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TOXOPLASMOSIS IN SHEEP

HAEMATOLOGICAL, SEROLOGICAL AND PARASITOLOGICAL STUDIES*

By

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WALDELAND, H.: *Toxoplasmosis in sheep. Haematological, serological and parasitological studies.* Acta vet. scand. 1977, *18*, 248—256. — Blood variables (cell values, contents of haemoglobin, minerals and proteins) were studied retrospectively in 13 lambs which had acquired toxoplasmosis during the summer, and in 2 lambs which had been infected during the perinatal period. Examination by immunoelectrophoresis was also performed. The lambs had been in experimental groups in a research project of blood values in sheep on pastures of different qualities. The groups comprised a total of 40 lambs, which had been bled once monthly. Examinations were also carried out in lambs experimentally infected with the RH strain of T. gondii, in lambs experimentally infected with a sheep strain of the parasite, and in a control group. No significant changes in the blood variables were found.

Meat samples from the experimentally infected lambs and their controls were examined parasitologically. T. gondii was recovered from all dye test positive (titre $\geq$ 1/16) lambs inoculated with the sheep strain, but not from any of the lambs inoculated with the RH strain. The results indicated that the RH strain does not produce cysts in the muscular tissue in sheep. Some observations indicated that this strain may protect against reinfection with other strains, but the number of individuals was too low for a statistical conclusion.

toxoplasma infection; haematology; sheep.

Serological examinations in this laboratory have shown that some sheep have acquired toxoplasmosis during experiments designed to estimate the influence of various factors on blood values. The question then arose whether the blood values routinely recorded during such experiments are influenced by a concurrent toxoplasma infection. *Michael et al.* (1972) estimated the haemoglobin and the haematocrit values and the number of

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erythrocytes and leucocytes in 2 experimentally infected sheep. The intention of the present work was to undertake a more extensive haematological and serological study both of naturally and of experimentally infected sheep during the early stage of infection.

Parasitological examination of muscles from naturally infected sheep has previously been recorded (*Waldeland* 1976 a). In the present work muscular tissue from experimentally infected sheep was examined to elucidate whether there is an association between recovery of Toxoplasma gondii and the strain of the parasite.

MATERIALS AND METHODS

Natural infection in lambs

A retrospective study was made of the blood variables of 13 lambs that had developed positive dye test (DT) titres ($\geq 1/16$, *Sabin & Feldman* 1948) during the summer, and in addition, the study comprised 2 other lambs with a perinatal infection. These 15 lambs had been included in 2 experimental groups that comprised a total of 40 twin lambs. The groups had been designed for a research project on blood values in lambs on pastures of different qualities (*Överås & Pestalozzi* 1974). In this retrospective study the 25 non-infected lambs served as controls. The lambs had been bled and weighed once monthly from they were about 8 weeks old and till the age of about 6 months.

Experimental infection in lambs

The experiment was designed with 40 lambs, all of which had negative DT titres before the experiment started. The lambs were equally divided into 4 groups, and the lambs in 3 of the groups were inoculated with T. gondii as recorded in Table 1.

The lambs were about 3 months old when the experiment started and were kept on pasture together with their dams until slaughtering about 4 months later. They were bled and examined clinically before the inoculation and 2, 6, 9, 30 and 64 days later. During this period the lambs were also weighed weekly. A final examination for toxoplasma antibodies was done just before slaughtering. The body temperatures were recorded before inoculation and once daily for 10 days. Samples of muscular tissue for parasitological examination were taken at slaughtering.

Table 1. Design for experimental infection in lambs with 2 different strains of Toxoplasma gondii.

Group no.	Number of lambs	Route of infection	Strain of T. gondii	Inoculum, dose
I	10	subcutaneously	human origin (RH)	300,000 trophozoites* in 1 ml 0.9 % saline
II	10	subcutaneously	sheep origin	50 cysts** in 1 ml 0.9 % saline
III	10	perorally	sheep origin	50 cysts** in 15 ml 0.9 % saline
IV	10	not inoculated		

* Harvested from mice inoculated intraperitoneally 3 days previously.
** Harvested from the brains of mice inoculated 4 months previously with brain suspension from an aborted lamb.

Laboratory examinations

The following examinations were performed: Packed red cell volume was estimated in a microhaematocrit centrifuge ("Cellocrit 2", AB Lars Ljungberg & Co., Stockholm) at 12,000 × g for 10 min. Red cell counts and leucocyte counts were performed with an automatic cell counter ("Celloscope", AB Lars Ljungberg & Co., Stockholm) as described by *Winter* (1965, 1966), and *Kvarstein* (1967). The determinations of haemoglobin, total serum proteins, serum iron, total iron binding capacity, differential leucocyte counts, reticulocyte counts and counts of red cells with Heinz bodies were done by the method used by *Överås* (1969, 1974)*. Inorganic phosphorus in serum was estimated with a spectrophotometer ("Beckman DU", Beckman Instruments, München) according to the method given by *Zeiss-Opton* (1951). Potassium and sodium in plasma were determined by flame emission spectroscopy, and the serum contents of calcium, magnesium, copper and zinc were determined by atomic absorption spectroscopy with an atomic absorption/emission spectrophotometer ("Unicam SP 90", Unicam Instruments Ltd., Cambridge) according to the manufacturer's manual. A commercial control serum ("Seronorm", Nyegaard & Co. A/S, Oslo) was routinely used.

Zone electrophoresis of serum was carried out on cellulose

* Differential leucocyte counts were done only of the experimentally infected lambs. Counts of reticulocytes and red cells with Heinz bodies were done only of the naturally infected lambs.

acetate membranes ("Sepraphore III", Gelman Instruments Company, Ann Arbor) in the Beckman microzone chamber ("Microzone Cell", Beckman Instruments, Inc., Palo Alto) according to the manufacturers' manuals. Immunoelectrophoresis of serum was done as described by *Hirschfeld* (1960). The sheep antiserum was produced in rabbits injected with pooled sera from 5 sheep by the procedure given by the same author.

The DT was performed by a micromethod (*Waldeland* 1976 b). All sera from each lamb were stored at —20°C until they could be examined on the same day with the same batch of antigen and accessory factor serum.

Examination of muscular tissue for T. gondii was done as previously described (*Waldeland* 1976 a).

RESULTS

Natural infection in lambs

Most of the 13 lambs developed positive DT titres during August, i.e. when they were about 4 months old. The titres ranged from 1/16 to 1/512. No significant changes in the blood values nor of the growth rates were found in the infected lambs compared with the controls. The zone electrophoresis and the immunoelectrophoresis showed normal patterns.

Experimental infection in lambs

Examination of blood films showed that 1 lamb in Group II and 1 lamb in Group IV were infected with Eperythrozoon ovis. The blood data from these 2 lambs were therefore excluded from the statistical analysis, as were the blood data from 7 other lambs for the following reasons: Two of the controls developed positive DT titres during the last 2 months before slaughtering. One lamb inoculated subcutaneously with the sheep strain of T. gondii (Group II) and 4 lambs inoculated perorally (Group III) did not develop positive titres during the first 64 days after inoculation. Later on 2 of these 5 lambs developed positive titres.

The geometrical mean titres in the 4 groups with the remaining 31 lambs are shown in Fig. 1. Positive titres were recorded 9 days after inoculation in some lambs inoculated subcutaneously (Groups I and II), the highest titres being observed in lambs inoculated with the RH strain of the parasite. Positive titres were found 1 week later in lambs inoculated perorally (Group III).

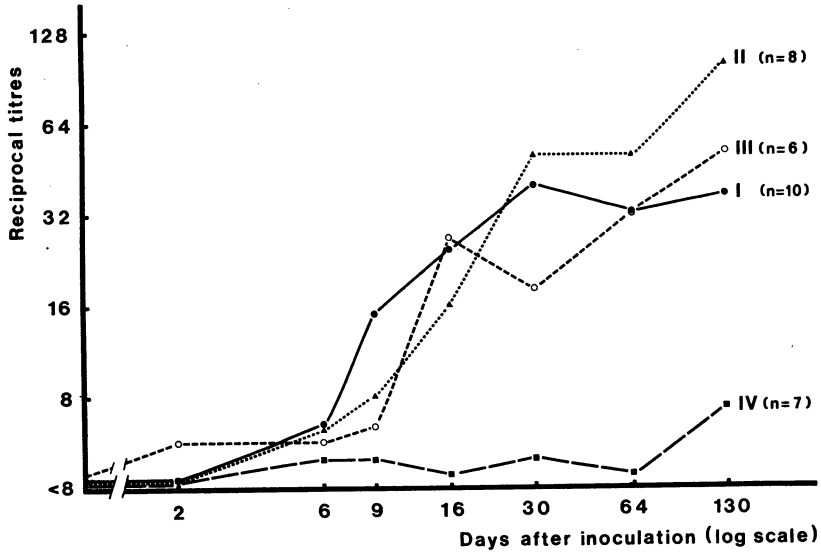

Figure 1. Dye test titres in 3 groups of lambs (I—III) inoculated with Toxoplasma gondii as recorded in Table 1, and in group 1 of not inoculated controls (IV). Lambs which acquired toxoplasmosis by natural infection, lambs with Eperythrozoon ovis infection, and lambs with no serological evidence of infection following inoculation are excluded.

The body temperatures of the lambs in Group I were moderately elevated, up to about 1 °C, from the 4th to the 7th day after inoculation. Moderately elevated temperatures were also recorded on the 9th day after inoculation in some lambs in Group III. The temperatures of the lambs in Group II remained stable. Some lambs in Group I showed dullness, deprived appetite and frequent respiration while the temperatures were elevated.

No distinct changes in the blood values nor in the electrophoretical patterns were found in the lambs which developed positive DT titres compared with the control group.

The results of the parasitological examination are recorded in Table 2. All lambs from which T. gondii was recovered had DT titres of $\geq$ 1/32 at slaughtering. The parasite was not recovered from any of the 10 lambs inoculated with the RH strain. All of the latter developed positive DT titres after inoculation, and had still titres up to 1/128 at slaughtering. Except these, all lambs from which the parasite was not recovered were DT negative.

Table 2. Recovery of Toxoplasma gondii by inoculation on mice from muscular tissue of 40 lambs, 30 of which were inoculated with the parasite as recorded in Table 1.

Animals	Recovery of Toxoplasma gondii							
	Group I		Group II		Group III		Group IV	
	+	—	+	—	+	—	+	—
Lambs that developed toxoplasma antibodies shortly after inoculation		10	8*	1	6			
Lambs that developed toxoplasma antibodies between 2 and 4 months after inoculation			1		1		2	
Lambs with no serological evidence of toxoplasma infection						3		8*
Total		10	9	1	7	3	2	8

+ : T. gondii recovered.
— : T. gondii not recovered.
* One lamb was infected with Eperythrozoon ovis.

DISCUSSION

The infection was not apparent in the naturally infected lambs. In the experimentally infected lambs only slight symptoms of unthriftiness were observed during the febrile period. These findings are mainly in agreement with observations in non-pregnant sheep reported in the available literature. *Smith* (1961) observed respiratory embarrassment that persisted for about 2 weeks in experimentally infected lambs. However, the rapid febrile response in his experiment indicated a heavier infection, probably caused by intraperitoneal inoculation of a greater number of the parasite than in the present work.

In the present investigation no significant changes in the blood values were found. The normal leucocyte counts are in contrast with those in 2 experimentally infected sheep recorded by *Michael et al.* (1972). This may be explained by the more severe course of infection in their experiment. In the present work the DT titres indicated that the experimental infection was comparable with a natural infection. It should also be mentioned

that no significant changes in blood values have been found in 20 ewes examined within 3 days after abortion in this laboratory (*Waldeland*, unpublished data). According to the present results inapparent toxoplasmosis is of negligible consequence for the blood data obtained during grazing experiments.

Both in Group I and in Group III positive DT titres were found in some lambs 5—7 days after elevated temperatures were recorded. The rapid antibody response in Group II where febrile reactions were not observed is therefore noteworthy. The question then arises if cysts, when given perorally, induces a later but heavier parasitaemia than when introduced parenterally. However, there was no correlation between the body temperatures and the DT titres, and the mean titres of lambs inoculated subcutaneously were higher than in lambs inoculated perorally except at one sampling. It is interesting to note that inoculation with trophozoites of the RH strain induced the most rapid antibody response, whereas inoculation with cysts of a sheep strain seemed to induce a more continuous antibody production.

The results of the parasitological examination indicate that the RH strain of T. gondii does not persist in sheep, at least not in the cystic stage, and therefore in this respect differs from sheep strains. This is consistent with a previous examination of 9 sheep with DT titres in the range from 1/16 to 1/128 at slaughtering, all of which had been inoculated with the RH strain 1 year earlier (*Waldeland*, unpublished data), and also with the examination of the 2 sheep by *Michael et al.*

The serological examination showed that of a total of 15 lambs previously DT negative, 4 acquired the infection during the period between the last 2 samplings, i.e. during the last 2 months before slaughtering. T. gondii was recovered from all of these 4 lambs. As all lambs were kept together, one should expect that they were exposed to the same source of infection, and that the parasite therefore also should have been recovered from some lambs in Group I. These findings therefore seem to indicate that a non-persisting strain of T. gondii may induce immunity against reinfection with other strains, although a statistically significant conclusion was not justified because of the low number of individuals. Further studies should be carried out to elucidate this presumption.

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SAMMENDRAG

Toxoplasmose hos sau. Hematologiske, serologiske og parasittologiske undersøkelser.

Blodverdier (celleverdier, hemoglobin-, mineral- og proteininnhold) ble undersøkt retrospektivt hos 13 lam som var blitt naturlig infisert med Toxoplasma gondii i løpet av sommeren, og hos 2 lam som var blitt infisert i tiden omkring fødsel. Immunelektroforese av serumprøvene ble også utført. Lammene hadde være fordelt på 2 grupper med til sammen 40 dyr i forsøk med beiter av ulik kvalitet, og var blitt undersøkt med en måneds mellomrom. Undersøkelser ble også foretatt av lam eksperimentelt infisert med RH stammen av T. gondii, av lam eksperimentelt infisert med en sauestamme av parasitten, samt av en kontrollgruppe. Det ble ikke funnet signifikante forandringer i blodverdiene i samband med infeksjonen.

Kjøttprøver fra de eksperimentelt infiserte lammene og fra kontrollgruppen ble undersøkt parasittologisk. T. gondii ble påvist hos alle dye test positive (titer $\geq$ 1/16) lam podet med sauestammen av parasitten, men ikke hos lam podet med RH stammen. Resultatene tydet på at RH stammen ikke danner cyster i muskulaturen hos sau. Noen observasjoner i forsøket tydet på at denne stammen av T. gondii kan beskytte mot infeksjon med andre stammer, men antall individer var for lavt til at det kunne trekkes en sikker konklusjon.

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