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## Lack of cross-protection between *Cowdria ruminantium* and *Ehrlichia phagocytophila*

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Des souches de *Cowdria ruminantium* isolées au Sénégal et en Afrique du Sud et qui se distinguent antigéniquement, ont été comparées dans des essais d'immunité croisée chez des chèvres avec une souche d'*Ehrlichia phagocytophila* d'origine néerlandaise. On a montré une absence complète d'immunité croisée entre *E. phagocytophila* et *C. ruminantium* malgré le fait que, précédemment, une certaine présence de déterminants antigéniques communs ait été observée. *Mots clés* : Chèvre - Rickettsie - *Cowdria ruminantium* - *Ehrlichia phagocytophila* - Immunité croisée.

late from Senegal caused severe reactions in most goats immunised with the Ball 3 vaccine stock, fatal in 3 out of 10 cases (8). Moreover, we have recently reported that distinct antigenic differences occur in goats using four South African stocks (Kümm, Kwanyanga, Welgevonden and Ball 3) and the isolate from Senegal (7). DU PLESSIS *et al* (6) have also reported antigenic heterogeneity between various isolates of *C. ruminantium*. In this paper we investigated whether a Dutch isolate of *E. phagocytophila* afforded protection in goats against four antigenically different stocks of *Cowdria* and vice versa.

### INTRODUCTION

Heartwater of ruminants, caused by the rickettsia *Cowdria ruminantium* and transmitted by *Amblyomma* ticks occurs in Africa, south of the Sahara, as well as in the Caribbean region (1, 11). Tick-borne fever of ruminants, caused by the rickettsia *Ehrlichia* (*Cytoecetes*) *phagocytophila*, is widely spread in Europe, where it is transmitted by *Ixodes ricinus* ticks. Serological cross-reactivity between *Cowdria* and *E. phagocytophila* has been reported using the indirect fluorescent antibody test (IFA) based on mouse macrophages infected with the Kümm stock of *Cowdria* (2, 4). Moreover, bilateral cross reactions have been detected with IFA between the Senegal stock of *Cowdria* and *E. phagocytophila* (9).

The aim of this investigation was to determine whether there is any cross-protection between *Cowdria* and *Ehrlichia*. A meaningful comparison between *Cowdria* and *Ehrlichia* in cross-immunity trials requires the use of more than one *Cowdria* isolate, since distinct antigenic differences between *Cowdria* isolates have been reported. For instance, certain South African stocks, such as Kümm, Kwanyanga and Welgevonden have shown no or incomplete cross-immunity in mice (3, 5, 10). Also, an iso-

### MATERIALS AND METHODS

#### Rickettsial stocks

The characteristics of the *Cowdria* and *Ehrlichia* isolates used are given in table I. The *Cowdria* isolates have been previously described ; Senegal stock (8), Kümm stock (5), Kwanyanga (10) and Welgevonden (3). *E. phagocytophila* was isolated in 1977 from the Dutch Island of Ameland by subinoculating blood pooled from 23 sheep into a splenectomized sheep (13). Infected blood from this sheep was cryoprotected with 10 % dimethylsulphoxide (DMSO) and frozen in liquid nitrogen. This *Ehrlichia* isolate was further characterized by VAN MIERT *et al* (14).

#### Cross-protection trials

Cross-protection experiments between stocks of *C. ruminantium* and *E. phagocytophila* were carried out in adult Dwarf and Saanen goats, born and bred in the Netherlands, which have been shown to be highly susceptible to heartwater (12).

Immunization against heartwater was carried out by an infection and treatment method : three goats (Nos 8756, 8763 and 8764) were injected intravenously with 2 ml of a thawed infective blood stabilate (table II). All frozen material proved infectious in control goats (table I). After the body temperature had risen to 41 °C on the second or third day of the febrile reaction, animals were treated with oxytetracycline (Terramycin LA, 20 mg/kg, Pfizer, England). The administration of the drug was repeated after 48 h if the high temperature persisted. Homologous

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TABLE I Characteristics of four isolates of *Cowdria ruminantium* and one isolate of *Ehrlichia phagocytophila* in untreated goats.

Designation	Number of goats	Incubation period (days)	Maximum temperature (°C)	Febrile period (days)	Days to death mean (range)	Mortality rate
Senegal	12	11.7 ± 1.8	41.2 ± 0.5	4.4 ± 1.2	16.2 (13-20)	12/12
Kümm	6	12.8 ± 1.9	41.7 ± 0.3	4.8 ± 1.2	20.0 (15-25)	5/6
Kwanyanga	15	11.1 ± 1.7	41.5 ± 0.5	5.2 ± 1.9	14.0	2/15
Welgevonden	5	10.0 ± 1.2	41.3 ± 0.6	3.2 ± 0.6	13.2 (12-15)	5/5
<i>Ehrlichia</i> (Ameland)	10	4.8 ± 1.2	41.7 ± 0.3	4.7 ± 1.0	—	0/10

TABLE II Cross-immunity trials between *Cowdria ruminantium* and *Ehrlichia phagocytophila*.

Goat number	Immune status	Challenged/infected with	Incubation period (days)	Maximum temperature (°C)	Febrile period (days)	Challenged with	Incubation period (days)	Maximum temperature (°C)	Febrile period (days)	Days to death
8756	C.R. (Senegal) <sup>a</sup>	<i>E. phagocytophila</i>	3	41.3	7 <sup>b</sup>	ND	—	—	—	—
8763	C.R. (Kümm) <sup>a</sup>	<i>E. phagocytophila</i>	5	41.9	5 <sup>b</sup>	ND	—	—	—	—
8764	C.R. (Kwanyanga) <sup>a</sup>	<i>E. phagocytophila</i>	4	41.6	4 <sup>b</sup>	ND	—	—	—	—
8769	Naive	<i>E. phagocytophila</i>	3	42.0	7 <sup>c</sup>	C.R. (Senegal)	11	41.2	4	15
8751	Naive	<i>E. phagocytophila</i>	5	40.2	2	C.R. (Senegal)	9	41.1	2	11
8733	Naive	<i>E. phagocytophila</i>	5	41.9	5 <sup>c</sup>	C.R. (Kümm)	10	41.8	9	Recovered
8734	Naive	<i>E. phagocytophila</i>	5	40.6	2	C.R. (Kümm)	13	41.2	4	17
8727	Naive	<i>E. phagocytophila</i>	3	41.3	6 <sup>c</sup>	C.R. (Kwanyanga)	11	41.7	5	Recovered
8806	Naive	<i>E. phagocytophila</i>	4	41.7	3	C.R. (Welgevonden)	9	41.5	4	13

<sup>a</sup>: C.R. = *Cowdria ruminantium* challenged with homologous stock of *Cowdria* before challenge with *Ehrlichia*.

<sup>b</sup>: parasitaemia ranging between 32 % and 52 % of neutrophils was reached between day 4 and day 7 p.i.

<sup>c</sup>: parasitaemia ranging between 45 % and 60 % was reached between day 4 and day 6 p.i.

challenges were given after one month and challenges with *Ehrlichia*, one month later. Reactions after homologous or heterologous challenge were not treated.

Infections with *E. phagocytophila* were carried out with 2 ml of thawed blood stabilate, administered intravenously into ten goats (table I). None of the animals required treatment, since tick borne fever usually produces a non-fatal infection in goats. Six out of these ten goats were challenged one month later with blood stabilate infected with *Cowdria* (table II).

All animals were monitored throughout the experiments by daily rectal temperature records and clinical inspection. Blood smears were prepared in *Ehrlichia*-infected goats only during the febrile responses to detect rickettsial inclusion bodies in neutrophilic granulocytes. Febrile reactions of 40 °C or more were considered to be significant. Smears were made of brain cortex of any goat which had died, stained with Giemsa and examined for rickettsial inclusion bodies within endothelial cells.

## RESULTS AND DISCUSSION

Three goats (Nos 8756, 8763, 8764) immunised against *Cowdria* (Senegal, Kümm or Kwanyanga) and subsequently challenged with *Ehrlichia*, developed rickettsiaemia, ranging between 32 and 52 % of neutrophils infected with *Ehrlichia* during the febrile response (table II). Three other goats (nos 8769, 8733 and 8727) which were first infected with *Ehrlichia* and subsequently challenged with *Cowdria* (Senegal, Kümm or Kwanyanga), developed rickettsaemia within the same range (54 - 60 %) (table II). Mean incubation period, maximum temperature and duration of the febrile responses to *Ehrlichia* in goats previously immunised against *Cowdria* were not different from reactions in naive goats (table II).

Challenge of goats with *Cowdria* caused the death of four out of six animals previously infected with *Ehrlichia*. Two goats died after challenge with *Cowdria* (Senegal), and two after challenge with Kümm and Welgevonden iso-

lates, respectively. The two remaining animals recovered from the challenge infection with *Cowdria* (Kümm and Kwanyanga). It is however unlikely that their recovery can be attributed to partial cross-protection afforded by *Ehrlichia*, since only 2 out of 15 naive goats succumbed to *Cowdria* (Kwanyanga) infection and 5 out of 6 died after infection by the Kümm stock of *Cowdria* (table I). It appears therefore that bilateral serological cross-reactions between *Cowdria* isolates and *E. phagocytophila* (9) are not linked with protective immunity as demonstrated here by the absence of cross-protection between four different stocks of *Cowdria* and *Ehrlichia*. In addition it was found that goats immune to *E. phagocytophila* were not protected against challenge with two other stocks of

*Cowdria*, namely the South African vaccine stock Ball 3 and the Um Banein stock isolated from the Sudan (data not shown).

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Antigenically distinct stocks of *Cowdria ruminantium* from Senegal and South Africa were compared with a Dutch isolate of *Ehrlichia phagocytophila* in cross-immunity trials in goats. There was a complete absence of cross-immunity between *E. phagocytophila* and *C. ruminantium*, despite previous observations that both rickettsial organisms have certain antigenic determinants in common. *Key words* : Goat - Rickettsia - *Cowdria ruminantium* - *Ehrlichia phagocytophila* - Cross-immunity .

**JONGEJAN (F.), WASSINK (L.A.).** Ausencia de protección cruzada entre *Cowdria ruminantium* y *Ehrlichia phagocytophila*. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (4) : 425-428

Se compararon diferentes grupos de *C. ruminantium* provenientes de Senegal y Africa del Sur, con aislamientos holandeses de *E. phagocytophila*, en estudios de inmunidad cruzada en cabras. No se encontró ninguna inmunidad cruzada entre *E. phagocytophila* y *C. ruminantium*, a pesar de que en previas observaciones ambos organismos mostraron ciertos determinantes antigenicos en común. *Palabras claves* : Cabra - Rickettsia - *Cowdria ruminantium* - *Ehrlichia phagocytophila* - Inmunidad cruzada.

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