Acta vet. scand. 1977, *18*, 227—236.

From the State Veterinary Research Station for Small Ruminants, Høyland, Sandnes, Norway.

TOXOPLASMOSIS IN SHEEP

LONG-TERM EPIDEMIOLOGICAL STUDIES IN FOUR BREEDING FLOCKS*

By
Harald Waldeland

WALDELAND, H.: *Toxoplasmosis in sheep. Long-term epidemiological studies in four breeding flocks.* Acta vet. scand. 1977, *18*, 227—236. — The epidemiology of toxoplasmosis in sheep was studied during a period of 3½ to 6 years in 4 flocks in which abortions from the infection previously had occurred.

The epidemiological pattern indicated that a heavy contamination of the environment may persist for about 2 years. Even in flocks with an apparently normal reproductive performance, toxoplasmosis may cause barrenness, abortion or delivery of dead lambs in 1—2 % of the breeding ewes.

The prevalence of infected sheep increased with the age. The incidence of infection was significantly lower in 6—12 months old lambs than in mature ewes.

The incidence of infection was higher on lowland pastures than on mountain pastures in not populated areas. On lowland pastures the incidence of infection was lower during the summer than during the winter.

toxoplasma infection; epidemiology; sheep.

In an investigation on the prevalence of toxoplasma antibodies in sheep from 4 different parts of Norway, dye test (DT) positive individuals (titres $\geq 1/16$, the *Sabin & Feldman* (1948) dye test) were found in 79 to 90 % of the breeding flocks (*Waldeland* 1976 b). The mean frequency of DT positive mature ewes in the 4 regions ranged from 42 to 50 %. The question then arose whether the high frequency of infected breeding flocks and the relatively low frequency of DT positive mature sheep were due to cyclical endemics or to a continual and low incidence of infection.

Later investigations (*Waldeland* 1977) indicated that the source of infection may be connected to a particular part of the

* This work was supported by grants from the Norwegian Research Council for Science and for the Humanities.

farm or part of the pastures. A relevant question is therefore to what extent the grazing pattern may influence the incidence of infection.

To elucidate these matters, the serological observations made during a period of 3½ to 6 years were studied in 4 flocks in which abortions from toxoplasmosis had previously occurred.

MATERIALS AND METHODS

The 4 flocks comprised a total of about 400 head during the winter. The number of sheep and the period of observation in each flock are given in Table 1.

Table 1. Observation period and number of breeding sheep in 4 flocks. The replacements constituted from 1 fourth to 1 third of the total number in the flocks nos. 1—3, and about 1 seventh of the total number in flock no. 4.

Flock no.	Observation period, years	Number of breeding sheep
1*	6	185—200
2	3½	56—77
3	3½	50—68
4	3½	70—77

* Flock no. 1 belonged to the State Veterinary Research Station for Small Ruminants.

The management was the same as in most flocks near this laboratory (*Waldeland* 1977), except that flock no. 3 was kept on farm leys and cultivated lowland pastures throughout the year.

Blood samples were collected in the spring and in the autumn, giving a total of 4606 samples. In the spring the samples were collected shortly before lambing. In the autumn the sheep in flock no. 1 were bled shortly after they returned from mountain pastures in September, and in the other flocks shortly before the mating season. Due to mustering errors, some ewes were not bled at each sampling of the flocks.

The individual sheep in the flocks nos. 1, 2 and 3 were identified by ear-tags, and the reproductive performance of each ewe was recorded. In flock no. 4 the identification of the individual sheep was unreliable, and no written records were kept.

Laboratory examinations

The sera were stored at —20°C until examined for toxoplasma antibodies as previously described (*Waldeland* 1976 a). Samples from sheep that were DT positive at their first bleeding and from DT positive sheep that previously had been negative were tested twice.

RESULTS

The prevalence of DT positive sheep ≥ 1 year old in the 4 flocks was within the range of 22 to 97 % during the observation period (Fig. 1). The highest prevalence was recorded in flock no. 4 about 1 year after the abortions. The incidence of

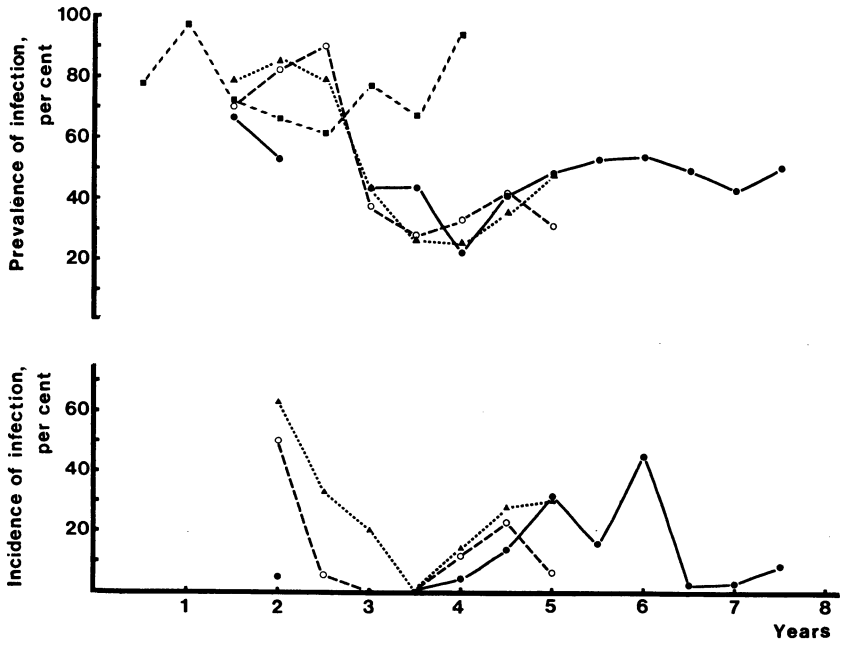

Figure 1. Prevalence of sheep ≥ 1 year old with dye test titres ≥ 1/16 in the flocks nos. 1—4 during the observation period (upper half of the figure), and incidence of infection (change from dye test negative (titres < 1/16) to dye test positive (titres ≥ 1/16) reaction) in the flocks nos. 1—3 (lower half of the figure). The abscissa indicates the time after abortions from toxoplasmosis was first diagnosed. Flock no. 1 was not examined in the autumn 2½ years after the abortions.

●———● Flock no. 1. ▲·····▲ Flock no. 3.
o– – –o Flock no. 2. ■– – – –■ Flock no. 4.

infection between each sampling of the flocks nos. 1—3 ranged between 0 and 64 % as recorded in the same figure. The highest incidence was found in flock no. 3 about 2 years after the abortions.

Both in flock no. 2 and in flock no. 3 there was a marked decline in the prevalence of DT positive sheep and in the incidence of infection between 2 and 3 years after the toxoplasma abortions. In flock no. 1 there was an increase in the incidence of infection from the 4th year after the abortions and 2 years on.

The incidence of overt toxoplasmosis. In the flocks nos. 1, 2 3, a total of 59 of 773 ewes > 1 year old were barren, aborted or delivered dead lambs during the observation period. There was no notable difference in the fertility rate between the flocks. As shown in Table 2, about 25 % of 65 ewes that developed positive

Table 2. Reproductive performance of ewes > 1 year old that converted from dye test negative to dye test positive during the pregnancy, compared with the performance of ewes that were either negative or positive throughout the pregnancy.

Dye test titres during the pregnancy	Number of mated ewes	Number of ewes that were barren, aborted or delivered dead lambs
Positive, ≥ 1/16	453	25 (6)
Negative, < 1/16	255	18 (7)
Conversion from negative to positive	65	16 (25)
Total number	773	59 (8)

Figures in brackets: Percentage.

DT titres during the pregnancy did not give birth to live lambs, compared with about 6 % of 708 ewes that were either DT positive or negative throughout the gestation ($P < 0.001$, chi-square test).

The prevalence of infection in relation to age. The prevalence of DT positive sheep in the flocks nos. 1, 2 and 3 increased with the age. In each of the 3 flocks, the total frequency of DT positive sheep ≥ 1 year old was nearly the same as among sheep aged 3—3½ years. The prevalence of positive sheep in relation

to age in the flock with the highest number of breeding sheep and the longest observation period (flock no. 1) is shown in Fig. 2.

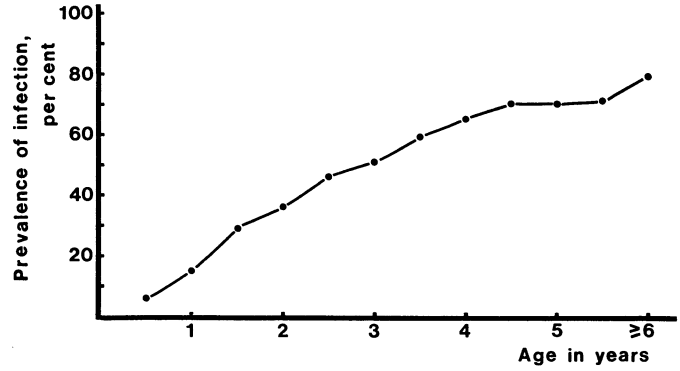

Figure 2. Prevalence of infection in relation to age in sheep in flock no. 1.

The incidence of infection in lambs compared with adult sheep. A total of 320 adult sheep and 240 lambs which were DT negative in the autumn in the flocks nos. 1, 2 and 3 were examined the following spring. As shown in Table 3, a total of 65 ewes (20 %) developed positive DT titres during the winter, compared with 29 (12 %) of the lambs ($P < 0.025$). In each

Table 3. Incidence of infection in ewes from 3 flocks during the winter, compared with the incidence in lambs. The examination comprised 5 pregnancy seasons in flock no. 1, and 4 seasons in the other 2 flocks.

Flock no.	Ewes > 1 year old		Lambs	
	DT- in the autumn	conversion from DT- to DT+ during the winter	DT- in the autumn	conversion from DT- to DT+ during the winter
1	221	43 (19)	155	19 (12)
2	70	10 (14)	52	4 (8)
3	29	12 (41)	33	6 (19)
Total number	320	65 (20)	240	29 (12)

Figures in brackets: Percentage.
DT- : Dye test negative.
DT+: Dye test positive.

of the flocks the incidence of infection was about twice as high among adult sheep as among lambs.

The incidence of infection on mountain pasture compared with the incidence on lowland pasture. About half of the sheep in flock no. 1 were kept on mountain pastures at an altitude of about 950 m in a not populated area during the summer, whereas the remainder stayed on permanent, fertilized grassland in the coastal area at an altitude of less than 100 m. The incidence of infection was about twice as high among ewes kept on lowland grazings compared with ewes kept on mountain pastures (Table 4), but the difference was not statistically significant. The prevalence of DT positive lambs on lowland grazings was significantly higher than on mountain grazings ($P < 0.05$).

Table 4. Infection in ewes and lambs kept on lowland pastures during the summer, compared with infection in ewes and lambs kept on mountain pastures. All the ewes were dye test negative in the spring when examined shortly before lambing. The lambs were examined only in the autumn.

	Number of ewes		Number of lambs	
Pasture	total	conversion from DT- to DT+ during the summer	total	DT+ in the autumn
Lowland	148	17 (11)	450	34 (8)
Mountain	165	10 (6)	349	13 (4)

Figures in brackets: Percentage.

The incidence of infection during the winter compared with the incidence during the summer. As recorded in Table 5, a total of 17 (11 %) of 148 ewes in flock no. 1 developed positive DT

Table 5. Incidence of infection in ewes $\geq$ 1 year old during the winter compared with the incidence during the summer on lowland pastures (flock no. 1).

	Number of ewes	
Period of the year	total	conversion from DT- to DT+
Summer	148	17 (11)
Winter	221	43 (19)

Figures in brackets: Percentage.

titres on lowland grazings during the summer, compared with 43 (19 %) of 221 ewes during the winter. In flock no. 3 where the sheep were kept on lowland pastures throughout the year, 12 (41 %) of 29 ewes became DT positive during the winter compared with 6 (21 %) of 28 ewes during the summer. The differences were not statistically significant.

DISCUSSION

The epidemiological pattern in the 4 flocks varied. A marked increase in the incidence of infection occurred in flock no. 1 from the 4th year after the abortions and 2 years on. In the flocks nos. 2 and 3 there was a decline in the incidence of infection and in the prevalence of toxoplasma antibodies during the 3rd year after the abortions, and in flock no. 4 there was some decline in the prevalence of toxoplasma antibodies during the 2nd year after the abortions and an increase during the 3rd and the 4th year. These findings are difficult to interpret, but one possible explanation of the observations in the flocks nos. 1, 2 and 3 is that a heavy contamination of the environment may persist for about 2 years. This is supported by earlier observations (*Frenkel* 1974) which showed that toxoplasma oocysts may survive in soil for at least 12 months. No analogous evidence was found in flock no. 4, but it is difficult to compare the observations from this flock with the results from the others because of the exceptionally low proportion of replacements.

As recorded in Table 2, about 6 and 7 % respectively of the ewes that were either DT positive or negative throughout the pregnancy were either barren, aborted or delivered dead lambs, whereas the frequency among 65 ewes that apparently acquired the infection during the pregnancy was about 18 % higher. This indicates that a total of about 12 of the assumed pregnancies were terminated by embryonic death, abortion or stillbirth due to toxoplasma infection. In addition, in flock no. 1 the parasite was isolated from the aborted foetuses of 2 ewes that were DT positive throughout the pregnancy. One of these had also aborted from toxoplasmosis during the previous pregnancy. In 2 other DT positive ewes that aborted in the same flock, there were significant rises in the titres ($\geq 1/512$). The loss from toxoplasmosis may accordingly be assessed to between 1 and 2 % of the 773 pregnancies in the flocks nos. 1, 2 and 3. In flock no. 4 detailed information of the reproductive performance was not ob-

tained, but it is noteworthy that there were about 10 % barren ewes during the last pregnancy in this investigation, i.e. during the same period when there was an increase in the prevalence of DT positive sheep.

The prevalence of DT positive sheep increased with age. This indicates that the flocks had been exposed to the parasite each year throughout the lifetime of the oldest ewes. The mean frequency of DT positive mature sheep in each flock was about the same as among ewes of the mean age, i.e. 3—3½ years. Although the epidemiological pattern may vary, it seems probable that a serological survey of sheep of this age may give a good estimate of the prevalence of infection in the sheep population within an area.

The higher incidence of infection in mature ewes than in lambs during the winter is difficult to explain. The lambs in the flocks nos. 1 and 2 were kept indoors during a longer period than the older sheep to prepare them for breeding. Ewes more than 1 year old may accordingly have been exposed to contaminated pastures during a longer period than the lambs. It should also be noted that the mature ewes in the 3 flocks were fed more silage than the lambs during the winter, and that a previous observation indicated that Toxoplasma gondii may be transmitted via the silage (*Waldeland* 1977).

In flock no. 3 there was a low mating frequency among the lambs, and this may be correlated with a lower incidence of infection as reported from New Zealand (*Anon.* 1965, 1966, 1967). However, this is not quite in agreement with results reported by other authors (*Galuzo* 1966, *Hartley* 1966). The question then arises to what extent the different rates of infection were influenced by physiological and immunological factors and by the management. Further work is required to elucidate this.

The incidence of infection in ewes on mountain pastures was lower than in ewes on lowland grazings, but a statistically significant conclusion could not be made. However, the prevalence of infection in lambs showed a significant difference. Both these types of pastures were in areas with a moist climate. Earlier authors have pointed out that the altitude seems to be of importance for the prevalence of toxoplasma antibodies in man (*Jacobs* 1956, 1963, *Walton et al.* 1966). However, based on our present knowledge, the most reasonable explanation seems to be that the sheep were kept on mountain pastures in a not populated

area free of or with very few cats. Similar differences have been found in sheep on islands with no cats compared with sheep on islands with cats (*Munday* 1972).

The incidence of infection on lowland pastures was nearly twice as high during the winter as during the summer both in flock no. 1 and in flock no. 3, but the number of sheep was too low to justify a statistical conclusion. However, it can be seen from Fig. 1 that there was a tendency of a higher incidence or a sharper increase in the incidence of infection during the winter each year in flock no. 1. In flock no. 3, the decline in the incidence of infection in the first part of the observation period was more marked during the summer than during the winter. This seasonal difference is consistent with observations by *Hartley* (1966) and *Munday* (1970). No similar comparison could be made in flock no. 2.

The higher incidence of infection during the winter could not be explained by the closer contact between infected and susceptible sheep, as only 1 of 28 susceptible ewes acquired the infection in flock no. 1 when housed together with 76 DT positive ewes during the winter the 4th year after the abortions. This is in agreement with a previous investigation (*Waldeland* 1977). However, it is possible that the risk of infection is greater during the winter because the sheep graze the herbage closer to the ground than during the summer. Ingestion of oocysts of T. gondii may accordingly occur more frequently. It may also be of importance that the sheep during the winter are kept near the farm yards where contamination with oocysts from cats may be heavier than on more distant pastures. Further investigations are required to elucidate this.

REFERENCES

Anon.: Annual Reports of Ruakura and Wallaceville Animal Research Stations and Whatawhata Hill Country Station 1963—64. Dept. Agric., Wellington, New Zealand 1965, p. 38.

Anon.: Animal Research in the New Zealand Department of Agriculture. Annual Reports, 1964—65. Wellington, New Zealand 1966, p. 47.

Anon.: Research in the New Zealand Department of Agriculture. Annual Report of Research Division 1965—1966. Wellington, New Zealand 1967, p. 51.

Frenkel, J. K.: Breaking the transmission chain of Toxoplasma: A program for the prevention of human toxoplasmosis. Bull. N. Y. Acad. Med. 1974, *50*, 228—235.

Galuzo, I. G.: Toxoplasmosis of animals. Int. Congr. Parasit. I., Roma 1964. Proc. Milano 1966, p. 204—206.

Hartley, W. J.: Some investigations into the epidemiology of ovine toxoplasmosis. N.Z. vet. J. 1966, *14*, 106—117.

Jacobs, L.: Propagation, morphology, and biology of Toxoplasma. Ann. N.Y. Acad. Sci. 1956, *64*, 154—179.

Jacobs, L.: Toxoplasma and toxoplasmosis. Ann. Rev. Microbiol. 1963, *17*, 429—450.

Munday, B. L.: The epidemiology of toxoplasmosis with particular reference to the Tasmanian environment. Thesis, University of Melbourne, Melbourne 1970.

Munday, B. L.: Serological evidence of Toxoplasma infection in isolated groups of sheep. Res. vet. Sci. 1972, *13*, 100—102.

Sabin, A. B. & H. A. Feldman: Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (Toxoplasma). Science 1948, *108*, 660—663.

Waldeland, H.: Toxoplasmosis in sheep. The reliability of a microtiter system in Sabin and Feldman's dye test. Acta vet. scand. 1976 a, *17*, 426—431.

Waldeland, H.: Toxoplasmosis in sheep. The prevalence of Toxoplasma antibodies in lambs and mature sheep from different parts of Norway. Acta vet. scand. 1976 b, *17*, 432—440.

Waldeland, H.: Toxoplasmosis in sheep. Epidemiological studies in flocks with reproductive loss from toxoplasmosis. Acta vet. scand. 1977, *18*, 91—97.

Walton, B. C., I. de Arjona & B. B. Benchoff: Relationship of Toxoplasma antibodies to altitude. Amer. J. trop. Med. Hyg. 1966, *15*, 492—495.

SAMMENDRAG

Toxoplasmose hos sau. Epidemiologiske langtidsstudier i 4 flokker.

Epidemiologiske undersøkelser ble foretatt i løpet av 3½ til 6 år i 4 saueflokker hvor det tidligere hadde forekommet toxoplasma-abort.

Det epidemiologiske mønster tydet på at en sterk kontaminering av miljøet kan ha en varighet på omkring 2 år. Selv i flokker hvor reproduksjonen tilsynelatende var normal, viste det seg at toxoplasmose kunne resultere i infertilitet, abort, eller dødfødte lam hos 1—2 % av søyene.

Forekomsten av infiserte sauer øket med alderen. Infeksjonshyppigheten var signifikant lavere hos 6—12 måneder gamle lam enn hos voksne sauer.

Infeksjonshyppigheten var høyere på lavlandsbeite enn på fjellbeite. På lavlandsbeite var hyppigheten lavere om sommeren enn om vinteren.

(Received December 30, 1976).

Reprints may be requested from: Harald Waldeland, the State Veterinary Research Station for Small Ruminants, Postboks 248, 4301 Sandnes, Norway.