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Q FEVER IN CATTLE AND SHEEP IN SOUTHERN AFRICA

A PRELIMINARY REPORT

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ABSTRACT

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In the course of a study initiated to elucidate the role of known abortifacient agents in ruminants in Southern Africa, *Coxiella burnetii* was identified in smears prepared from bovine and ovine placental tissues obtained from 12 farms. The organism was also isolated in embryonated eggs, and specific antibodies were demonstrated in sera of animals originating from these farms. In 7 of the 12 herds in which Q fever was diagnosed, other pathogens were identified concomitantly.

Résumé

LA FIÈVRE Q CHEZ LE BOVIN ET LE MOUTON EN AFRIQUE DU SUD: UNE COMMUNICATION PRÉLIMINAIRE

Au cours d'une étude destinée à déterminer le rôle des agents microbiens susceptibles à entrainer l'avortement chez les ruminants en Afrique du Sud, les auteurs ont pu mettre en évidence le microbe Coxiella burnetii *au moyen de frottis préparés à partir du placenta de vaches et de brebis provenantes de 12 élevages. Le microbe a pu être isolé sur oeufs embryonnés et des anticorps spécifiques ont pu être mis en évidence dans le sérum des animaux provenants de ces élevages. Les auteurs ont pu identifier des microbes pathogènes concurrents chez 7 sur 12 troupeaux infectés de la fièvre Q.*

INTRODUCTION

Derrick (1937) was the first to report on the occurrence of Q fever in abattoir workers in Queensland, Australia, while Burnet & Freeman (1939) provided evidence that the causative organism was a *Rickettsia* which they succeeded in transmitting to laboratory animals. Owing to its differences from other rickettsias, the organism has since been assigned to a new genus, *Coxiella*, Swain 1965.

Since Derrick made his report, the infection has been shown to have a very wide distribution, being found in almost every country where it has been looked for (Babudieri, 1959).

The first clinical case of Q fever in man in South Africa was reported by Gear, Wolstenholme & Cort (1950), and a further case was documented by Saner & Fehler during the same year. A serological survey carried out by the South African Institute for Medical Research (Annual Report SAIMR, 1953) showed that Q fever was the most common rickettsial infection in man in this country. It appears that the sera of most adults in South Africa contain antibodies, and that the majority of clinical cases occur in immigrants and children.

Since overt disease due to *Coxiella* infections is rare in animals, Q fever is regarded as an inapparent infection in both wild and domestic animals (Babudieri, 1959). However, bronchopneumonia in sheep and perinatal deaths in sheep, goats and cattle have been ascribed to *C. burnetii* (Marmion & Watson, 1961; Tamarin, Rosenfield & Landau, 1964; Schaal, 1972).

There is abundant evidence that Q fever in man is derived from animals or animal products, and that the most common means of transmission is by airborne infections (Babudieri, 1959; Marmion & Stoker, 1958; Schaal, 1972; Mohr, 1965; Derrick, 1961; Schaaf, 1962; Delay, Lennette & Deome, 1950). Is has also been well documented that ticks and other ectoparasites disseminate the disease (Majerska & Brezina, 1968; Smith, 1940; Burgdorfer & Varma, 1967; Heisch, Grainer, Harvey & Lister, 1962; Giroud & Capponi, 1968), and that birds, too, may have a part in its spread (Syrucek & Raska, 1956). Lateral spread of *C. burnetii* infections amongst domestic animals, however, seems to be very rare (Babudieri, 1959).

Since June 1972, an extensive investigation has been undertaken at this Institute to determine to what extent known bacterial and viral agents are responsible for perinatal losses in sheep and cattle. The purpose of this paper is to record the diagnosis of Q fever in animals in Southern Africa.

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MATERIALS AND METHODS

Examination of foetal and placental tissues

Material from 780 sheep foetuses and 1 536 bovine foetuses was examined. With the exception of those for *C. burnetii* and Rift Valley fever virus, the methods employed for the identification of the various infectious agents involved have already been described (Ehret, Schutte, Pienaar & Henton, 1975; Schutte, McConnell & Bosman, 1971).

C. burnetii: Impression smears were prepared from foetal lung, spleen, kidney and several cotyledons. For the direct demonstration of the organism the smears were heat-fixed and stained by Stamp's modified Ziehl-Neelsen method (Stamp, 1951) and by the Gimenez method (Gimenez, 1964).

Placental tissues received from farms C, D, E, F and H (Tables 1 & 2) were further processed for the isolation of the organism in embryonated eggs, using the method advocated by Lennette (1964). In addition, placental tissue from a sheep which aborted on farm C was injected intraperitoneally into 4 guinea-pigs. Two of the guinea-pigs were killed 9 days after infection and the spleen and lungs from these animals were homogenized in Ten Broeck grinders and diluted with sterile phosphate buffer (0,1 M, pH 7,2). Ten-fold dilutions were made and 0,2 ml volumes of the resulting suspensions inoculated into 7-day-old embryonated eggs. The remaining 2 guinea-pigs were exsanguinated 35 days after inoculation and the sera stored at —20 °C for serological investigations.

TABLE 1 *C. burnetii* and other infectious agents demonstrated in ovine foetal material

Farm	District	Breed	Pathogen		
			C. burnetii	*C. psittaci*	RVF†
A	Ventersdorp	Merino	+	+	—
B	Lichtenburg	Dorper	+	+	—
C	Pretoria	Dorper	+	—	—
D	Karibib*	Dorper	+	—	n e
E	Bloemhof	Merino	+	—	+
F	Karibib*	Van Rooy	+	—	n e

* Material submitted by Dr A. Schmidt-Dumont, Regional Veterinary Laboratory, Windhoek
† RVF = Rift Valley fever virus
\+ = positive
— = negative
n e = not examined

TABLE 2 *C. burnetii* and other infectious agents demonstrated in bovine foetal material

Farm	District	Breed	Pathogen			
			C. burnetii	*C. psittaci*	RVF*	*S. typhimurium*
G	Bloemhof	Afrikaner	+	—	+	—
H	Bethal	Friesian	+	+	—	+
I	Pietersburg	Jersey	+	—	—	—
J	Pretoria	Friesian	+	—	—	—
K†	Witbank	Afrikaner cross	+	—	+	—
L	Bronkhorstspruit	Charolais	+	+	—	—

* RVF = Rift Valley fever virus
\+ = positive
— = negative
† Serological evidence of *B. abortus* and *L. pomona* infection also obtained

Rift Valley fever virus: Bovine and ovine foetal tissues from all the farms except D and F (Table 1) were examined for the presence of Rift Valley fever virus by the methods described by Findlay & Daubney (1931).

Serological tests

Sera from 2 of the above-mentioned guinea-pigs as well as from 10 ewes which aborted on farm C were tested for antibodies to *C. burnetii* with a complement fixation (CF) test*. A further 128 serum samples from sheep and cattle originating from 8 farms were tested for antibodies to *C. burnetii* by means of the micro-agglutination test (MA)** as described by Fiset, Ormsbee, Silberman, Peacock & Spielman (1969).

Results

Sheep

Q fever was diagnosed in sheep on 6 different farms by the examination of placental smears stained with Stamp's method (Table 1).

C. burnetii was isolated from placental tissue originating from farm C by injecting tissue suspensions into guinea-pigs. Organisms were isolated from these guinea-pigs by subinoculation of their tissues into embryonated eggs. A further isolate was made from ovine placental tissue received from farm F by direct inoculation into embryonated eggs. Attempts to isolate the organism from placental tissue from farms D and E were foiled by severe bacterial contamination.

The presence of a dual infection was revealed on farms A and B by the isolation of *C. psittaci* from foetal tissues of which placental smears were positive for *C. burnetii*. On farm E, Rift Valley fever virus was isolated from foetal tissues and *C. burnetii* from the placenta (Table 1).

CF antibodies specific for *C. burnetii* were demonstrated in all 10 sheep sera collected from farm C. Specific antibodies were also demonstrated in sera from guinea-pigs infected with placental tissue from a ewe which aborted on the same farm (Table 3). Agglutinating antibodies to *C. burnetii* were demonstrated in 45 of the 48 ovine sera received from farms C, D and E (Table 4).

Cattle

An examination of placental impression smears stained with Stamp's method identified *C. burnetii* in cattle from 6 different farms (Table 2). *C. burnetii* was isolated from bovine placental tissue from farm H by direct inoculation into embryonated eggs. Rift Valley fever virus was isolated from 2 of the bovine foetuses of which placental smears also proved to be positive for Q fever (Table 2). Two foetuses from farms H and L harboured both *C. burnetii* and *C. psittaci*. It is of interest that evidence of infection with *C. burnetii, B. abortus, L. pomona* and Rift Valley fever was found on farm K. Bovine foetal tissue received from farm H was infected with *C. burnetii, C. psittaci* as well as with *S. typhimurium*. Agglutinating antibodies to *C. burnetii* were demonstrated in 65 of the 80 bovine serum specimens received from 5 of the farms where Q fever was diagnosed by examination of placental impression smears (Table 4).

* Kindly conducted by Dr R. Swanepoel, Veterinary Investigation Centre, Salisbury
** The antigen used was made available by Dr W. Sixl, Hygiene Institute der Universität Graz, Austria

TABLE 3 Complement fixing antibodies to *C. burnetii* in sera from guinea-pigs infected with foetal material and from ewes from farm C

	Total No. tested	Total No. pos.	Total No. neg.	Titres of individual positive sera						
				1/4	1/8	1/16	1/32	1/128	1/256	1/1 024
Guinea-pigs	2	2	0	—	—	—	1	1	—	—
Ewes	10	8	2	—	1	4	1	—	1	1

TABLE 4 Agglutinating antibodies to *C. burnetii* in sheep and cattle sera

	Farm	Total No. tested	Total No. positive	Total No. neg.	MA titres of individual positive sera						
					1/4	1/8	1/16	1/32	1/64	1/128	1/256
Sheep	C	12	9	3	—	—	1	3	3	1	1
	D	27	27	—	—	—	7	7	9	4	1
	E	9	9	—	—	1	1	5	2	—	
Cattle	G	6	4	2	—	2	—	1	—	—	1
	H	20	17	3	4	5	4	2	2	—	—
	I	8	7	1	2	2	2	1	—	—	—
	K	14	13	1	1	6	1	3	—	2	—
	L	32	24	8	—	4	2	6	8	3	1

Discussion

This investigation has provided direct and serological evidence of the presence of Q fever in sheep and cattle in Southern Africa. Although the overall incidence, as determined by direct examinations of placental and foetal tissues, was low, this does not necessarily reflect the true situation. All the cases were diagnosed by examination of placental impression smears and the infection rate may well have been higher if more placentas had been available for examination. The fact that these cases originated from farms spread over such a large area may indicate a much wider distribution in Southern Africa than is generally surmised. A serological survey in progress (Kurz, unpublished data) seems to favour the latter conclusion.

The isolation of *C. burnetii* from placental material is also of interest because the examination of impression smears of the material from farm C initially led to a tentative diagnosis of brucellosis. Only after attempts to isolate *Brucella* and to demonstrate specific antibodies had failed were methods introduced for the isolation and identification of *C. burnetii*.

The inexperienced investigator could easily be confused by the fact that, in smears stained by Stamp's method (Stamp, 1951), the small acid-fast (red staining) coccoid *Coxiella* organisms have a close morphological resemblance to *Brucella*. However, with some experience, the differences become apparent and a positive diagnosis is possible without the necessity of having to isolate the organism first. This close resemblance may be responsible for the fact that the disease has only lately been diagnosed.

Furthermore, it is interesting to note that, in 7 of the 12 herds in which Q fever was diagnosed, other pathogens known to cause perinatal losses were identified concomitantly. The isolation of *C. burnetii* together with other pathogens has already been reported. Although Marmion & Watson (1961) recorded the isolation of *C. burnetii* and *Vibrio fetus* from ovine foetal material, they believe that abortions in sheep may be induced by high doses of *Coxiella* alone. Tamarin, Rosenfeld & Landau (1964), who reported on the isolation of *C. burnetii* together with *C. psittaci*, suggest that uncomplicated infections with *C. burnetii* will not result in abortions and that additional factors had to operate concurrently before this takes place. Evidence accumulated in the present study seems to support their hypothesis.

Q fever constitutes a danger not only to ruminants but also, more importantly, to man. Man apparently contracts this disease most commonly by the inhalation of airborne *Coxiella* particles, and the placenta of animals constitutes a particularly rich source of infective material for the contamination of the environment. Welsch, Lennette, Abananti & Winn (1951) provided evidence that organisms may be present in the placental tissue, not only of serologically positive animals but also of negative ones. According to Schaal (1972) the ingestion of raw milk also constitutes a danger to man.

These sources must be responsible for the fact that Q fever is a very common infection in man in Southern Africa (loc. cit.), but evidence in support of this hypothesis is still meagre and further investigation is warranted.

It is also clear that studies to evaluate the influence of this disease on ruminant reproduction and production should be undertaken. Furthermore, vaccines which have proved of value elsewhere should be evaluated under local farming conditions.

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