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

## TOXOPLASMOSIS IN SHEEP

THE PREVALENCE OF TOXOPLASMA ANTIBODIES
IN LAMBS AND MATURE SHEEP FROM DIFFERENT PARTS
OF NORWAY\*

# By Harald Waldeland

WALDELAND, H.: Toxoplasmosis in sheep. The prevalence of Toxoplasma antibodies in lambs and mature sheep from different parts of Norway. Acta vet. scand. 1976, 17, 432—440. — The prevalence of toxoplasma antibodies (Sabin and Feldman's dye test) was examined in 1929 lambs and mature sheep from 4 different parts of the southern Norway. Blood samples were collected from 1377 lambs from 211 flocks at slaughtering during September and October in 1968 and 1969, and from 552 ewes from 65 flocks during the winters of 1970—1971 and 1971—1972. Toxoplasma antibodies were found in 20—39 % of the lambs, and in 42—50 % of the mature ewes. The prevalence of flocks with at least 1 dye test positive (titre  $\geq 1/16$ ) lamb ranged between 48 and 80 %, and of flocks with at least 1 dye test positive breeding ewe between 79 and 90 %. The results from each part of the country are discussed, and conclusions of regional differences in the epidemiology of Toxoplasma gondii are deduced. In spite of variations in the epidemiological pattern, no evidence was found of significant differences in the geographical distribution of the parasite.

toxoplasma infection; epidemiology; sheep.

A study of Toxoplasma gondii as a cause of reproductive loss in sheep during the period 1968—1973 showed that this parasite was the main cause of abortion in the south-western part of Norway (Waldeland 1976 a). The intention of the present work was to compare the prevalence of toxoplasma antibodies in this region with the prevalence in 3 other parts of the country, to elucidate possible geographical differences in the epidemiology and in the importance of the infection in sheep as related to reproductive failure.

<sup>\*</sup> This work supported by grants from The Norwegian Research Council for Science and for the Humanities.

### MATERIALS AND METHODS

#### Lambs

Blood samples of 1377 lambs, 5—7 months old, were collected at random from 211 flocks at slaughtering during September and October 1968 and 1969. The origin and flock distribution are recorded in Table 1.

Table 1. Origin and flock distribution of 1377 lambs 5—7 months old examined for toxoplasma antibodies.

	Number of						
Origin	lambs	flocks	lambs examined within each flock				
Rogaland	473	97	1—5				
Buskerud (Hallingdal)	217	30	1—24				
Hordaland (Voss)	348	26	237				
North and South Trøndelag	339	58	1—29				
Total	1377	211					

# Mature sheep

A total number of 552 samples from mature sheep from 65 flocks with no reproduction problem was examined. The samples were collected from the older breeding ewes during the winters of 1970—1971 and 1971—1972. Details of the material are given in Table 2.

Table 2. Origin and flock distribution of 552 breeding ewes examined for toxoplasma antibodies.

		f	
Origin	ewes	flocks	ewes examined within each flock
Rogaland	196	20	8—10
Buskerud (Hallingdal)	141	16	5—11
Hordaland (Voss)	121	19	410
South Trøndelag (Berkåk)	94	10	7—10
Total	552	65	

The blood samples from Rogaland were collected by members of the staff at this laboratory, and at the other places by the local veterinarians. The samples collected in Rogaland were centrifuged and the sera frozen within 5 hrs. after the bleeding. The majority of the blood samples from the other places were received within 4 days after the bleeding. The sera were stored at —20°C until examined for toxoplasma antibodies by a micromodification of the Sabin & Feldman (1948) dye test (DT) as described by Waldeland (1976 b).

A preliminary investigation was carried out to estimate possible changes in the titres during the transport. The DT titres in blood samples and sera stored for up to 10 days at room temperature (18—20°C) and at 4°C were compared with the titres in sera frozen immediately after sampling and stored at —20°C. Twenty-five sheep were selected to give titres in the range from 1/8 to 1/128, and the sera were examined 1, 4, 7, and 10 days after bleeding.

The daily fall of the titres during the 10-day period was 0.075 twofold dilution in blood and serum stored at room temperature, and 0.04 twofold dilution in blood and serum stored at 4°C, estimated by the method of least squares. The titres of the frozen sera remained very stable.

#### RESULTS

As shown in Table 3 the prevalence of DT positive lambs (titres  $\geq 1/16$ ) were approximately the same in Rogaland, Hordaland and Trøndelag, ranging from 20 to 26 %, while a significantly higher prevalence was found in lambs in Buskerud (P < 0.001, chi-square test).

Table 3.	Sabin and Feldman's dye test titres in 1377 lambs from
	4 different parts of the southern Norway.

Origin	Total number	U	Negative $< 1/16$		Positive $\geq 1/16$		Dye test titres					
	of sera						1/16		1/64		<b>≥</b> 1/256	
Rogaland	473	357	(75).	116	(25)	98	(21)	1/3	(3)	5	(1)	
Buskerud (Hallingdal)	217	132	(61)	85	(39)	64	(29)	19	(9)	2	(1)	
Hordaland (Voss) North and South	348	277	(80)	71	(20)	54	(15)	14	(4)	3	(1)	
Trøndelag	339	<b>251</b>	(74)	88	(26)	58	(17)	28	(8)	2	(1)	
Total	1377	1017	(74)	360	(26)	274	(20)	74	(5)	12	(1)	

Figures in brackets: percentage.

The frequencies of infected flocks, i.e. flocks with at least 1 DT positive lamb, ranged from 48 to 80 % as recorded in Table 4. The frequencies of infected flocks in the 4 counties differed significantly (P < 0.025), i.e. the frequency was highest in Buskerud and lowest in Rogaland.

Table 4. Toxoplasma antibodies (Sabin and Feldman's dye test) in 211 flocks of lambs from 4 different parts of the southern Norway, based on the examination of 1377 blood samples.

	Number of flocks								
Origin	al	l sampl	es	one or more samples					
	total	titre	< 1/16	titre	≥ 1/16	titre	<b>&gt; 1/64</b>		
Rogaland	97	50	(52)	47	(48)	15	(15)		
Buskerud (Hallingdal)	30	6	(20)	24	(80)	13	(43)		
Hordaland (Voss) North and South	26	8	(31)	18	(69)	9	(35)		
Trøndelag	58	22	(38)	36	<b>(62)</b>	18	(31)		
Total	211	86	(41)	125	(59)	55	(26)		

Figures in brackets: percentage.

To eliminate possible influences due to differences in the number of samples collected from the flocks, the prevalence of infection was also calculated from the first 5 samples examined from each flock. The results did not differ notably from the results recorded in Tables 3 and 4.

Table 5. Sabin & Feldman's dye test titres in 552 mature sheep from 4 different parts of the southern Norway.

Origin	Total	Negative < 1/16		Positive $\geq 1/16$		Dye test titres					
	number of sera					1/16		1/64		≥ 1/256	
Rogaland	196	102	(52)	94	(48)	49	(25)	41	(21)	4	(2)
Buskerud (Hallingdal)	141	82	(58)	59	(42)	42	(30)	16	(11)	1	(1)
Hordaland (Voss)	121	60	(50)	61	(50)	33	(27)	26	(21)	2	<b>(2)</b>
South Trøndelag (Berkåk)	94	<b>52</b>	(55)	42	(45)	30	(32)	12	(13)	0	
Total	552	296	(54)	256	(46)	154	(28)	95	(17)	7	(1)

Figures in brackets: percentage.

The frequencies of DT positive mature sheep ranged from 42 to 50 % as recorded in Table 5. The frequencies of sheep with titres  $\geq 1/64$  were 10-11 % higher in Rogaland and Hordaland than in Buskerud and Trøndelag, but no statistically significant differences were found between the 4 counties.

The frequencies of infected flocks of breeding ewes, i.e. flocks with at least 1 DT positive ewe, ranged from 79 to 90 % as can be seen from Table 6. The frequency of flocks in which at least

Table 6. Toxoplasma antibodies (Sabin and Feldman's dye test) in 65 flocks of breeding ewes from 4 different parts of the southern Norway, based on the examination of 552 blood samples.

Origin	Number of flocks								
	al	l sampl	les	one or more samples					
	total	titr	e < 1/16	titre	<b>≥ 1/16</b>	titre	≥1/64		
Rogaland (Hallingdal)	20	2	(10)	18	(90)	16	(80)		
Buskerud	16	<b>2</b>	(13)	14	(87)	7	(44)		
Hordaland (Voss)	19	4	(21)	15	(79)	12	(63)		
South Trøndelag (Berkåk)	10	2	(20)	8	(80)	4	(40)		
Total	65	1/0	(15)	55	(85)	39	(60)		

Figures in brackets: percentage.

1 ewe had DT titre  $\geq$  1/64 was markedly higher in Rogaland than in the other counties, but the differences were not statistically significant.

The prevalence of infected flocks of lambs compared with the prevalence of infected flocks of ewes were significantly different in Rogaland (P < 0.005), but not in the other counties.

#### DISCUSSION

The storage experiment indicated that some fall in the titres might have taken place during the transport, but this fall was probably of little importance as most of the samples were received within 4 days after the bleeding. It is possible that the decline of the titre levels might have been greater than indicated by this investigation. However, serum samples received by post from ewes that have aborted from toxoplasmosis, usually show titres at the same levels as samples processed shortly after bleeding.

The frequencies of DT positive mature sheep in the 4 regions were within the same range as reported from several other countries (Munday 1970). No reports of extensive serological studies of the prevalence of toxoplasma antibodies in lambs at marketing time have been found in the available literature, or the reports do not state whether lambs or mature sheep have been examined. In the present investigation, the prevalence of infection in lambs from 3 of the counties was 20—30 % lower than in mature sheep. This is consistent with long-term studies of infected flocks which show that the prevalence of toxoplasma antibodies increases with age (Waldeland 1977 a).

The prevalence of infected lambs in Buskerud was significantly higher than in the 3 other counties, and it is therefore surprising that the prevalence of infected breeding ewes in that region was lower than in any of the other counties. In Rogaland the situation was the contrary: In spite of a low frequency of infected lambs and particularly of infected flocks of lambs, this region had the highest proportion of infected breeding ewes. However, the frequencies of infected flocks of ewes did not differ significantly between the 4 counties.

These findings indicate that although toxoplasma infection is common in sheep in all these parts of the southern Norway, the epidemiological pattern may vary. These variations may be due to the management, which differs according to climatical and geographical conditions.

In Rogaland the relatively mild climate allows the sheep to be kept outdoors during daytime most of the winter. During the summer a large proportion of the flocks is moved onto mountain or hill pastures where they graze for about 3 months. The rest of the year most of the sheep graze on cultivated pastures or farm leys. The prevalence of toxoplasma antibodies in lambs on mountain pastures is low, even if there is a large proportion of infected ewes in the flocks (Waldeland 1977 a). The incidence of infection in the same flocks was considerably higher on cultivated and natural pastures in the lowland. Most of the sheep return from mountain pastures 2—4 weeks before marketing, and a low prevalence of toxoplasma antibodies in lambs compared with mature sheep in the present material was therefore to be expected.

In Buskerud the sheep are kept indoors during the winter, when the fields are covered with snow for several months. In the grazing period from the last part of May and till October, they are first moved onto partly forrested pastures near the farms, from where they successively move to not forrested pastures at higher altitudes. It is possible that differences between the fauna of this region and the fauna of the mountain and hill pastures in the south-western part of Norway may be of importance for the higher prevalence of latent toxoplasmosis in lambs in Buskerud. It is also possible that environmental conditions, such as the soil type and the amount of humus in the soil which are of importance for the longevity of coccidian oocysts (Soulsby 1968), in this county are favourable for the preservation of the infectivity toxoplasma oocysts. It may also be that the oocysts are preserved during the winter under the snow, which forms a good temperature insulation.

The DT titres in sheep decline proportionally with the time after the initial infection (Waldeland 1977 b). In addition to the present investigation which indicates that sheep in Buskerud are infected at a younger age than those in Rogaland, this may explain why the prevalence of DT positive mature sheep was lower in Buskerud, and that the difference was particularly evident at high titres.

A low frequency of high DT titres was found also among mature sheep in Berkåk in South Trøndelag where the climate and the management essentially are as in Hallingdal in Buskerud, but the frequency of infected lambs in North and South Trøndelag was nearly the same as in Rogaland. However, no general conclusions of the epidemiology in Trøndelag can be made from these results, as Berkås is only a small district within this part of the country, where the climate and the geographical conditions may differ from area to area.

The difference between the frequencies of infected flocks of lambs and infected flocks of ewes was about 40 % in Rogaland. This observation indicates that several flocks are little exposed to T. gondii during the summer and the autumn until marketing time, and that most of the infections occur during a later period.

The prevalence of infected flocks of mature sheep was about the same in Buskerud as in Rogaland, but, as the high prevalence of infected flocks of lambs in Buskerud showed that most flocks were exposed to the parasite during the grazing season, no conclusion can be made of seasonal differences in the incidence of infection in this county.

The frequencies of infected lambs and mature sheep were mainly the same in Hordaland as in Rogaland, as were the titre level of the DT positive individuals. However, the prevalence of infected flocks of ewes in Hordaland was only about 10 % higher than that of infected flocks of lambs, or about 20 % if estimated by the first 5 samples examined from each flock. Although the number of flocks examined is small, these findings indicate that a greater part of the infected flocks are exposed to T. gondii during the grazing period until marketing than in Rogaland. Allowing for some decline in the DT titres after the initial infection (Waldeland 1977 b), the prevalence of DT positive mature sheep agrees well with an incidence of infection of the order found in the lambs. Accordingly, the prevalence of DT positive 5-7 months old lambs in Hordaland should give a rough indication of the annual incidence of infection in the sheep population in that area.

From the findings discussed above the following deductions can be made:

In Rogaland a greater part of the flock infections occurs during the late autumn and winter, i.e. during the pregnancy season. This may be associated with the long grazing period after the sheep return from mountain or hill grazings where the incidence of infection is low, and may explain the great importance of toxoplasmosis as a cause of reproductive loss in this county.

In Hallingdal in Buskerud the incidence of infection during the summer is relatively high, resulting in a high rate of infected and thereby immune young individuals. Consequently, abortions from toxoplasmosis are probably not so frequent in this district as in Rogaland.

The incidence of infection in Voss in Hordaland is moderate during the grazing period until marketing time, and most of the flock infections occur during this period. This means that abortions from toxoplasmosis probably are less frequent than in Rogaland, but as a great proportion of the younger ewes are susceptible, the consequences from infection during the indoor period may be severe.

The prevalence of toxoplasma antibodies in sheep in North and South Trøndelag was mainly as in the other counties, but no conclusions about the epidemiology could be made as the samples were from places with different climatical and geographical conditions. In spite of these epidemiological differences, no evidence of significant differences in the geographical distribution of T. gondii was found.

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#### **SAMMENDRAG**

Toxoplasmose hos sau. Forekomst av Toxoplasma-antistoffer hos lam og voksne sauer fra forskjellige distrikt i Norge.

Forekomsten av Toxoplasma-antistoffer ble undersøkt ved hjelp av Sabin & Feldman's dye test hos 1929 lam og voksne söyer fra 4 forskjellige områder i Sør-Norge. Det ble samlet inn blodprøver fra 1377 lam ved slakting i perioden september-oktober 1968 og 1969, og fra 552 voksne søyer i løpet av vinteren 1970/71 og 1971/72. Prøvene fra lam stammet fra 211 flokker, og fra voksne søyer fra 65 flokker.

Hos 20—39 prosent av lammene og 42—50 procent av de voksne søyene ble det påvist antistoffer mot Toxoplasma gondii. Frekvensen av flokker med minst et dye-test positivt lam (titer  $\geq 1/16$ ) varierte fra 48 til 80 prosent, og frekvensen av flokker med minst ei positiv søye fra 79 til 90 prosent. Resultatene tyder på at det er regionale forskjeller i epidemiologien ved Toxoplasma-infeksjon hos sau. Til tross for variasjoner i epidemiologien, ble det ikke funnet forskjeller i den geografiske utbredelsen av parasitten.

## (Received November 11, 1976).

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