

From the State Veterinary Research Station for Small Ruminants,  
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TOXOPLASMOSIS IN SHEEP  
TOXOPLASMA GONDII IN MUSCULAR TISSUE,  
WITH PARTICULAR REFERENCE  
TO DYE TEST TITRES AND HAEMOGLOBIN TYPE\*

By

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WALDELAND, H.: *Toxoplasmosis in sheep. Toxoplasma gondii in muscular tissue, with particular reference to dye test titres and haemoglobin type.* Acta vet. scand. 1976, 17, 403—411. — The presence of *Toxoplasma gondii* in the diaphragm was correlated with the dye test titres in 174 sheep. In 94 of these the presence of the parasite was also correlated with the haemoglobin (Hb) type. *T. gondii* was recovered from 3 % of the sheep with titres  $< 1/16$ , compared with 30 % of those with titres  $1/16$  and 70 % of those with titres  $\geq 1/32$ . The results indicate that the distinction between serologically positive and negative individuals at a final serum dilution of  $1/16$  is justified. Some evidence was found that the parasite is easier to recover from dye test positive mature sheep than from dye test positive lambs.

Of the 174 sheep, 143 were examined at random among 186 sheep culled or cast for age during a 4-year period from 1 flock in which the prevalence of *Toxoplasma* antibodies was representative for flocks in the southern Norway, and *T. gondii* was recovered from 53 (37 %) of these. It was concluded that 10—15 % of the lamb carcasses and 25—37 % of the carcasses from mature sheep in this country have *T. gondii* in their muscles detectable by the peptic digestion technique.

A possible genetical influence on the infection was indicated by the higher frequency of recoveries of *T. gondii* from sheep with Hb type B than from sheep with the Hb types A or AB, but the number of individuals with Hb type B was too small to demonstrate statistically significant differences.

The epidemiological importance of infected sheep carcasses is discussed.

toxoplasma infection; meat inspection; haemoglobin type; sheep.

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The potential role of meat animals as a source of infection with *Toxoplasma gondii* has been well established since *Jacobs & Melton* (1957) developed a method to recover the parasite from meat samples. Unfortunately, this method is too laborious for large-scale surveys. Previous reports indicate that the prevalence of *T. gondii* in meat from sheep is correlated with the titres for *Toxoplasma* antibodies (*Jacobs et al.* 1963, *Work* 1967), but the correlation has apparently not been systematically examined. The intention of the present work was to elucidate this correlation, in order to provide a basis for further epidemiological conclusions from serological surveys which are easier to perform. As an additional basis for such conclusions, meat from most mature sheep slaughtered during a 4-year period was examined in an experimental flock with prevalence of *Toxoplasma* antibodies representative for flocks in the southern Norway.

Older ewes may often have a long-standing infection and relatively low titres of *Toxoplasma* antibodies (*Waldeland* 1977), and it was therefore also necessary to examine the influence of age on the presence of *T. gondii* in meat.

It was also the intention to elucidate whether there are genetical differences in sheep concerning the prevalence of *T. gondii* in meat. For this purpose the relation between the haemoglobin (Hb) phenotype and the presence of the parasite in the muscles was examined.

#### MATERIALS AND METHODS

The examination was carried out during the period 1968—1973 in a flock of 120—130 mature ewes. Two and a half years before the investigation started, 4 of 120 ewes aborted from toxoplasmosis. The prevalence of dye test (DT) (*Sabin & Feldman* 1948) positive (titres  $\geq 1/16$ ) mature sheep in the flock ranged between 40 and 53 % during the 5-year period.

Meat samples from a total of 174 sheep of the "Rygja" and the "Dala" breeds were examined. The samples were each year collected in the autumn. Fourteen samples were from lambs about 6 months old, the remaining 160 were from sheep aged 1½ to 8½ years, which were cast for age or culled for other reasons. During 1 season 31 samples were selected from DT positive sheep, whereas during the remaining 4 years when a total of 186 mature sheep were slaughtered, samples were taken at random from 143 of these.

The meat samples consisted of 100 g of the diaphragm muscle from each individual. The samples were stored at 4°C until the processing, which was done within 24 hrs. by the peptic digestion technique (Jacobs & Melton 1957), modified as recommended by Work (1967). The final sediment was resuspended in 6–10 ml physiological saline, and 1.0–1.3 ml of this suspension was inoculated into each of 5–6 mice. The mice brains were examined after 6–10 weeks for encysted *Toxoplasma* as described by Beverley & Watson (1961).

Blood samples of the sheep were collected during the last 24 hrs. before slaughtering, and examined for *Toxoplasma* antibodies by a micromodification of the Sabin and Feldman dye test (Waldeland 1976a). Serological examination of the mice was carried out prior to inoculation and when they were killed.

The Hb type was determined in 94 of the 174 sheep by electrophoresis on cellulose acetate membranes in the "Beckman Microzone Electrophoresis System"\* as described in the manufacturer's manual.

## RESULTS

Cysts of *T. gondii* were found in mice inoculated from 69 meat samples. The presence of cysts in the mice brains coincided with DT positive reactions of the mice sera at a final serum dilution of 1/4.

The results from the inoculations are compared with the DT titres of the sheep in Table 1. The parasite was recovered from about 58 % of the sheep with DT titres  $\geq$  1/16, and from about 3 % of the sheep with DT titres  $<$  1/16.

\* Beckman Instruments, Palo Alto, California, USA.

Table 1. Detection of *Toxoplasma gondii* in meat of 174 sheep, grouped according to their titres of *Toxoplasma* antibodies (Sabin and Feldman's dye test).

Detection of <i>T. gondii</i>	Dye test titres							Total number
	$<$ 1/16	1/16	1/32	1/64	1/128	1/256	1/512	
Positive	2	11	21	27	7	1		69
Negative	56	25	9	10	4		1	105
Attempted	58	36	30	37	11	1	1	174

Thirty-three of the 58 sheep with DT titres  $< 1/16$  had a titre of  $1/8$ , the remaining 25 had lower titres. The inoculations were positive from about 30 % of the sheep with a DT titre of  $1/16$ , compared with 70 % from those with higher titres.

No significant differences were found between lambs, sheep selected by previous serological examination, and culled and cast sheep examined at random, concerning the inoculation results in relation to the DT titres.

*T. gondii* was recovered from 53 (37 %) of the 143 culled sheep examined at random during the 4-year period. These 143 sheep were on the average about 1 year older than the remaining sheep in the flock. The results from the inoculations are recorded in Table 2 according to the age of the sheep. The prevalence of sheep in Table 2 with positive DT titres was about 64 % (91 of 143). The relation between the titre values and the inoculation results was mainly the same within all age groups.

Table 2. Detection of *Toxoplasma gondii* in meat from 143 sheep aged  $1\frac{1}{2}$  to  $8\frac{1}{2}$  years, examined at random among 186 sheep culled or cast for age from the same flock during a 4-year period.

Age (years)	Detection of <i>T. gondii</i>	
	attempted	positive
$1\frac{1}{2}$	23	4 (17)
$2\frac{1}{2}$	33	6 (18)
$3\frac{1}{2}$	20	7 (35)
$4\frac{1}{2}$	14	6 (43)
$5\frac{1}{2}$	13	9 (69)
$6\frac{1}{2}$	21	13 (62)
$7\frac{1}{2}$	17	7 (41)
$8\frac{1}{2}$	2	1 (50)
Total	143	53 (37)

Figures in brackets: percentage.

The prevalence of *T. gondii* in meat from sheep with different Hb types is recorded in Table 3.

Of the 63 DT positive sheep, *T. gondii* was recovered from all of 4 individuals with Hb type B, compared with 15 of 26 (58 %) and 21 of 33 (64 %) respectively of those with Hb types A and AB. The detection of the parasite in 2 sheep with DT titres  $< 1/16$

recorded in Table 1 was in ewes with the Hb types AB and B respectively.

Table 3. Detection of *Toxoplasma gondii* in meat of 94 sheep, grouped according to their haemoglobin type. Sixty-three of the sheep had dye test titres  $\geq 1/16$ , the remainder had titres  $< 1/16$ .

Haemoglobin type	Detection of <i>T. gondii</i>	
	attempted	positive
A	47	15
AB	40	22
B	7	5
Total	94	42

#### DISCUSSION

The results presented in Table 1 show that *T. gondii* was recovered from about 58 % of 116 DT positive sheep. This is of the same order as reported by *Jacobs et al.* (1963) who detected the parasite in about 67 % of 34 sheep with DT titres  $\geq 1/16$ , and by *Work* (1967) who recovered the parasite from 7 of 9 sheep with titres  $\geq 1/50$  but not from any of 22 sheep with titres  $\leq 1/10$ .

In the present work *T. gondii* was recovered from a considerably higher proportion of sheep with DT titres  $\geq 1/32$  than from sheep with a titre of  $1/16$ , but the most marked difference in the inoculation results was found between sheep with DT titres  $\geq 1/16$  and those with titres  $< 1/16$ . These results indicate that a distinction between serologically positive and negative individuals at a final serum dilution of  $1/16$  is justified. The 2 individuals with titres  $< 1/16$  from which *T. gondii* was recovered were both old ewes which had DT titres  $\geq 1/16$  1 year earlier. This indicates that the ewes had a long-standing infection with *T. gondii* and that the serum contents of *Toxoplasma* antibodies had declined to a scarcely detectable level at the time of slaughtering.

The parasite was not detected in an individual with a DT titre of  $1/512$ . Unfortunately, the serum sample collected the day before slaughtering was the only one examined from this sheep, but the high titre value indicated a recently acquired infection

(Waldeland 1977). It is therefore probable that this sheep was slaughtered before stages of *T. gondii* resistant enough to withstand the pepsin-HCl treatment were formed. This presumption is supported by negative inoculation results also from a sheep with a DT titre of 1/128, which was slaughtered in the acute stage of the infection as proved by retrospective examination of 3 serum samples collected during the last month before slaughtering.

According to these findings it is probable that *T. gondii* is easier to recover from DT positive sheep cast for age than from DT positive lambs about 6 months old, as older sheep are more likely to have a long-established infection. Unfortunately, the present investigation comprised too few lambs to confirm this hypothesis.

As demonstrated in Table 2 the frequency of positive inoculations increased with age up to 5—6 years, which shows that the prevalence of latent toxoplasmosis increases with age. This is in accordance with the higher prevalence of DT positive individuals among the slaughtered sheep than in the flock as a whole, as the slaughtered sheep on the average were about 1 year older than the remainder.

In an investigation on the prevalence of *Toxoplasma* antibodies in sheep in Norway (Waldeland 1976 b), serological evidence of the infection was found in about 46 % of the mature ewes, and in about 26 % of the lambs. The distribution of the different titres in the DT positive mature ewes was comparable with those recorded in Table 1, but the DT positive lambs had a higher frequency of low titres. The serological examination of the present flock showed that the prevalence of infection was representative for mature sheep in this country, and the frequency of positive inoculations in Table 2 therefore probably also applies to carcasses from mature sheep as a whole. The results from the serological survey (Waldeland 1976 b) together with the results presented in Tables 1 and 2, accordingly lead to the conclusion that *T. gondii* is present in 25—37 % of the carcasses from mature sheep and in 10—15 % of the lamb carcasses in this country. It should be noted that this estimation only refers to the presence of *T. gondii* in 100 g meat detectable by the peptic digestion technique. The virulence of the parasite for mice was low, which agrees with observations reported in the available literature, and on 6 occasions cysts of *T. gondii* were only found

in 1 of 6 mice. It is therefore probable that a greater frequency of sheep with *T. gondii* in the muscular tissue could be found by using greater samples and inoculation of a higher number of mice.

The results recorded in Table 3 show that the frequency of recovery of *T. gondii* was highest from sheep with Hb type B and lowest from sheep with Hb type A, but the differences were not statistically significant. It is noteworthy that the parasite was recovered from all of 4 DT positive sheep with Hb type B, whereas the frequencies of recovery from DT positive sheep with Hb type A or AB were in the range from 58 to 64 %. Unfortunately, the number of DT positive individuals with Hb type B was insufficient to justify a statistical conclusion. However, added to observations by *Waldeland* (1977) which suggest a relationship between the Hb type and the titre values during the first months after the initial infection, the present results indicate that genetic factors may influence the course of the infection. The prevalence of the different Hb types may differ between the various breeds (*Efremov & Brænd* 1965), and it is therefore possible that there is a higher correlation between positive DT titres and presence of *T. gondii* in the muscular tissue in sheep of breeds with a higher frequency of the Hb<sup>B</sup> gene.

Intestinal stages of *T. gondii* have only been found in some representatives of the Felidae, and the importance of sheep as a source of infection is therefore probably mainly restricted to transmission by ingestion of tissue cysts. *Toxoplasma* cysts in meat are not resistant to ordinary household procedures (*Work* 1968), and human infection from tissue cysts is therefore probably connected with handling or ingestion of raw or undercooked meat that has not been frozen. Accordingly, the risk of infection from meat varies with cultural habits, and in this country infected mutton is probably of little importance as a source of human infection. Infected pork or beef may in this respect be of greater consequence.

Some of the offal of slaughtered sheep is mixed in fodder for minks and foxes, and this represents an important source of infection for these animals as no precaution such as heating or freezing of the fodder is compulsory. Surplus fodder is sometimes fed to pigs which thus may acquire the infection, as may dogs, cats, rats, birds and other animals that pilfer some of the fodder. Sheep slaughtered on the farms, aborted foetuses and

placentas, and sheep which have died on the pastures also represent a source of infection to other animals. Sheep themselves are probably strongly exposed to infection by ingestion of oocysts of *T. gondii* on contaminated pastures, as they graze the herbage close to the ground. It is therefore likely that sheep are of great importance in the epidemiology of toxoplasmosis, by contributing to maintain a widespread distribution of the parasite.

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## SAMMENDRAG

*Toxoplasmose hos sau. Påvisning av Toxoplasma gondii i sauekjøtt, med særlig henblikk på dye-test titer og haemoglobintype.*

Forekomst av *Toxoplasma gondii* i diafragma-muskulatur ble undersøkt hos 174 sauer og sammenholdt med sauenes dye-test titre, og hos 94 av disse sauene ble forekomsten også sammenholdt med haemoglobin (Hb)-typen. Parasitten ble påvist hos 3 % av sauene med titre < 1/16, hos 30 % med titer 1/16, og hos 70 % av sauene med høyere

titre ( $\geq 1/32$ ). Resultatene tyder på at serologisk positive og negative sauer vanligvis kan skilles etter reaksjonen ved en serumfortynning på 1/16. Enkelte observasjoner indikerte at det er lettere å påvise parasitten hos voksne dye-test positive sauer enn hos dye-test positive lam.

Av de 174 sauene ble 143 undersøkt vilkårlig blant 186 sauer som ble utsjaltet fra samme avlsflokk i løpet av en 4-års periode. Frekvensen positive dye-test titre i denne flokken lå nær gjennomsnittet hos voksne sauer i det sørlige Norge. Hos 53 (37 %) av de 143 sauene ble *T. gondii* påvist i muskulaturen. Konklusjonen av undersøkelsen var at 10—15 % av alle lammeslakt og 25—37 % av slaktene fra voksne sauer her i landet har *T. gondii* i muskulaturen i stort nok antall til at den kan påvises med den metode som her ble anvendt.

Parasitten ble påvist med høyere frekvens hos sauer med Hb-type B enn hos sauer med Hb-type A eller AB, og dette tyder på at genetiske faktorer kan ha innvirkning på infeksjonen. Antall dyr med Hb-type B var imidlertid for lite til at det kunne trekkes noen sikker konklusjon.

Den epidemiologiske betydningen av infiserte saueslakt og sauekadavre er diskutert.

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