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TOXOPLASMOSIS IN SHEEP

INFLUENCE OF VARIOUS FACTORS ON THE ANTIBODY CONTENTS*

By

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WALDELAND, H.: *Toxoplasmosis in sheep. Influence of various factors on the antibody contents.* Acta vet. scand. 1977, *18*, 237—247. — The contents of toxoplasma antibodies were estimated by a micro-modification of the dye test for up to 3 years after the initial stage of infection both in ewes which had aborted and in ewes with normal pregnancies. During the first 2 years the titres in ewes which had aborted were significantly higher than in ewes with normal pregnancies. The investigation indicated that dye test titres $\geq$ 1/512 usually occur during the first stage of infection, and are mainly found in ewes with clinical toxoplasmosis.

The dye test titres in lambs due to antibodies transferred with the colostrum were up to 4 twofold dilutions higher than in their dams during the first 2 days after birth. Later the titres declined, and at the age of about 2 months only 3 of 29 lambs had higher titres than their dams. After the age of about 3 months maternally derived antibodies were not detected.

The contents of toxoplasma antibodies in sheep with listeric encephalitis were nearly the same as found by a serological survey of the local sheep population. The examination indicated that the dye test titres in sheep are little influenced by conditions that may affect the defence mechanism.

Sheep with haemoglobin type B had significantly higher dye test titres than sheep with the haemoglobin types A and AB when examined less than about 6 months after they had acquired the infection. No association was found between the susceptibility to toxoplasma infection and the haemoglobin type.

toxoplasma infection; antibody formation; sheep.

The prevalence of infection with Toxoplasma gondii in sheep is high (*Waldeland* 1976 a), and toxoplasma antibodies may accordingly be found incidentally in both healthy sheep and sheep with various diseases. In young lambs the presence of toxoplasma antibodies may be due to absorption from the colostrum. A diagnosis based on dye test (DT) examination (*Sabin & Feld-*

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man 1948) is therefore dependent on knowledge of the changes in titre values both after the initial infection and after absorption of colostral antibodies. The intention of the present work was to define the pattern of these changes.

Cell-mediated immunity is of great importance in infection with T. gondii as well as in infection with Listeria monocytogenes. It is recognized that conditions associated with an impaired defence mechanism may predispose for listeriosis (*Hyslop* 1975). Listeric encephalitis is a common sheep disease in Norway, and studies at this research station indicate that listeric infection rarely results in clinical disease unless the sheep have been subjected to predisposing factors (*Grönstöl* 1977). To elucidate if the contents of toxoplasma antibodies are influenced by such factors or by the listeric infection, the DT titres in all sheep with listeric encephalitis submitted to this research station were compared with titres recorded in the healthy sheep population in the same area.

A previous investigation indicated that genetical factors may influence the presence of T. gondii in muscular tissue (*Waldeland* 1976 b). In the present paper the DT titres in relation to the haemoglobin (Hb) type are recorded.

MATERIALS AND METHODS

The sheep were of the 2 Norwegian breeds "Rygja" and "Dala". The investigation was designed as follows:

1. *DT titres in ewes after the initial stage of infection*

Ewes which had aborted. A total of 128 ewes were bled 1—3 times during the first 12 months after abortion from toxoplasmosis, giving 212 samples. The ewes were from 25 flocks where T. gondii was the only microorganism detected in aborted materials examined as previously described (*Waldeland* 1976 c). In addition, a total of 52 samples were collected from 13 ewes which were bled twice yearly during the period 1½ to 3 years after toxoplasma abortion.

Ewes with normal pregnancies. Fourty-four ewes were bled twice yearly during a period of up to 3 years after they developed positive DT titres ($\geq 1/16$). Totally, 191 samples were examined. The ewes were from a flock which had been examined by the DT every spring and autumn.

2. *Influence of maternal antibodies on DT titres in lambs*

Blood samples of 52 lambs from 31 ewes were collected before they were allowed to suckle, and at 24 and 48 hrs. after birth. Later samplings were done irregularly, but most lambs were bled 4 times during the following 4 months. The ewes were bled at the same time as their offspring, giving a total of 549 samples. Fourteen of the lambs were from DT negative ewes, whereas the remainder was born to 22 ewes selected among DT positive sheep.

3. *DT titres in sheep with listeric encephalitis*

Samples were collected from 46 mature sheep and 20 lambs 7—11 months old submitted to this research station because of listeric encephalitis. The titres in these sheep were compared with titres previously recorded in the healthy sheep population (*Waldeland* 1976 a).

4. *DT titres in relation to the haemoglobin type*

A flock of about 190 breeding sheep was examined twice yearly during a 5-year period. The frequencies of individuals with the Hb types A, AB and B in this flock were about 60, 30 and 10 % respectively. Totally, 1772 samples were examined.

Laboratory examinations

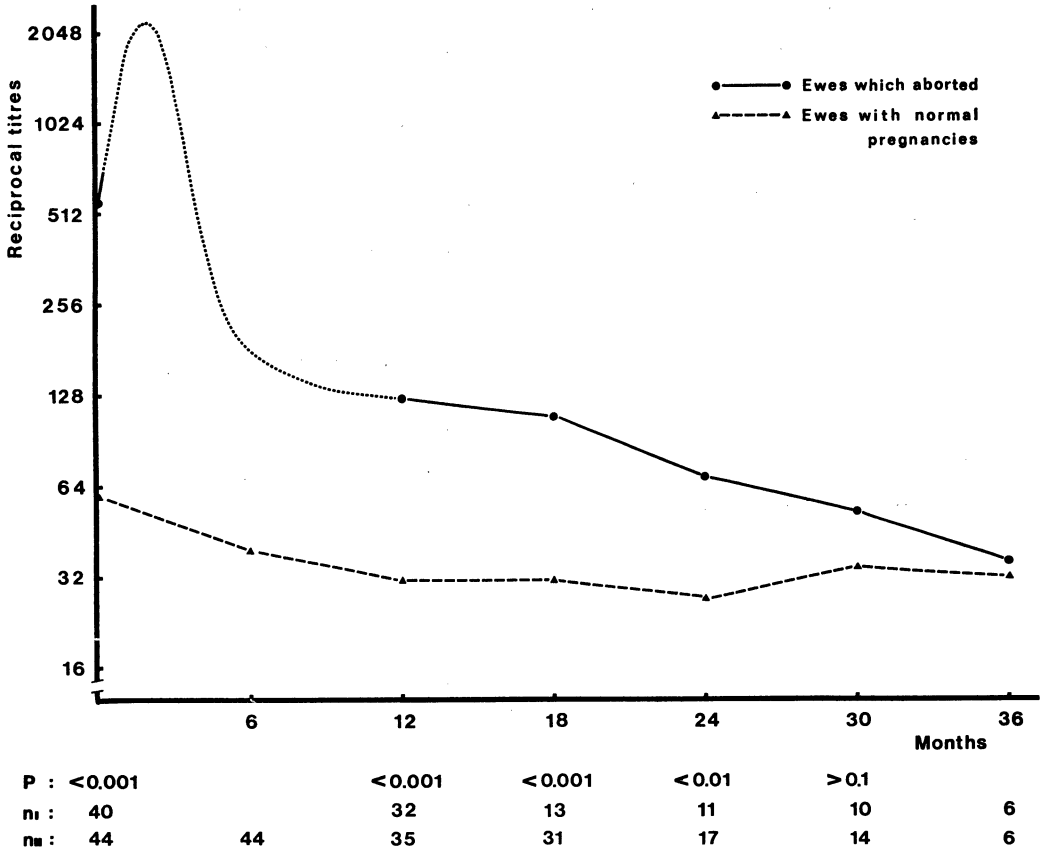
The examination for toxoplasma antibodies was done by a micromodification of the DT (*Waldeland* 1976 d). The sera from the newborn lambs and their dams were stored at —20°C until all sera from each ewe and her lambs could be examined at the same time with the same batch of accessory factor serum and antigen preparation.

The Hb typing was done as previously recorded (*Waldeland* 1976 b).

RESULTS

1. *DT titres in ewes after the initial stage of infection*

Ewes which had aborted. Fourty ewes examined within 1 week after abortion had DT titres ranging from 1/32 to 1/8192, with a geometrical mean of 1/548. Titres $\geq$ 1/4096 were observed in 2 of these ewes, whereas similar titres were found in 6 of 9 ewes examined 9—10 weeks after abortion. Later the titres de-



P :	<0.001		<0.001	<0.001	<0.01	>0.1	
n_I :	40		32	13	11	10	6
n_{II} :	44	44	35	31	17	14	6

Figure 1. Geometrical mean of dye test titres in ewes which had aborted from toxoplasmosis, compared with the mean titre in ewes with normal pregnancies. The latter ewes had been dye test negative (titres $< 1/16$) when examined 6 months earlier. The abscissa gives the time after abortion or after the first dye test positive sample was recorded. n_I gives the number of ewes which had aborted, and n_{II} gives the number of ewes with normal pregnancies. P gives the statistical significance of differences in titres (Student's t-test) between the 2 groups of ewes. During the first 12 months the mean titre in ewes which had aborted was estimated from a total of 212 samples.

clined as illustrated in Fig. 1. In 32 ewes examined 12 months after abortion the titres ranged from 1/16 to 1/512, with a geometrical mean of 1/125. The decline of the mean titre continued during the following 2 years at a rate of about 0.9 twofold dilutions each year.

Ewes with normal pregnancies. The geometrical mean titre at the first DT positive sampling was 1/57. During the following year there was a decline of the mean titre of nearly 1 twofold dilution (Fig. 1), but later only small changes in the titres were observed. In most ewes the DT titres ranged between 1/16 and 1/128 during the observation period; only 7 of the 191 samples showed a titre of 1/256. No difference was found between the 23 ewes which developed positive DT titres during the pregnancy compared with the 21 ewes which developed positive titres during the non-pregnancy.

The DT titres in the ewes which had aborted were significantly higher than in the ewes with normal pegnancies during the first 2 years after the infection was detected (Fig. 1), but later the titres were nearly the same in the 2 groups.

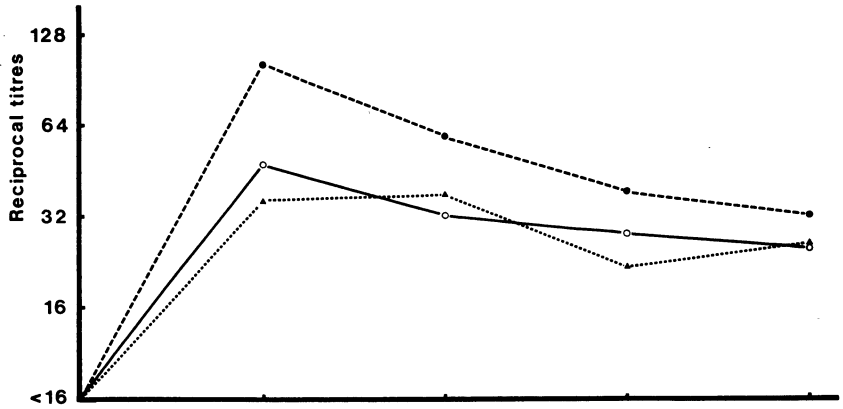
2. *Influence of maternal antibodies on DT titres in lambs*

Positive titres were not detected in any of the 14 lambs from the 9 DT negative ewes.

All of the 36 lambs from 21 of the 22 DT positive ewes had titres of $< 1/16$ before suckling, but during the following 2 days their titres ranged from 1/16 to 1/1024. On the average, the DT titres in the lambs were a little more than 1 twofold dilution higher than in their dams during the first 2 weeks after birth. A maximum difference of 4 twofold dilutions higher was observed in 2 single lambs. In relation to their dams, the titres in 7 single lambs were slightly but not significantly higher than in 29 twin or triplet lambs.

At the age of 6—10 weeks, only 3 of 29 lambs had higher DT titres than their dams. Positive DT titres were not found in any of the lambs at the age of 3 months or older.

One of the 2 lambs from the last of the 22 DT positive ewes had a titre of 1/16 before suckling, whereas its twin was DT negative. Otherwise, the titres in these lambs did not differ from the titres in the other lambs from DT positive ewes during the first 2 weeks after birth. After the age of 4 weeks their titres were from 1 to 3 twofold dilutions higher than in their dam, ranging from 1/64 to 1/256.



n_A :	40	40	32	30	25
n_{AB} :	26	26	18	14	16
n_B :	9	9	7	4	3

Figure 2. Geometrical mean titres after conversion from dye test negative to positive reactions in sheep with different haemoglobin (Hb) types. Sheep with Hb type B had significantly higher titres than sheep with Hb type A or AB at the examination at 6 months ($P < 0.02$ and $P < 0.001$ respectively), but later the differences were not statistically significant.

o——o Mean titre in sheep with Hb type A.
▲·····▲ ——— „ ——— AB.
●----● ——— „ ——— B.
n_A : Number of sheep with Hb type A.
n_{AB}: ——— „ ——— AB.
n_B : ——— „ ——— B.

3. *DT titres in sheep with listeric encephalitis*

Nineteen (41 %) of the 46 mature sheep were DT positive with titres ranging from 1/16 to 1/256. Of the 20 lambs, 6 (30 %) were DT positive with titres from 1/16 to 1/512. No notable differences were found between the titres in these sheep compared with the titres in the healthy sheep population.

4. *DT titres in relation to the haemoglobin type*

No significant differences in the prevalence of positive DT titres or in the incidence of infection were found between sheep of the Hb types A, AB and B.

A total of 75 sheep which previously had been DT negative developed positive titres during the investigation. The number of individuals and the geometrical mean titres in the different Hb groups are recorded in Fig. 2. Thirty-one of these sheep were culled from the flock during the first 18 months after the infection was detected, leaving a number of 44 individuals at the last examination. The mean titre in sheep with Hb type B was more than 1 twofold dilution higher than in sheep with the Hb types A and AB at the first DT positive sampling ($P < 0.02$ and $P < 0.001$ respectively). Later the differences declined, but 6 months after the infection was detected the mean titre in sheep with Hb type B was still about 0.7 twofold dilutions higher than in sheep with the other Hb types.

The incidence of abortion or barrenness in ewes which developed positive titres during pregnancy was about the same in each of the Hb groups.

DISCUSSION

The results recorded in Fig. 1 show that the ewes with overt toxoplasmosis had significantly higher geometrical mean titres during the first 2 years after the initial stage of infection than the ewes with normal pregnancies. Experimental studies by *Watson & Beverley* (1971) indicated that abortion usually is the result of infection during the first 3—4 months after mating, and that infection during the last month of gestation is unlikely to cause abortion. In the present investigation the first samples of ewes which had aborted were therefore probably taken between 1 and 3 or 4 months after they had acquired the infection, as most of the abortions occurred during the last month of pregnancy. The ewes with normal pregnancies were bled every 6 months, and might have acquired the infection any time between 2 samplings. In relation to the initial infection, the ewes which had aborted were therefore probably not bled more than 2 months later than the ewes with normal pregnancies. This difference in time should have little influence on the difference in mean titres recorded in Fig. 1.

The difference in mean titres indicated that the higher antibody contents are associated with abortion. The question then arises whether the higher titres are induced by a better antigenical stimulus from the proliferation of T. gondii in the foetus and the placenta as discussed by *Beverley & Watson* (1971), or

if abortion and high titres ultimately are the results of a heavier initial infection or infection with a more virulent strain of the parasite. However, if the strain and the infective dose were important, one should expect high titres also in some ewes infected during the non-pregnancy. In the present investigation the DT titres in ewes infected during the summer or early autumn were low, and titres at about the same level have also been found by monthly examination of lambs naturally infected during the summer (*Waldeland* 1977). The most reasonable explanation of higher titres in ewes after abortion seems therefore to be that the infection induces a better antibody response by proliferation of the parasite in the foetus and the placenta. This explanation may also apply to the antibody response from infection during late pregnancy, as high titres have been found in ewes with T. gondii in the foetal placenta following inoculation during the last month before lambing (*Watson & Beverley*).

The geometrical mean titre in ewes examined shortly after abortion was 1/548, whereas the highest titre in ewes with a normal pregnancy was 1/256. At this research station titres of $\geq$ 1/512 in ewes with a normal pregnancy are nearly exclusively found in flocks where other ewes have aborted from toxoplasmosis during the same pregnancy season. On these occasions the ewes with a normal reproductive behaviour have probably acquired the infection at a late stage of gestation. These observations indicate that titres $\geq$ 1/512 in mature sheep are associated with a recently acquired infection and are mainly found in ewes with overt toxoplasmosis. This conclusion is mainly in agreement with observations by *Hartley & Moyle* (1968). It should be noted that the serological examination in the present work was done by a micromodification of the DT which usually shows titres from 1 to 2 twofold dilutions lower than by the conventional technique (*Waldeland* 1976 d).

The examination of newborn lambs and their dams showed that all lambs from DT positive ewes were positive after suckling, whereas all lambs from DT negative ewes were negative. However, it is possible that some ewes with titres $<$ 1/16 may have sufficient antibodies in their colostrum to produce positive titres in their lambs (*Munday* 1970). In the present investigation the effect of the transmission mechanism was illustrated by the up to 4 twofold dilutions higher titres in the lambs than in their dams.

The fading of maternally derived antibodies over a period of about 3 months is in accordance with observations by *Hartley* (1966), but *Munday* found still positive DT titres in a few 6 months old lambs. In the present investigation the titres in the ewes ranged up to 1/128, and it is probable that positive titres may be found during a longer period in single lambs from ewes with higher antibody contents. However, the present results show that serological examination at about 2 months after birth may indicate whether the lambs are infected, as only 3 of 29 lambs had higher titres than their dams at that time. The twin lambs of which 1 was DT positive before suckling were probably infected during the perinatal period, as their titres increased also after the age of 2 days, i.e. at a time when colostral antibodies are not absorbed (*Brambell* 1970). Toxoplasma antibodies were still detected 3 years after birth in 1 of these lambs which was kept for breeding.

The DT titres in lambs and mature sheep with listeric encephalitis did not differ noteworthy from the titres found by a serological survey of healthy sheep from this part of the country (*Waldeland* 1976 a). These findings indicate that the DT titres in sheep with latent toxoplasmosis are little influenced by listeric infection and by conditions that may affect the defence mechanism. Similar titres were also found in 144 other sheep with various diseases submitted to this research station. It should also be mentioned that a remarkable stability in the DT titres was demonstrated by monthly examination of ewes kept on different feeding and housing conditions during pregnancy and lactation (*Waldeland*, unpublished data).

The antibody production was higher in sheep with Hb type B than in sheep with the other Hb types during the first period of the infection, although no difference was found in the susceptibility to the infection. The higher antibody contents in sheep with Hb type B was not of any consequence for the diagnostical interpretation of DT titres in ewes which abort, as the highest titre in sheep with Hb type B was 1/256. It is possible that the higher titres were due to a better antigenical stimulus, as there seems to be a heavier dissemination of the parasite in muscular tissue in sheep with this Hb type compared with sheep with the other Hb types (*Waldeland* 1976 b). Further studies are required to elucidate these genetical differences.

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SAMMENDRAG

Toxoplasmose hos sau. Faktorer som påvirker antistoff-nivået.

Serumprøver fra sauer som hadde abortert på grunn av toxoplasmose, og fra sauer med normal drektighet, ble undersøkt ved hjelp av en mikromodifikasjon av Sabin & Feldman's dye test i opp til 3 år etter at de var blitt infiserte. De første 2 årene var det signifikant høyere titre hos sauer som hadde abortert, sammenliknet med sauer med normal drektighet. Undersøkelsen tydet på at titre $\geq$ 1/512 er

vanligst på et tidlig stadium av infeksjonsforløpet i forbindelse med klinisk toxoplasmose.

Dye test titrene hos lam etter overføring av antistoff med kolostrum var opp til 4 doble fortynninger høyere enn hos morsøyene de første 2 dagene etter fødselen. Senere falt titerverdiene gradvis, og ved ca. 2 måneders alderen hadde bare 3 av 29 lam høyere titre enn morsøyene. Etter ca. 3 måneders alderen ble det ikke påvist overførte antistoff.

Titerverdiene hos sau med listeria-encephalitt var omtrent som påvist ved serologisk undersøkelse av den friske sauepopulasjon innen samme område. Undersøkelsen tydet på at dye test titre hos sau er lite påvirket av forhold som kan ha betydning for sjukdomsresistensen.

Sau med hemoglobintype B hadde signifikant høyere titre enn sau med hemoglobintypene A eller AB ved undersøkelse innen 6 måneder etter at de ble infiserte. Det ble ikke funnet noen sammenheng mellom mottakelighet for infeksjonen og hemoglobintype.

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