells (50 µl/well) in Nunclon microwell plates (Nunc Intermed, Roskilde, Denmark) and was incubated for 60 min at 37°C. A cell suspension (5-10x10⁵ cells/ml) of primary bovine turbinate cells in Eagle's Minimal Essential Medium (MEM) supplemented with 5% foetal calf serum (FCS) was thereafter added in volumes of 50 µl in each well. The cultures were checked for cytopathic changes and final readings

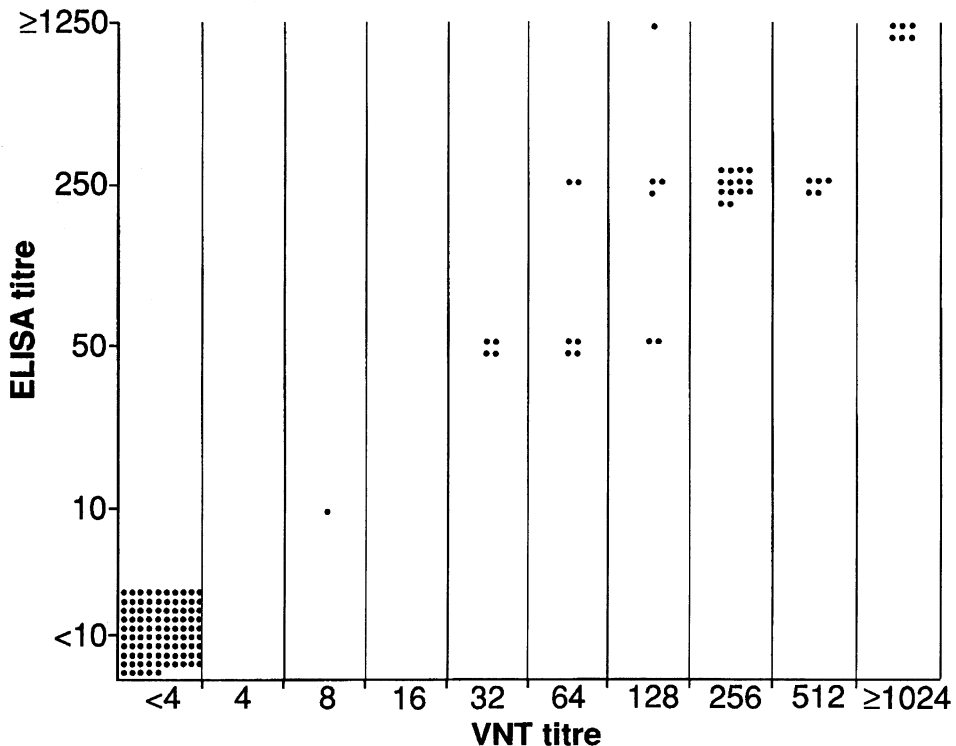


Figure 2. Comparison of antibody titres to border disease virus, detected by ELISA and VNT, in 137 sheep sera.

were made 3 to 4 days later when complete cytopathic effect (CPE) was seen in the control wells. The titres are expressed as the reciprocal of the highest serum dilution that prevented CPE in at least 2 of the 3 replicated cultures.

ELISA. An ELISA (SVANOVA Biotech AB, Uppsala, Sweden) applying a monoclonal antibody (MAb) to bovine immunoglobulin (Ig)G which crossreacts with ovine IgG, was used for detection and titration of antibodies to BDV. The ELISA test was performed according to the kit-procedure. The BVDV-strain Ug-59 (*Borgen* 1963) was used as antigen in the ELISA. Five-fold dilutions of serum samples starting from 1:10 were tested in volumes of 100 µl per well and incubated for 1 h at 37°C. After 3 washings with PBS-T, 100 µl of the lyophilized HRP-conjugated MAb (reconstituted in PBS-T) was added per well and incubated for 1 h at 37°C. The plates were then washed as described above. The substrate to the enzyme was added in a volume of 200 µl/well. After 10 min, 50 µl 2M H₂SO₄ was added to stop the reaction. The absorbance value at 450 nm was measured with a Titertec microplate reader (Flow Lab., Irvine, Scotland). Serum titres were determined as the highest serum dilution giving an absorbance value above 0.20 (0.20 = mean A₄₅₀ +2 S.D. of 87 sheep sera negative for virus-neutralizing antibodies to BDV).

Results

Comparison between VNT and ELISA for detection of antibodies to BDV

Of 137 sheep sera analysed by both VNT and ELISA, all sera with a VNT titre <4 (n =95) proved negative in the ELISA (titre <1:10). All 42 sera with a VNT titre of ≥4 proved positive in the ELISA (titres ranging

Table 1. Prevalence of antibodies to *T. gondii* in sheep of different ages.

Age (Years)	Sheep tested <i>n</i>	Seropositive animals	
		<i>n</i> _{pos}	% of <i>n</i>
1	413	31	8
2	97	30	31
3	60	19	32
4	51	12	24
≥5	83	40	48

Total	704	132	19

between 1:10 and 1:1250) (Fig. 2).

Serological findings and reproductive performance in flocks studied

Toxoplasma. On the first sampling occasion, 132 (19%) of the 704 sheep tested had antibodies to *T. gondii*. In 12 (28%) of the 54 flocks, all tested sheep were seronegative, and in 42 flocks, 4-78% of the animals tested were seropositive. The geographic distribution of seropositive and seronegative flocks is shown in Fig. 1. The proportion of seropositive animals increased with age from 8% (31 of 413) among animals younger than 1 year to 48% (40 of 83) in animals older than 4 years (Table 1).

During the observation period, seroconversion was observed in 2 rams and 2 ewes from 4 different farms, giving an incidence rate of 0.7%. Their endpoint titres in IFAT ranged between 1:320 and 1:1280. Clinical symptoms due to the infection were not observed in any of these 4 animals.

Border disease. From the 704 samples taken prior to the breeding period, 7 (1%) BD-antibody positive ewes (titre 1:50) were found in 5 different flocks. With 1 exception, these ewes were older than 1 year. Their titres remained constant between the 2 sampling occasions, except in 2 ewes where the

titre decreased from 1:50 to 1:10. Thirteen seronegative ewes in different age groups from 3 different flocks (7, 5 and 1 sheep from each flock), seroconverted during the observation period. Their titres ranged between 1:50 and 1:250; clinical symptoms due to the infection had not been observed.

No flock was found having a prevalence above 13%. In all, the antibody prevalence rose from 1% pre-mating to 2.9% at lambing, with an incidence rate of 1.9% during the gestation period.

At 22 of the 54 farms studied, both cattle and sheep were kept, either housed together or in separate buildings, and/or grazing on common pasture. In 5 out of 6 flocks where seropositive sheep were found, the sheep had been in contact with cattle. Furthermore, in all 3 flocks where seroconversion was demonstrated, cattle were bred, and in 1 of these flocks a heifer persistently infected with BVDV was found (unpublished data).

The proportion of BDV-positive flocks was found to be significantly higher, $p = 0.035$ (Fisher's test), among flocks with cattle contact, than among cattle-free flocks.

Reproductive performance. The total frequencies of barrenness, abortion and stillbirth in the 54 flocks studied were 2.8%, 0.02% and 2.6% respectively, whereas the mean flock frequencies ($\% \pm SD$) were 2.3 ± 6.2 , 0.2 ± 1.3 and 2.4 ± 2.1 , respectively.

Among the 7 flocks where seroconversion to either *T. gondii* or BDV was observed, the reported incidence of stillbirth did not exceed 2%, and in only 2 flocks did the reported incidence of abortion or barrenness exceed 3%. In 1 of these 2 flocks where 1 ram seroconverted to *T. gondii*, 1 barren ewe was reported, resulting in a rate of 6% barrenness. In the other flock, where 1 older ewe also seroconverted to *T. gondii*, the

majority of the ewe lambs were barren, giving a rate of 42%. In this flock, 20% of the ewe lambs were examined, but no seroconversion to either *T. gondii* or BDV was detected among them.

Five flocks were subject to gestation failures such as barrenness or abortions with frequencies exceeding 5%, or more than 8% of lambs being stillborn. Except for the flock with 42% barrenness mentioned above, no seroconversion against *T. gondii* or BDV was observed, and no animals seropositive to BDV were found in these 5 flocks. Finally, in the 3 flocks for which no information was available, seroconversion to *T. gondii* or BDV was not observed.

Discussion

For serological surveys, the ELISA technique is a most valuable tool, facilitating the analysis of large quantities of samples. The method has been widely used to detect antibodies to *T. gondii* in many species, including sheep (Ugglå & Buxton 1990). Since the IFAT is generally considered to give results in good agreement with the Sabin Feldman dye test (Munday & Corbould 1971), which is regarded as a reference test (Ugglå & Buxton 1990), the IFAT was used as a complement to the ELISA in this study.

For the detection of antibodies to BDV, an already established BVDV-ELISA was evaluated on sheep sera. The excellent agreement between the results obtained with the ELISA and with the micro VNT demonstrates that this ELISA can be used on ovine sera when a conjugate is used that reacts with ovine IgG1.

The prevalence of *Toxoplasma* infection in sheep has been studied in numerous serological surveys conducted in different parts of the world. Results vary considerably, from 0 to 96% seropositive, with a median preva-

lence of 30% (Blewett 1983, Fayer 1981). In the present study, antibodies to *T. gondii* were detected in 19% of the sheep tested, while in an earlier Swedish survey a prevalence of 60-70% was found (Uggla and Hjort 1984). Then, as well as in the present investigation, a large proportion of the animals examined were lambs 6-7 months old. The increasing seroprevalence with age is therefore unlikely to have influenced the results. However, since the selection of flocks and sheep differ between the 2 investigations, no further conclusions about the disparity could be drawn.

The prevalence of antibodies to *T. gondii*, increases with age of the animals and can be used to calculate the rate of infection (Blewett 1983). When applied to the results presented here, the method gives an annual seroconversion rate of approximately 10%, which contrasts with the very low incidence of seroconversion (0.6%) observed during the gestation period studied. One possible interpretation is that in the flocks studied, sheep acquired *Toxoplasma* infection predominantly during summer while grazing. In Norway, however, Waldeland (1977a) observed the contrary, namely a higher incidence of infection during winter. This was assumed to be due to a heavier contamination of the fields close to the farms, where the sheep were kept in winter, than on the more distant pastures grazed during summer. In Sweden, sheep are usually kept indoors during the winter, but since both grain (Plant *et al.* 1974) and silage (Waldeland 1977b) have been reported as sources of *Toxoplasma* infection, outbreaks are also likely to occur among housed sheep.

Serological surveys regarding BD conducted throughout the world have shown prevalence rates ranging between 5 and 40% (Barlow & Patterson 1982, Everman *et al.* 1981), but in

individual flocks the prevalence may be close to 100% (Sawyer *et al.* 1986). The results of the present investigation suggest that the flocks studied were mainly BDV-free and the prevalence, 1-3%, is remarkable low compared to recent observations regarding BVDV in Swedish heifers with a prevalence of 41% (Alenius *et al.* 1986). However, in other countries, marked differences in prevalence between various geographical regions have been reported (Everman *et al.* 1981, Alvarez *et al.* 1989), which is why, especially in certain regions and also in certain flocks, BD may be more common than indicated by the present study.

The major mode of transmission of BDV is reported to be contact between sheep (Nettleton 1988). Live vaccines have also been reported to be a transmission route for both BVDV and BDV, and recently BDV in goats was shown to emanate from a contaminated orf-vaccine (Løken *et al.* 1991). In the present study, BDV was found to be significantly more common on farms with both sheep and cattle production, than on those raising sheep only. This observation, together with the relatively high prevalence of BVDV-infected cattle in Sweden (Alenius *et al.* 1986), and the low prevalence among sheep, strengthens the assumption that sheep can become infected with BVDV by cattle and that cattle are an important source of pestivirus infection for sheep. However, once persistently infected lambs are born, they serve as an additional source of infection for other sheep and for other ruminants as well (Løken 1991, Carlsson, in manuscript).

A common feature of the 2 pathogens studied is that the severity and type of clinical manifestation are dependent on the gestational stage at which infection occurs. The 15 ewes seroconverting to either *Toxoplasma* or BDV in this study, probably contracted the

infection at a stage of gestation when their foetuses were no longer sensitive to damage by the microorganisms. Hence no gestation failure was observed.

The overall incidence of ovine gestation failure in Sweden is low. In 1987 the reported total frequencies of barrenness, abortion and stillbirth were 1.4%, 0.06% and 3.1%, respectively (Anon. 1987). For the flocks participating in the present survey, the corresponding frequencies were 2.8%, 0.02% and 2.6%. When one considers that the relatively high rate of barrenness found in this study was due to the 42% barrenness rate observed in one single flock, the flocks studied can be regarded as representative of the Swedish sheep population as a whole, as regards reproductive disorders. In the five flocks where gestation failure occurred, factors other than *Toxoplasma* or BDV were the most likely cause. However, due to the sporadic nature of gestation failure in general, the results from this study do not allow of any definite conclusions about the relative importance of the two pathogens studied.

To conclude, the observed prevalence and incidence of *Toxoplasma* and BDV infections were relatively low. Even though the flocks examined were not randomly selected, the geographic distribution, size, breed and health status of the flocks were relatively representative of the sheep population in Sweden (Anon. 1987). Thus, the results obtained indicate that a large proportion of the Swedish sheep population is susceptible to both infections. Sheep seropositive to *Toxoplasma* were found in many flocks, suggesting that the parasite is widely spread throughout the country. The results obtained also indicate that cattle, which are commonly infected with BVDV, are an important source of BDV infection in sheep. Therefore, in situations of reproductive disorders in

sheep, increased clinical and diagnostic attention to *Toxoplasma* and BDV is suggested. Due to the risk of transmission of BVDV from cattle to sheep it must be recommended that the 2 species should not be kept together, and especially not during the first half of gestation. The study also showed that the ELISA tested is suitable for the serological diagnosis of BD in sheep.

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Sammanfattning

Toxoplasmos och border disease hos får i Sverige: Seroprevalens och incidens under en dräktighetsperiod.

Serumprover från 704 får i 54 svenska fårbesättningar analyserades vid 2 tillfällen, vid betäckning och vid lamning, avseende förekomsten av antikroppar mot *Toxoplasma gondii* och border disease virus (BDV). För detta ändamål utvärderades på fårsera en ELISA, som tidigare utvecklats för påvisande av antikroppar mot bovin virus diarrhoe virus hos nöt. Mycket god överensstämmelse mellan resultaten från denna metod och virusneutralisationstest förelåg. Vid det första provtagningstillfället visade sig 132 (19%) får i 42 besättningar ha antikroppar mot *T. gondii* och 7 (1%) får i 5 besättningar mot BDV. Mellan de 2 provtagningarna serokonverterade 4 djur mot *T. gondii* och 13 mot BDV, motsvarande 0,7% incidens för *Toxoplasma*

infektion respektive 1,9% för BDV-infektion. Några kliniska symtom till följd av infektionerna iaktogs ej och de reproduktionsstörningar som noterades i 5 besättningar berodde med största sannolikhet på andra orsaker än de 2 studerade infek-

tionsämnena. Andelen besättningar med BDV-antikroppspositiva får var signifikant högre bland besättningar där fåren haft kontakt med nötkreatur än bland sådana där kontakt mellan de bägge djurslagen ej förekom.

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