

# Persistence of *Ehrlichia phagocytophila* Infection in Two Age Groups of Lambs

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**Stuen S, Bergström K: Persistence of *Ehrlichia phagocytophila* infection in two age groups of lambs. Acta vet. scand. 2001, 42, 453-458.** – Tick-borne fever (TBF) is caused by the rickettsiae *Ehrlichia phagocytophila* and is a common disease in sheep in tick (*Ixodes ricinus*) infested areas in Norway. Earlier investigations have shown that some sheep could remain infected for several months after the primary infection. In this study, the persistence of *E. phagocytophila* after experimental infection was investigated in 2 age groups of lambs. Six lambs (1-2 weeks old) and 14 lambs (6-8 months old) were inoculated intravenously with an ovine strain of *E. phagocytophila* and thereafter examined clinically (including daily body temperature recording) and by haematological and serological (*E. equi* antibodies) methods for the next 4 months. At the end of this period, the lambs were examined for a TBF infection by blood smear investigation and blood inoculation studies. The infection was demonstrated in 19 (95%) of the 20 lambs.

*granulocytic Ehrlichia; tick-borne fever; sheep; age.*

## Introduction

Tick-borne fever (TBF) caused by *Ehrlichia phagocytophila* and transmitted by the tick *Ixodes ricinus* is an old and very common disease in sheep from the coast of southern Norway (Stuen 1997, 1998). This disease is characterized by high fever, inclusions in circulating neutrophils, reduced milk yield, abortion and reduced fertility in rams (Woldehiwet & Scott 1993). However, the most serious problem associated with TBF in sheep is the immunosuppression that may dispose to secondary infections, such as *Staphylococcus aureus* pyaemia and *Pasteurella hemolytica* septicaemia (Brodie *et al.* 1986, Stuen 1996).

Earlier studies indicate that older lambs and adults may be persistently infected with *E. phagocytophila* for several months; one adult sheep has been found infected 25 months after the initial infection (Foggie 1951, Stuen *et al.* 1998). Experimental blood inoculation trials

with *E. phagocytophila* have shown that 1-2-week-old lambs react with less clinical symptoms than older animals (Stuen *et al.* 1992, Stuen 1993). The purpose of the present study was to investigate whether young lambs also become persistently infected with *E. phagocytophila*, and to compare the rate of persistence of the infection in these lambs with the rate of persistence in older lambs.

## Materials and methods

Forty lambs of the Dala and Rygja breeds were used in this study. Twenty lambs were inoculated intravenously on day 0 with 1 ml of a whole blood dimethyl sulphoxide stabilate of an *E. phagocytophila* strain (GenBank accession number M73220) originally isolated from a sheep (Stuen *et al.* 1992). Six lambs were 12-14 days old, while 14 lambs were 6-8 months old at the start of the study. In addition, 20

lambs of the same age were followed simultaneously as uninfected controls. None of them had previously been on *I. ricinus*-infested pasture and were kept indoors during the whole experimental period of 5 months.

Rectal temperatures were measured daily at the same hour in the morning in all lambs throughout the experimental period. The incubation period was defined as the period between inoculation and the first day of fever ( $\geq 40.0^\circ\text{C}$ ). The duration of fever was recorded as the number of days with elevated body temperature ( $\geq 40.0^\circ\text{C}$ ). The magnitude of fever was calculated as the area under the temperature curve for each lamb as described by Woldehiwet & Scott (1982).

Blood samples were collected daily into EDTA during the fever period following the inoculation of infected blood, and then weekly for the first month, and finally 4 months after the inoculation. In addition, EDTA-blood samples were collected from individual lambs on days when rectal temperatures above  $40.0^\circ\text{C}$  were recorded. Hematological values including total and differential leucocyte counts were determined electronically (Technicon H1<sup>®</sup>, Miles Inc., USA) and blood smears were prepared and stained with May-Grünwald Giemsa. Four hundred neutrophils were examined on each smear by microscopy and the number of these cells containing *Ehrlichia* inclusions was recorded.

Serum samples were collected on days 0, 30 and 120 and analysed by an indirect immunofluorescence antibody assay (IFA) to determine the antibody titre to *E. equi* (Artursson *et al.* 1999). Briefly, 2-fold dilutions of sera were added to slides precoated with *E. equi* antigen (Protatek International and Organon Teknika). Bound antibodies were visualised by fluorescein-isothiocyanate (FITC)-conjugated rabbit-anti-sheep immunoglobulin (Cappel, Organon Teknika) under fluorescent light. Sera were screened for antibodies at dilution 1:40. If positive, the serum was further diluted and retested. A titre of 1.6 ( $\log_{10}$  reciprocal of 1:40) or more was regarded as positive.

After 4 months, the inoculated lambs were treated intramuscularly with 50 mg corticosteroid (Prednisolonacetat vet<sup>®</sup>, Hoechst or Prednisolon<sup>®</sup>, Leo) daily for 4 consecutive days. On the day after the last treatment, each of the 20 susceptible control lambs was inoculated intravenously with 200-250 ml citrate-blood taken from the previously inoculated animals, each of the 20 donors providing blood to 1 control, respectively. The clinical and haematological reactions of the donor and susceptible lambs were observed during the next 3 weeks. Statistical calculations were done by Statistix<sup>®</sup>, version 4.0 (Analytical Software).

Table 1. Mean and standard deviation (std) of different clinical variables in *Ehrlichia phagocytophila* infected lambs. The lambs were inoculated with *E. phagocytophila* infected blood when they were 12-14 days old and 6-8 months old, respectively.

Age	N	Incubation period (days)	Max. temperature ( $^\circ\text{C}$ )	Duration of fever (days)**	Magnitude of fever ( $\text{mm}^2$ )*	Infected neutrophils ( $10^9 \text{ litre}^{-1}$ ) (day 6)	Numbers of neutrophils ( $10^9 \text{ litre}^{-1}$ ) (day 14)**
12-14 days	6	$4.0 \pm 0.58$	$41.40 \pm 0.141$	$4.3 \pm 1.11$	$364 \pm 102$	$1.35 \pm 0.853$	$0.68 \pm 0.154$
6-8 months	14	$4.3 \pm 0.60$	$41.62 \pm 0.204$	$8.6 \pm 2.02$	$702 \pm 232$	$1.51 \pm 0.590$	$0.31 \pm 0.061$

n number of animals

\* ( $p < 0.05$ , two-sample t-test)

\*\* ( $p < 0.01$ , two-sample t-test)

Table 2. Number of fever relapses and antibody immunofluorescent (IFA) titre to *E. equi* in twenty lambs during the first 4 months of an *E. phagocytophila* infection. The lambs were inoculated with *E. phagocytophila* infected blood when they were 12-14 days old and 6-8 months old, respectively.

Age	n	Number of fever relapses ***	IFA- titre ( $\log_{10}$ )		Number of lambs found infected with <i>Ehrlichia</i> after 4 months
			(day 30) ***	(day 120)	
12-14 days	6	3.17 ± 0.898	2.25 ± 0.270	1.90 ± 0.00#	6 of 6 (100%)
6-8 months	14	1.14 ± 0.833	2.99 ± 0.335	2.20 ± 0.233	13 of 14 (93%)

# only one lamb was found seropositive

\*\*\* ( $p < 0.001$ , two-sample t-test)

## Results

All primary infected lambs reacted with fever and infected neutrophils (rickettsemia) during the first 14 days after inoculation with *E. phagocytophila*. No other clinical signs were recorded, besides 1 or 2 days of reduced appetite in the older lambs. Different clinical variables are shown in Table 1.

After the primary fever period the infected lambs showed fever relapses of 1 to 3 days' duration. The number of relapses varied significantly between the 2 age groups (Table 2). During these fever relapses, *Ehrlichia* inclusions were found in the peripheral blood by blood smear examination. Temperatures above 40°C were not recorded in the control lambs.

All lambs reacted with an antibody titre following inoculation with *E. phagocytophila*, although 2 of the 6 younger lambs had low positive titres (1:160 in both) already at the start of the study. The antibody titre to *E. equi* varied significantly between the 2 lamb groups at 1 month after *E. phagocytophila* inoculation (Table 2).

Twelve susceptible lambs inoculated with blood from the older lambs and 5 lambs inoculated with blood from the younger lambs reacted with fever and rickettsemia 2-5 days after blood transfusion. In addition, 2 donors, 1 in each group, were found *Ehrlichia* positive by blood smear evaluation 12-14 days later. Altogether,

13 of the 14 previously infected lambs in the older group (93%), and all of the 6 younger lambs (100%) were infected at 4 months after primary inoculation, respectively (Table 2).

Of the 20 previously infected lambs, only 3 lambs reacted with fever of 1 or 2 days' duration associated with corticosteroid treatment and blood losses.

## Discussion

All lambs reacted with fever and rickettsemia as a result of an *E. phagocytophila* infection. The clinical response to TBF was less severe in young lambs compared with older lambs. This is in accordance with earlier observations in experimentally *E. phagocytophila* infected lambs (Stuen *et al.* 1992, Stuen 1993).

The number of fever relapses varied significantly between the 2 age groups during the first 4 months of the infection. In an earlier study where eight 3-week-old lambs were infected with *E. phagocytophila* and regularly examined for 2 months, the lambs had a mean number of fever relapses of  $3.62 \pm 0.484$  (Stuen 1990). Unfortunately, the persistence of infectivity in these lambs was not investigated.

The cause of fever relapses in *E. phagocytophila* infected lambs is unknown, but the relapses may indicate a recurrence of blood rickettsemia. In a previous study (Stuen *et al.*

1998), no direct relation was found between fever relapses and recurrence of rickettsemia, since fever was recorded in only 21% of the times where infected neutrophils was detected in the blood of *E. phagocytophila* infected lambs. That study also indicated that recurrence of rickettsemia did not cause an increase in the IFA-titre, as was also observed in the present work.

All infected lambs reacted with seroconversion measured 30 days following inoculation. Strong serological cross-reactions between *E. equi*, *E. phagocytophila* and the agent causing human granulocytic ehrlichiosis (HGE) have been reported (Dumler *et al.* 1995, Nicholson *et al.* 1997, Pusterla *et al.* 1997). The sensitivity of the present test may have been increased by use of a more proper antigen (Bjoersdorff *et al.* 1999, Walls *et al.* 1999), but unfortunately homologous antigen was not available.

One month after inoculation, the antibody titre was significantly higher in the older lambs when compared with the younger lambs. Although 2 young lambs were seropositive at the start of the inoculation due to colostral antibodies, an earlier study indicates that maternal antibodies do not normally reduce the production of antibodies in experimentally *E. phagocytophila* infected lambs (Stuen *et al.* 1992). In addition, 5 of the 6 younger lambs were seronegative 4 months following inoculation. This may indicate that the immunological reaction to an *E. phagocytophila* infection is stronger and of longer duration in 6-8-month-old lambs compared with that of 2-week-old lambs.

Four primary inoculated lambs, which were found seronegative 4 months after the initial infection, transmitted *E. phagocytophila* to susceptible lambs by blood transfusion. In addition, another seronegative lamb was found infected 12 days later. This indicates that seronegative lambs may be infected, and that

serology is not a good criterion for assessing recovery from a persistent state of an *E. phagocytophila* infection. However, antibodies may have been detected with a more sensitive test, i.e. by use of *E. phagocytophila* as antigen.

Four months after the inoculation, *E. phagocytophila* was found in the peripheral blood of all except 1 lamb. The present study therefore indicates that almost all *E. phagocytophila* infected lambs are persistently infected for at least 4 months, and that this persistence is age independent.

The present results indicate that clinical signs and serological response are not related to the rate of persistence. Only one strain of *E. phagocytophila* was used in this study, and no difference in clinical signs of TBF has earlier been observed in the Dala and Rygja breeds (Stuen personal information). However, both significant breed differences to *E. phagocytophila* infection and different strains of *E. phagocytophila* that evoke different clinical and immunological reactions have been found in sheep (Scott 1983, Foggie 1951).

It should be mentioned that granulocytic *Ehrlichia* infection is found to persist in other species such as dogs (Egenvall *et al.* 2000) and red deer (Stuen *et al.* 2001), but not in horses and cattle (Madigan 1993, Pusterla *et al.* 1998). Only 3 of 19 (16%) persistently infected lambs reacted with fever associated with high doses of corticosteroids and blood losses. This indicates that stress induced by such treatments is not enough to cause fever relapses in the majority of infected lambs.

In conclusion, the present study indicates that all ages of lambs are of epidemiological importance for the maintenance of *E. phagocytophila* infection in *I. ricinus* populations. However, the mechanism on how granulocytic *Ehrlichia* evades the immune response in lambs and other persistently infected animals is unknown.

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## Sammendrag

Persistens av granulocytter *Ehrlichia*-infeksjon hos 2 aldersgrupper av lam.

Granulocytter *Ehrlichia*-infeksjon (sjodogg) hos sau er vanlig i Norge i områder med skogflått, *Ixodes ricinus*. Tidligere undersøkelser har vist at enkelte sauer kan være infisert i flere måneder etter primærinfeksjonen. I denne undersøkelsen ble persistens av *E. phagocytophila* etter en eksperimentell poding undersøkt i 2 aldersgrupper av lam. Seks lam

(1-2 uker gamle) og 14 lam (6-8 måneder gamle) ble podet intravenøst med en ovin stamme av *E. phagocytophila* og deretter fulgt klinisk, hematologisk og serologisk (antistoffer mot *E. equi*) i 4 måneder. Etter

denne perioden ble lammene undersøkt for en fortsatt sjodogg-infeksjon ved hjelp av blodutstryk og blodpoding på mottagelige lam. Infeksjon ble påvist hos nitten (95%) av de tjue lammene.

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