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Infectivity and cross-immunity studies of *Theileria lestoquardi* and *Theileria annulata* in sheep and cattle: I. In vivo responses

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Abstract

In a series of experiments, sporozoite stabilates of a *Theileria lestoquardi* (Lahr) and a *T. annulata* (Ankara) stock prepared from *Hyalomma anatolicum anatolicum* ticks, were used to examine the infectivity of both parasite species for sheep and cattle and to study the development of cross-immunity between these parasite species. In the first experiment sheep and cattle were inoculated with *T. lestoquardi* sporozoites. Surviving animals and naive sheep and cattle were, in the second experiment, inoculated with *T. annulata*. In the third experiment, naive sheep and sheep previously infected with *T. annulata*, were inoculated with *T. lestoquardi*. The following responses to inoculations were monitored: clinical and haematological signs of infection, appearance of parasitic stages of the parasites in lymph node biopsies and in peripheral blood and serological response to *T. lestoquardi* and *T. annulata* schizont antigens. While *T. lestoquardi* readily infected sheep and caused severe disease, it did not infect cattle. On the other hand, *T. annulata* infected both cattle and sheep. However, whereas cattle became severely affected, infected sheep showed mild clinical symptoms only and piroplasms did not develop. Despite their different behaviour in the host species examined, cross-immunity studies suggested that the parasite species are very closely related. Experiments in sheep indicated that *T. lestoquardi* infection protected against subsequent *T. annulata* infection. On the other hand, recovery from *T. annulata* infection did not prevent infection by sporozoites of *T. lestoquardi*, resulting in the establishment of schizonts and their

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subsequent development into piroplasms, although it protected against the major clinical effects of *T. lestoquardi* infection.

Keywords: *Theileria annulata*; *Theileria hirci*; *Theileria lestoquardi*; Cattle-protozoa; Sheep-protozoa; Experimental infections

1. Introduction

Tick-borne protozoan parasites of the genus *Theileria* infect wild and domestic ruminants. In their mammalian hosts, the parasites have a complex life cycle, with sporozoites transforming into schizonts in white blood cells of the mononuclear lineage, followed by the development of piroplasms in erythrocytes. Identification of the *Theileria* species and characterisation of parasite stocks are based on different criteria such as vector specificity, parasite morphology, host specificity and pathogenicity, serological cross-reaction as well as cross-protection to other species within the genus (Uilenberg, 1981; Irvin and Morrison, 1987; Morzaria, 1989).

In cattle, the most important species are *T. annulata* and *T. parva*, causing tropical theileriosis and East Coast fever, respectively, myelo- and lymphoproliferative diseases that in susceptible cattle usually are fatal. Of the species infecting small domestic ruminants, *T. lestoquardi* (syn. *T. hirci*) transmitted by *Hyalomma anatolicum anatolicum* ticks is regarded as the only pathogenic one (Hooshmand-Rad and Hawa, 1973a, b; Morel and Uilenberg, 1981).

The parasite presently described as *T. lestoquardi* of small ruminants resembles *T. annulata* of cattle in various biological respects. They share at least one vector tick, and thus their known distribution overlaps at least partially, with *T. lestoquardi* being reported from south-eastern Europe, northern Africa, the Near and Middle East and India (Levine, 1985). The schizont stage of both parasite species can, like in several other species of *Theileria*, be cultured in vitro (Hooshmand-Rad and Hawa, 1975; Brown, 1987) and they have antigenic similarities in the indirect fluorescent antibody test (IFAT) (Leemans et al., 1997). In addition, *T. annulata* sporozoites can infect sheep peripheral blood mononuclear cells (PBMC) in vitro (Steuber et al., 1986; Entrican et al., 1991).

To analyse differences and similarities between these parasite species in more detail, comparative experiments based on sporozoite stabilates of a previously uncharacterised stock of *T. lestoquardi* (Lahr) and a well-known *T. annulata* stock (Ankara) (Schein et al., 1975) were carried out. The infectivity of both parasite species for sheep and cattle was studied comparing clinical, parasitological and haematological parameters as well as antibody reactions in IFAT. Preliminary cross-immunity studies were also performed, in which sheep and cattle were challenged with the reciprocal parasite species as used for the initial inoculations.

2. Materials and methods

2.1. Experimental design

A series of experimental infections was carried out in which the infectivity of *T. lestoquardi* and *T. annulata* for sheep and cattle was studied. All experimental

infections, comprising eight calves (C1–C8) and 14 sheep (S1–S14), were initiated by the subcutaneous inoculation of sporozoite stabilates as described by Brown (1987).

In the first experiment (Exp I), the susceptibility of sheep and cattle to infection with *T. lestoquardi* sporozoites was compared in six sheep (S1–S6) and six calves (C1–C6). Two sheep (S7–S8) and two calves (C7–C8) served as uninfected controls.

The second experiment (Exp II) was carried out to determine (i) whether sheep could act as hosts for *T. annulata* and (ii) whether previous exposure of sheep and cattle to *T. lestoquardi* protected against *T. annulata* challenge. Six weeks after the start of Exp I, four naive sheep (S7–S10) and two naive calves (C7–C8) as well as four sheep (S1–S3 and S6) and six calves (C1–C6) from Exp I were inoculated with *T. annulata* sporozoites.

The final experiment (Exp III) was designed to study whether sheep that had undergone *T. annulata* infection developed immunity to *T. lestoquardi* sporozoite challenge. This experiment, comprising the four sheep that had initially been introduced as naive animals in Exp II (S7–S10) and four more sheep (S11–S14) as susceptible controls, was initiated 7 weeks after the start of Exp II.

2.2. Experimental animals

Eight female Swedish Friesian calves, 4–6 months old at the start of the examination period, were obtained from a bovine viral diarrhoea virus (BVDV) and bovine leukaemia virus-free farm. Fourteen male sheep of the Swedish landrace, 6 months old when the experiments started, came from a Border disease and Maedi-Visna-free flock. All animals arrived 1 week before initiation of the experiments and were housed and kept according to routine procedures at the Department of Ruminant Medicine and Veterinary Epidemiology at the Swedish University of Agricultural Sciences (SLU). Sheep were sheared and dewormed upon arrival. At the end of each challenge experiment or earlier to avoid severe suffering, animals were killed by intravenous injection of barbiturates.

2.3. Sporozoite stabilates

Different cryo-preserved sporozoite stabilates, prepared as described by Brown (1987) from ground-up tick supernatant of laboratory-reared 3-day prefed *H. a. anatolicum* ticks infected with *T. annulata* (Ankara) (Schein et al., 1975) or *T. lestoquardi* (Lahr), were used for inoculations. The latter stock was originally isolated from ticks feeding on a sheep undergoing natural *T. lestoquardi* infection in Lahr, Iran, and has subsequently been maintained at the Razi Institute in Tehran (Hooshmand-Rad, 1995, pers. comm.).

In Exp I, animals were inoculated at the left side of their necks with 1 ml each of two different *T. lestoquardi* batches, resulting in a final dose of 4.5 tick equivalents per animal (te/animal), whereas in Exp II, animals were inoculated at the right side of their necks with 0.5 ml of a *T. annulata* stabilate (1 te/animal). In Exp III, 2 ml of one batch of *T. lestoquardi* stabilate at 4 te/animal, applied at the left side of the neck, was used for

each sheep. The dosages of the stabilates used for the establishment of experimental infections were determined on the basis of in vivo and in vitro infectivity studies carried out earlier (Brown et al., 1998).

2.4. Clinical evaluation of infection

Animals were monitored daily for clinical signs of infection for 3–5 weeks from the day of inoculation (Day 0). The following parameters were recorded: body temperature, general condition, appetite, consistency of faeces, presence of cough, conjunctival and nasal discharge and the size of palpable lymph nodes. Rectal temperatures over 39.4°C in calves and over 39.8°C in sheep in association with schizont parasitosis were considered as febrile reactions to *Theileria* infections. Severity of reactions was classified as inapparent, mild, moderate or severe according to recommendations from a workshop on *T. parva* (Anon., 1989).

2.5. Parasitological evaluation of infection

Needle biopsies from lymph nodes draining the site of inoculation (left or right prescapular lymph nodes) were taken on alternate days from the first day of fever or when the lymph nodes were enlarged until they diminished in size and were analysed for the presence of schizont-infected cells in Giemsa-stained cytospin smears. The remaining cells were cultured to detect growth of parasite-infected cells (for results of these latter experiments see Leemans et al. (1999)). Development of schizont- and piroplasm-parasitaemia was followed every other day by examination of Giemsa-stained blood smears for 1 month and thereafter once a week.

2.6. Haematological evaluation of infection

From the start of each experiment, packed red blood cell volumes (PCV) were measured every other day by the microhaematocrit centrifuge method in blood collected by jugular vein puncture in EDTA containing vacutainer tubes (Becton-Dickinson, Meylan Cedex, France). PCV values below 28% for calves and below 22% for sheep were considered sub-normal. From Day 10 onwards through clinical reaction, total white blood cell (WBC) counts were determined at the Department of Clinical Chemistry, SLU, according to standard procedures. In both sheep and cattle, WBC values of below 4×10^9 cells/l were regarded as subnormal.

2.7. Serological evaluation of infection

Reactions to primary and challenge infections were analysed by measurement of the antibody responses to *T. lestoquardi* and *T. annulata* schizont antigens by IFAT as described earlier (Leemans et al., 1997). Sera of all animals were tested and their endpoint titres determined from the following occasions; from Exp I sera of Days 0, 28 and 42; from Exp II sera of Days 0, 21 and 49 and from Exp III sera of Days 0 and 28.

3. Results

3.1. Reactions of sheep to primary T. lestoquardi inoculation (Exp Ia)

The main clinical, parasitological and haematological reactions of sheep to *T. lestoquardi* infection are summarised in Table 1. In all inoculated sheep, enlarged lymph nodes were seen at the side of inoculation from Days 5–6 onwards, followed by an increase in the size of the contra-lateral lymph node and the development of fever in the second week after inoculation. Moreover, a range of other clinical symptoms was observed in five of the six sheep. These symptoms, varying in severity among individuals, included loss of condition, intermittent diarrhoea, coughing and listlessness. One sheep was killed on Day 17 and another died on Day 22, whereas the others gradually recovered. Two sheep received supportive oral treatment with a glucose/electrolyte solution for ruminal atony.

Schizont-infected cells were observed in lymph node biopsy smears of all six sheep and the highest parasitosis (9%) was observed on Day 11, the last day that lymph nodes were sampled during the clinical reaction period. Schizont-infected cells were seen in low numbers in peripheral blood from Days 10–14. Piroplasms were detected in all sheep and parasitaemia slowly increased until maximum levels of 5.5–32% were reached. In surviving sheep, piroplasms were still demonstrated on Day 42.

All animals showed a drop in WBC counts with lowest values ranging from 2.9–6.8×10^9 cells/l. Mean lowest WBC count fell to 55% of mean pre-infection value. PCV levels diminished in all sheep except the one that was killed on Day 17. In the fourth week of infection, the mean PCV value of five sheep decreased to 60% of mean pre-infection level.

In the two non-inoculated control sheep no changes were observed in any of the clinical parameters, but mean WBC fell to 89% and mean PVC to 82% of mean pre-infection values.

Table 1
Primary reactions of sheep to inoculation with *T. lestoquardi* (Exp Ia)

Clinical, parasitological and haematological parameters	Sheep number					
	S1	S2	S3	S4	S5	S6
First day of fever >39.8°C	14	12	12	10	12	9
Total number of days with fever	10	10	8	11	6	17
Max. temperature (°C)	41.4	41.5	41.4	41.6	41.8	41.8
First day of schizont detection in lymph node	9	7	9	9	9	9
First day of piroplasm detection	10	12	12	12	12	12
Peak piroplasm parasitaemia (%)	5.5	5.5	6.0	32.0	10.6	9.5
Fall in WBC (%)	18	57	55	62	40	35
Fall in PCV (%)	27	39	42	48	18	45
Survival (s) or day of death/euthanasia	s	s	s	22	17	s

3.2. Reactions of cattle to primary T. lestoquardi inoculation (Exp Ib)

Very slightly enlarged draining lymph nodes were seen on Days 7–10 in all six calves following *T. lestoquardi* inoculation. This was the only clinical sign considered to be related to the inoculation of the sporozoite stabilate. Neither schizonts nor piroplasms were seen in Giemsa-stained blood or lymph node smears and no significant decreases were recorded in PCV or WBC counts.

However, temperatures above 39.4°C were recorded intermittently in all calves including both non-inoculated controls. Moreover, all calves developed a persistent cough and one calf showed slight diarrhoea before inoculation. Analysis of pre-inoculation- and Day 28 sera for the presence of antibodies to adeno-, BVDV-, corona-, parainfluenza- and respiratory syncytial virus showed seroconversion in all calves to corona virus while no changes in titres to any of the other infectious agents were demonstrated. Therefore, clinical symptoms including febrile reactions were regarded to be the result of a concomitant corona virus infection that the calves had inadvertently become exposed to.

3.3. Reactions of cattle to T. annulata inoculation (Exp IIa)

A summary of the main clinical, parasitological and haematological observations in calves undergoing *T. annulata* infection is presented in Table 2. All calves inoculated with *T. annulata* became ill from Day 10 onwards and developed severe theileriosis with high fever and generalised lymph node enlargement. The condition of three of the calves previously inoculated with *T. lestoquardi* and of the two control animals undergoing primary *T. annulata* infection deteriorated progressively and these animals were killed. In the other three previously inoculated calves, symptoms varied in severity but these calves recovered.

In all calves schizont-infected cells were seen, after their first detection on Day 5, in increasing numbers in lymph node biopsy smears at all later sampling occasions.

Table 2
Reactions of naive cattle and of cattle previously inoculated with *T. lestoquardi* to inoculation with *T. annulata* (Exp IIa)

Clinical, parasitological and haematological parameters	Calf number							
	Naive		Previously inoculated					
	C7	C8	C1	C2	C3	C4	C5	C6
First day of fever >39.4°C	6	6	5	5	6	4	6	5
Total number of days with fever	11	10	18	24	10	28	9	12
Max. temperature (°C)	41.7	>42.0	41.7	41.6	41.9	>42.0	41.9	41.9
First day of schizont detection in lymph node	5	7	5	7	5	7	7	7
First day of piroplasm detection	10	10	10	10	10	10	10	10
Peak piroplasm parasitaemia (%)	81.2	81.0	42.5	21.8	93.8	60.6	66.0	74.8
Fall in WBC (%)	76	74	43	66	75	73	88	65
Fall in PCV (%)	71	61	57	45	65	69	50	57
Survival (s) or day of death/euthanasia	17	15	s	s	16	s	14	16

Piroplasms were first observed on Day 10 and parasitaemias thereafter rapidly increased in all animals.

WBC counts dropped in all calves with lowest values ranging from 1.2–4.7 $\times 10^9$ cells/ml. Mean lowest WBC count fell to 30% of mean pre-infection level and no differences were noted between challenged individuals and those undergoing primary infection. All calves became anaemic and lowest PCV counts of 10–18% were recorded 14–16 days post-inoculation in challenged and control calves. The mean lowest PCV value was 41% of mean pre-inoculation level.

3.4. Reactions of sheep to T. annulata inoculation (Exp. IIb)

The reactions of sheep to inoculation of *T. annulata* sporozoite stabilate are shown in Table 3. No clinical symptoms except a rise in body temperature and varying degrees of lymph node enlargement were observed in any of the sheep inoculated with *T. annulata*. These reactions were all more pronounced in the sheep after primary inoculation with *T. annulata* compared to reactions in those previously inoculated with *T. lestoquardi*.

Following primary inoculation of *T. annulata*, pre-scapular lymph nodes at the side of inoculation were markedly increased in size in all four sheep between Days 5–14. A slight increase of the contra-lateral node was observed in one sheep and fever lasting for 3–4 days developed in three animals. In challenged sheep, an obvious increase in size of the draining lymph node was seen in one sheep. This was also the only challenged sheep in which slight fever was observed.

Schizont-infected cells were rarely observed in two sheep following primary inoculation of *T. annulata*, whereas high numbers of mononuclear blast cells were seen in direct smears of lymph node biopsies of all four sheep. Piroplasms were never demonstrated in any of these sheep. In challenged sheep schizont-infected cells were never detected, but rare piroplasm-infected erythrocytes were sometimes still seen.

Table 3
Reactions of naive sheep and of sheep previously inoculated with *T. lestoquardi* to inoculation with *T. annulata* (Exp IIb)

Clinical, parasitological and haematological parameters	Sheep number							
	Naive				Previously inoculated			
	S7	S8	S9	S10	S1	S2	S3	S6
First day of fever >39.8°C	8	8	7	–	–	–	–	–
Total number of days with fever	4	5	3	-	–	–	–	3
Max. temperature (°C)	40.1	41.2	40.0	–	–	–	–	40.0
First day of schizont detection in lymph node	5	–	7	–	–	–	–	–
First day of piroplasm detection	–	–	–	–	2	–	6	2
Peak piroplasm parasitaemia (%)	–	–	–	–	<0.5	–	<0.5	<0.5
Fall in WBC (%)	25	24	7	12	103[a]	15	23	127[a]
Fall in PCV (%)	14	–	15	19	9	–	17	7
Survival (s) or day of death/euthanasia	s	s	s	s	s	s	s	s

[a] Increase in WBC expressed as percentage of pre-infection level.

Table 4
Reactions of naive sheep and of sheep previously inoculated with *T. annulata* to inoculation with *T. lestoquardi* (Exp III)

Clinical, parasitological and haematological parameters	Sheep number							
	Naive				Previously inoculated			
	S11	S12	S13	S14	S7	S8	S9	S10
First day of fever >39.8°C	13	10	12	17	–	10	–	2*
Total number of days with fever	8	14	7	3	–	4	–	11
Max. temperature (°C)	41.1	41.2	>42.0	40.7	–	40.3	–	40.5
First day of schizont detection in lymph node	9	9	9	11	7	11	11	11
First day of piroplasm detection	12	12	12	12	12	12	12	12
Peak piroplasm parasitaemia (%)	11.8	39.6	8.6	6.2	5.8	1.8	0.6	5.0
Fall in WBC (%)	60	57	86	76	49	59	43	60
Fall in PCV (%)	53	53	10	39	23	30	11	49
Survival (s) or day of death/euthanasia	s	26	19	s	s	s	s	s

[a] Fever in this animal was biphasic with the second peak starting on Day 15.

A fall in WBC counts was seen in most sheep but values always remained well above minimum normal levels for sheep, and during Exp I a decrease to similar levels was observed in the non-inoculated sheep. A fall in PCV levels comparable to the fall seen in the latter sheep during Exp I was also observed during Exp II in sheep of both groups, but below-normal PCV values were never seen.

3.5. Reactions of sheep to T. lestoquardi inoculation (Exp III)

The main reactions of sheep to primary or challenge inoculation of *T. lestoquardi* stabilate are presented in Table 4. Reactions of sheep to primary inoculation were comparable to those observed during Exp I (Table 1), with the exception of one sheep in which fever developed somewhat later, reached a lower maximum level and lasted for a shorter period. Besides fever and lymph node enlargement which were observed in all four sheep, three animals clearly lost condition and became dull. One sheep developed severe diarrhoea whereas another developed ruminal atony. These sheep were killed on Days 19 and 26, respectively.

In sheep that were previously inoculated with *T. annulata*, fever was demonstrated in two sheep and in both animals it was substantially lower than in three of the four control sheep undergoing primary infection. In all challenged sheep draining lymph nodes increased in size, but to a lesser extent than in the controls. Moreover, changes in size were first seen some days later than in the controls and no changes were detected in the nodes at the contra-lateral side. No other clinical signs of disease were observed.

Schizont-infected cells were detected in lymph node biopsy smears of all control sheep undergoing primary *T. lestoquardi* infection, and such cells were also seen in low numbers in peripheral blood. Schizonts were also demonstrated in very low numbers in biopsies from the contra-lateral side when first looked for on Day 11. Piroplasm parasitaemia reached somewhat higher levels than during Exp I. In all challenged sheep, schizont-infected cells were detected in low numbers in lymph node biopsies at the side

of inoculation and in some sheep also in very low numbers in biopsies of the contralateral node. In none of the challenged sheep were schizont-infected cells seen in peripheral blood smears. Piroplasms were first seen at the same time as in control sheep, but at lower levels. Parasitaemias in two challenged sheep always remained substantially lower than in the control sheep, but in two other sheep maximum piroplasm levels were comparable to those in the controls.

Leukopenia (values ranging from 1.0–3.9 $\times$ 10^9 cells/ml) developed during the second week of infection in three sheep undergoing primary infection but also in three of the challenged sheep. In control sheep, lowest mean WBC values dropped to 29% of pre-infection levels and in challenged sheep to 41%. With the exception of the sheep that was killed on Day 19, all controls became anaemic with lowest mean PCV values falling to around 60% of mean pre-infection levels. Challenged sheep showed considerable individual variation: whereas two sheep showed a slight decrease in PCV values, the other two sheep developed anaemia, which in one animal was severe.

3.6. Serological findings

The antibody responses to *T. lestoquardi* antigen of sheep sera collected during Exps. I–III are summarised in Table 5. On Day 28, an antibody reaction was demonstrated in sera of all sheep that were inoculated with *T. lestoquardi* (Exp I), and by Day 42 these levels had increased further. In all sera, cross-reactions of a level similar to *T. annulata* antigen were seen (data not shown). Three weeks after primary inoculation of *T. annulata* (Exp II), all sheep showed an antibody response to *T. annulata* antigen with titres ranging between 1 : 640–1 : 2560 (data not shown). Titres to *T. lestoquardi* antigen were somewhat lower (Table 5). After challenge with *T. annulata* to sheep that previously had

Table 5
Antibody reactions of sheep sera in the IFAT applying *T. lestoquardi* schizont antigen

Sheep no.	Reciprocal endpoint titres							
	ExpI[a]			Exp II[b]			Exp III[c]	
	d0	d28	d42	d0	d21	d49	d0	d28
S1	<40	320	10 240	10 240	10 240			
S2	<40	320	2 560	2 560	5 120			
S3	<40	160	2 560	2 560	5 120			
S6	<40	80	10 240	10 240	10 240			
S7				<40	160	640	640	10 240
S8				<40	640	160	160	20 480
S9				<40	640	640	640	5 120
S10				<40	640	640	640	>20 480
S11							<40	160
S14							<40	160

Sera were collected on different days (d_n) following primary or challenge inoculation of sheep with sporozoites of *T. lestoquardi* or *T. annulata*.
[a] Exp I = period following inoculation with *T. lestoquardi* on Day 0.
[b] Exp II = period following inoculation with *T. annulata* 42 days after the start of Exp I.
[c] Exp III = period following inoculation with *T. lestoquardi* 49 days after the start of Exp II.

been inoculated with *T. lestoquardi*, antibody titres to *T. lestoquardi* antigen either showed a minimal increase compared to pre-challenge titres or had the same titre but with a somewhat stronger intensity of fluorescence. Antibody reactions to *T. lestoquardi* antigen of naive sheep infected with *T. lestoquardi* during Exp III were comparable to the responses seen during Exp I, with all sheep seroconverting and antibody levels on Day 28 being of the same magnitude. On the other hand, much higher titres were recorded in sheep that had previously been inoculated with *T. annulata*, and endpoint titres on Day 28 were comparable to those recorded in sheep on Day 42 during Exp I.

No antibody response was detected in any of the calves inoculated with *T. lestoquardi* (Exp I) in sera of Days 0, 28 and 42 when tested against *T. lestoquardi* or *T. annulata* schizont antigens. In contrast, an antibody response to *T. annulata* schizont antigen was demonstrated in sera from Day 14 in five of the calves inoculated with *T. annulata* (Exp II), with endpoint titres varying between 1 : 40–1 : 160. By Day 28 the three surviving calves were all seropositive to *T. annulata* antigen with titres of 1 : 320–1 : 1280. At this time sera cross-reacted with the *T. lestoquardi* schizont antigen at a dilution of 1 : 40.

4. Discussion

While *T. lestoquardi* readily infected the sheep and in this host species caused a myelo- and/or lymphoproliferative disease, no indications were found that calves were infected by *T. lestoquardi*. Furthermore, it was demonstrated that the sheep, which is not considered to be a natural host of *T. annulata* (Uilenberg, 1981; Robinson, 1982), can become infected with this parasite. However, the developmental cycle of *T. annulata* in the sheep seemed to be incomplete since no piroplasms were detected. Cross-protectivity studies in sheep suggested that some degree of reciprocal cross-immunity developed between *T. lestoquardi* and *T. annulata*.

Apart from preliminary in vivo and in vitro experiments reported by Brown et al. (1998) that formed the basis for the experiments described here, this is the first time that *T. lestoquardi* infections have been established using sporozoites obtained from ticks as described for other *Theileria* species (Brown et al., 1973). All naive sheep that were inoculated with sporozoites of the Lahr stock of *T. lestoquardi* developed malignant ovine theileriosis. Generalised lymph node enlargement and high fever were the most prominent symptoms of the *T. lestoquardi* infection. Another consistent finding was a marked fall in WBC counts, often resulting in leukopenia that lasted for several days. Anaemia was also recorded in all sheep that survived for more than 3 weeks. Although individuals showed a rather wide variation in range and magnitude of other clinical symptoms, the first appearance of parasites was quite uniform in all sheep. Schizont-infected cells were first seen in lymph nodes draining the site of inoculation but were later also demonstrated in peripheral blood and in lymph nodes at the contra-lateral side. Like in other *Theileria* infections, *T. lestoquardi*-infected sheep remained carriers of piroplasms for more than 8 weeks.

While infection with *T. lestoquardi* was demonstrated in all sheep, no signs of infection were ever observed in any of the calves. Calves reacted neither clinically nor serologically following the inoculation of *T. lestoquardi* sporozoites and no develop-

mental stages of the parasite were ever detected. Sheep and cattle thus differ significantly in their susceptibility to infection with *T. lestoquardi*, and although the numbers of cattle tested were too low to fully exclude the possibility, our data indicate that cattle do not become infected with this parasite. An attempt to infect cattle with a parasite of sheep described as *T. hirci* has been reported earlier (Raghvachari and Madhava Krishna Reddy, 1959). While sheep were successfully infected by mechanical transmission of blood, their attempt to infect a calf failed. Moreover, neither Brown et al. (1998) nor Kirvar et al. (1998), were able to demonstrate *T. lestoquardi* infection in cattle inoculated with the same stock of the parasite as used here.

All calves, independent of previous exposure to sporozoites of *T. lestoquardi*, developed severe theileriosis after inoculation with sporozoites of *T. annulata*. No major differences in clinical signs were observed between calves undergoing primary *T. annulata* infection and those previously inoculated with *T. lestoquardi*. Also, prepatent periods to detection of schizonts and of piroplasms as well as antibody responses to schizont antigens were the same in both groups of calves. The clinical, parasitological and haematological reactions observed here were in agreement with those reported for calves undergoing primary *T. annulata* infection (Gill et al., 1977; Pipano et al., 1981; Innes et al., 1989; Preston et al., 1992). Serological responses were also comparable to earlier observations (Burridge et al., 1974; Pipano et al., 1977). It was concluded that previous inoculation of *T. lestoquardi* in calves in no way influenced the course of subsequent *T. annulata* infection.

Reactions to *T. annulata* inoculation of naive sheep ranged from mild to virtually inapparent with considerable swelling of the pre-scapular lymph node at the side of inoculation being the most obvious reaction. Infection was proven by the demonstration of schizont-infected cells, albeit in two of four animals only. However, the fact that all sheep reacted serologically following primary inoculation of *T. annulata* sporozoites, indicated that all sheep had become infected. Infection of all sheep was later confirmed by culture of schizont-infected cells (Leemans et al., 1999). Of particular interest is the fact that in none of these sheep were piroplasms ever observed, indicating that *T. annulata* is unable to complete its life cycle in sheep. However, piroplasms may have developed at levels too low to be detected by microscopical examination of peripheral blood and no attempts were carried out to analyse whether ticks applied onto *T. annulata*-infected sheep were able to pick up infection.

To our knowledge, this is, apart from the preliminary studies by Brown et al. (1998), the only report of successful infection of sheep with sporozoites of *T. annulata*. Cross-infectivity studies have been widely used to study the host range of different *Theileria* species (Uilenberg, 1981), but very few reports are available on attempts to infect sheep with *T. annulata* (Sergent et al., 1945; Neitz, 1957) and these were unsuccessful. However, several authors have shown that sheep PBMC can become infected in vitro with sporozoites of *T. annulata* and transform into continuously growing cell lines (Steuber et al., 1986; Entrican et al., 1991), and sheep have been inoculated with autologous in vitro infected *T. annulata* cells (Steuber et al., 1986). Interestingly, these authors found no erythrocytic stages in sheep inoculated with such autologous cells, whereas mild symptoms of theileriosis, including demonstration of piroplasms, were observed in cattle inoculated with in vitro established *T. annulata* schizont-infected sheep cells.

The question whether previous *T. lestoquardi* infection of sheep protected against subsequent infection with *T. annulata* could not be conclusively answered, although some findings indicated that this was the case. Slight differences were observed between primary infected and challenged sheep in clinical reactions, of which the increased sizes of the draining lymph nodes in sheep undergoing primary *T. annulata* infection were the most prominent findings. Moreover, schizonts were detected in some of the latter sheep, but not in any of the sheep challenged with *T. annulata* following *T. lestoquardi* infection. Serologically, challenged sheep responded only very weakly, with antibody titres remaining the same or increasing one dilution step only, also suggesting a limited multiplication of the parasites in sheep.

When compared to sheep undergoing primary *T. lestoquardi* infection, previous *T. annulata* infection protected sheep from the major clinical consequences of *T. lestoquardi* infection. However, this protection was shown to be partial only and did not occur in all animals, since substantial differences were shown in individual parasitological and haematological parameters. It may be of interest that the sheep reacting strongest to *T. lestoquardi* challenge inoculation was the only animal that had not shown a temperature reaction to primary *T. annulata* infection. Moreover, both schizonts and piroplasms were demonstrated in all challenged sheep, albeit in some of them at lower levels than in sheep undergoing primary *T. lestoquardi* infection. Although these parasites in theory could be either *T. lestoquardi* or *T. annulata*, the fact that piroplasms also developed, which were never seen during primary *T. annulata* infection of sheep, made it more likely that they were *T. lestoquardi*. Also, all challenged sheep reacted serologically with a strong secondary antibody response. Thus, previous infection with *T. annulata* did not prevent sheep from infection with *T. lestoquardi*.

Analysis of serum samples from cattle and sheep used in the present experiments, confirmed our earlier observations of a high degree of cross-reactivity of *T. lestoquardi*- and *T. annulata*-specific antisera with their reciprocal schizont antigens (Leemans et al., 1997). Since we showed here that sheep can become infected with *T. annulata* also, IFAT based on schizont antigens will not be useful for differentiation of infections caused by either of the parasite species in sheep.

In conclusion, this series of experiments provided evidence that a significant difference exists between *T. lestoquardi* and *T. annulata* in their capacity to infect sheep and cattle. While it was demonstrated that sheep can become infected with *T. annulata*, our data strongly indicate that cattle do not become infected with *T. lestoquardi*. Moreover, our data suggested that in sheep partial cross-immunity developed of *T. annulata* to *T. lestoquardi* and vice versa, indicating a very close relationship between both parasite species.

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