

Q FEVER IN BRITAIN: ISOLATION OF *RICKETTSIA BURNETI* FROM THE TICK *HAEMAPHYSALIS PUNCTATA**

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INTRODUCTION

Q fever is endemic in south-east England, and epidemiological studies suggest that consumption of infected cows' milk and contact with parturient sheep or their products provide ample opportunity for infection of man (Marmion, Stoker, McCoy, Malloch & Moore, 1953; Marmion, Stewart, Richmond, Barber & Stoker, 1954). There is no evidence that any patients are infected by tick bite, but the tick, *Haemaphysalis punctata*, is commonly found on sheep and cows in the area, and has been investigated to find out if it harbours *Rickettsia burneti*.

Despite the isolation of *R. burneti* from many species of ticks collected on sheep and cows in various parts of the world (Derrick, 1953; Berge & Lennette, 1953), it has been difficult to assess the importance of these parasites for transmission of infection amongst domestic animals. A recent review (Marmion, 1954) discusses in detail the reasons for this difficulty which, in brief, is due mainly to the existence of a means of transmission of the rickettsia among cattle and sheep, depending not on ticks, but on the excretion of the organism from the genital tract of the animals at parturition and on its dissemination in aerosols, in dust or on fomites to other animals susceptible to infection by their respiratory and, possibly, alimentary tracts. Moreover, apart from the fact that infection can probably spread in the absence of ticks, there is little positive information on the efficiency of infected ticks as vectors of Q fever among domestic animals under natural conditions.

In spite of this uncertainty as to the significance of ticks as vectors among domestic animals, it nevertheless seemed important to establish whether or not our native ticks are infected.

This paper reports the isolation of *R. burneti* from *H. punctata* and also includes some observations on the technical problem of distinguishing between rickettsiae inside a tick and the contamination of its external surface with rickettsiae excreted by the host animal and contaminating its own skin or wool.

DISTRIBUTION OF TICKS IN GREAT BRITAIN

Ixodes ricinus (the sheep tick) is the only tick which is common and widely distributed throughout the British Isles. It lives on a variety of warm-blooded hosts,

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especially sheep, cows, deer, hares and hedgehogs. At least eight other *Ixodes* species have also been found in small numbers, mainly on small mammals and birds. Apart from *H. punctata*, the only other ticks reported have been *Dermacentor reticulatus*, found infrequently in Devon and Wales, and *Argas reflexus* and *A. vespertilionis*, said to be present on bats.

H. punctata was first reported in the Romney Marsh by Nuttall & Warburton (1915) and, although its presence has now been confirmed, to the exclusion of *I. ricinus*, in many parts of Kent, very few specimens have been found anywhere else. *H. punctata* is commonly found on sheep pastured on the short grass of the reclaimed salt marshes around the south-east coast. Bracken and other rank vegetation are not found and this may explain the absence of *I. ricinus*.

Previously, unsuccessful attempts had been made to isolate *R. burneti* from *I. ricinus* collected in Scotland, Cumberland and Devon, and from a few specimens of *D. reticulatus* from Devon, but two batches of *H. punctata* collected from the Sheppey and Gravney marshes of north Kent produced antibodies to *R. burneti* when inoculated into guinea-pigs and mice (Marmion *et al.* 1953). This was not repeatable when the batches were inoculated into further animals, however, and in any event it was not possible to exclude external contamination of the ticks.

The severe floods of 1953 then covered the areas where these ticks had been collected, thereby making further collections impossible for some years. In 1953 a systematic survey of the Romney Marsh area of southern Kent was started to find out if sheep were infected and might constitute a source of Q fever in that area. During the course of this investigation, one of the flocks with serological evidence of infection (flock 3 in the paper by Marmion *et al.* 1954) was found to be heavily infested with ticks and provided a convenient source of collection in the spring of 1954. Efforts were also made at that time to obtain ticks from nine other flocks in the Romney Marsh by enlisting the help of their shepherds, and that of Mr F. J. L. Kett, F.R.E.S., who examined seven flocks on our behalf. Despite this, only 104 specimens were collected from sheep in flocks other than flock 3, so that tick infestation was evidently neither frequent nor general among Romney Marsh sheep in the spring of 1954.

GENERAL CONSIDERATIONS AND METHODS OF TESTING

The period of greatest tick infestation coincided with the lambing season, which in flock 3 lasted from March to the end of May. Previously, in 1953, some thirty-two of ninety-five ewes in this flock had been found to have complement-fixing antibody to *R. burneti* in their sera. It was for this reason that during the 1954 lambing season various specimens were collected from the sheep with the object of isolating *R. burneti*, which was subsequently obtained from the placenta and wool of one ewe and the placenta of another (Stoker, Brown, Kett & Marmion, 1955). At the same time specimens of *H. punctata* were collected from the ewes during the main lambing period (April and May) and from newborn lambs at 'docking' and 'cutting' (castrating). Subsequently, when the lambing season had drawn to a close the whole flock of ewes and lambs, together with some wethers, was searched on two

occasions with the assistance of Mr R. D. Brown, M.R.C.V.S., and Mr F. J. L. Kett, F.R.E.S. A total of 1762 ticks was obtained altogether.

The ticks were mostly found on the belly of the sheep round the navel, on the legs and in the region of the udder and vulva. Field observation by one of us (B.P.M.) led to the conclusion that the ticks, especially those taken from round the udder and vulva, might be contaminated externally with birth fluids, vaginal discharges and faeces, which might contain the rickettsia. The problem was therefore to distinguish, on the one hand, between rickettsiae on the external surface of the tick which might be contaminants picked up on its journey through the sheep's wool soiled in the way just described and, on the other hand, rickettsiae actually inside the tick, present in the conventional view, as the result of the tick ingesting an infected blood meal (but see Discussion for another explanation). The resistance of *R. burneti* to chemicals (Ransom & Huebner, 1951; Malloch & Stoker, 1952) prevented sterilization of the tick surface by this means, so we decided to compare the infectivity of washings from the intact ticks with the infectivity of suspensions of the tick tissues. It was felt that the demonstration of a much higher infectivity in the tissue suspensions than in the washings would suggest strongly that the rickettsiae were inside the tick and, by inference, that multiplication was taking place in its tissues. If, on the contrary, the infectivity of the tissues and the surface washings were similar, the rickettsiae might still be in the tick tissue (those on the outside being in the tick excreta), but the possibility could not be excluded that the isolation might be due to external contamination in the sheep's wool.

The ticks were, therefore, examined in the following way. After removal of residual pieces of wool, and identification with help from Dr A. D. Lees (Agricultural Research Council Unit of Insect Physiology), each lot of ticks was gently washed in a mortar with 8 ml. of penicillin horse serum broth (10 % horse serum in tryptic digest broth with 100 units of penicillin per ml.) at room temperature. This washing was then removed for storage. The ticks were then ground in sand with another 8 ml. of penicillin horse serum broth. The sand and debris were allowed to settle by gravity and 2 ml. quantities of the supernatant were used to inoculate guinea-pigs intraperitoneally. The remainder of the supernatant and the whole of the earlier washing were held at -20° C.

The tick suspensions were toxic for guinea-pigs and over a third of inoculated animals died within a few days of inoculation. If both animals inoculated with the same suspension died, this test was repeated in two or more guinea-pigs. If only one of the animals died, however, the test was not repeated.

The guinea-pigs were bled 42 days after inoculation and the serum tested for complement-fixing antibody against *R. burneti*. If antibody was present at 1 in 10 or above, the balance of the tick suspension used for the inoculation and the washing prepared from the same batch, were each titrated for infectivity by inoculation of groups of guinea-pigs with 2 ml. quantities of ascending tenfold dilutions in horse serum broth.

RESULTS

Flock 3

Of the 1762 specimens of *H. punctata* collected from this flock, 291 were engorged adult females and the remainder comprised unengorged adult males and a few unengorged nymphs. Engorged and unengorged ticks were tested separately according to source and time of collection in twenty-five separate pools each containing between sixteen and 371 ticks. *R. burneti* was isolated from two pools (nos. 85 and 273); the remainder were negative.

Pool 85 consisted of ninety-four unengorged ticks collected from ewes lambing between 7 and 13 April. It included ticks collected from a sheep which excreted *R. burneti* in the placenta. These ticks were present in a wool specimen from which *R. burneti* was also isolated. Both guinea-pigs inoculated with the suspension of these ticks developed Q fever antibodies at a dilution of at least 1 in 40.

Table 1. *Infectivity titrations of tick pools and washings*

Numerator denotes number of guinea-pigs developing Q fever antibody (all with titres of 1 in 40 or greater). Denominator denotes number inoculated.

Inoculum	Dilution of inoculum					
	Undil.	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵
Tick Pool 85						
Washings from intact ticks	1/1	2/3	0/3	0/3	—	—
Ground suspension	2/2	0/3	0/3	0/3	—	—
Tick Pool 273						
Washings from intact ticks	0/3	0/3	0/3	0/3	0/3	—
Ground suspension	2/2	3/3	3/3	2/3	2/3	0/2

The tick suspension and the surface washings from the intact ticks were then titrated for infectivity; the results are shown in Table 1, where it will be seen that the infectivity of both was very low. Since the tick tissue suspension did not have greater infectivity than the surface washing it was impossible to rule out contamination from the wool. The infectivity of the placenta and wool from the positive sheep were respectively 10^{4.5} and 10^{3.25} guinea-pig ID₅₀ per gm. (Stoker *et al.* 1955), so that contamination seems quite likely.

Pool 273 consisted of 371 unengorged ticks collected from wethers, ewes and lambs between 14 May and 11 June. Only five ewes lambed during this period and none of them was found to excrete *R. burneti*. Both guinea-pigs inoculated with this pool of ticks developed Q fever antibody at a dilution of at least 1 in 40, and the tick suspension and washings were subsequently titrated. The results in Table 1 show a striking contrast to those obtained with pool 85. The ground tissues of pool 273 are infective at a dilution of 10⁻⁴, whereas the washings from the unground ticks are non-infective. This strongly suggests that large numbers of rickettsiae were present in the tissue of some of the ticks and that the result was not due to contamination from the sheep. The finding would also support the view that the rickettsia was multiplying in the tick.

The yolk sacs of fertile hens' eggs were inoculated with a suspension of spleen from a guinea-pig previously inoculated with this tick suspension. Organisms morphologically resembling rickettsiae grew in the yolk sacs and antigen made from this reacted in complement-fixation tests with serum from a rabbit taken 30 days after inoculation with the Nine Mile strain of *R. burneti*.

Other flocks

The 104 specimens of *H. punctata* found in seven other flocks from Romney Marsh or its vicinity were tested in four pools, but *R. burneti* was not isolated.

DISCUSSION

Although ticks in Great Britain have not previously been found to harbour *R. burneti*, the organism has been reported in many tick genera and species from other countries. There is also experimental evidence that various species of tick will support multiplication of the rickettsia, transmit infection between experimental animals, and maintain infection from instar to instar, and occasionally from generation to generation, of the tick itself. (See reviews by Derrick, 1953; Weyer, 1953; Berge & Lennette, 1953.) The detection of *R. burneti* in *H. punctata* on infected sheep in south-east England is yet another example of the widespread infection of ticks.

H. punctata has a very local distribution in Great Britain, and it coincides with an area where infection with *R. burneti* is common amongst cows and sheep as well as humans. Attempts to isolate from the much more widespread *I. ricinus* have previously failed in this country, but it has not been possible to collect this tick from animals known to be infected with *R. burneti*.

From data obtained with other species of tick it is unlikely that *R. burneti* can persist indefinitely from generation to generation in *H. punctata* without passage through an intermediate mammalian host. *H. punctata* has been reported on one occasion from a hedgehog and possibly once from plovers in the Romney Marsh (Nuttall & Warburton, 1915), but all other collections in Kent have been made from sheep and cattle, so that these animals, which are known to be infected with *R. burneti*, presumably act as a source for the ticks.

The precise way in which the ticks are infected is, however, uncertain. Except for a few days following experimental inoculation, *R. burneti* has not been isolated from the blood stream of sheep and cows, so that opportunities for ingestion of the rickettsiae in a blood meal may be rare. As an alternative it is conceivable that ticks may sometimes be infected from external contamination of their mouthparts before attachment (e.g., in the rickettsia-laden wool of sheep), with subsequent ingestion of the contaminating organisms during engorgement.

This very possibility of external contamination of the ticks, however, has led to the precautions described in our report. They were taken because of the risk of 'isolation' from tick species (or other ectoparasites), which might be unsusceptible to natural infection with *R. burneti* and which might play no part in its natural history. Several previously reported isolations of *R. burneti* have been made from

ticks of various species collected from sheep, cows and goats. The significance of these isolations might perhaps be questioned without further knowledge of the risk of contamination from the host at the time of collection.

Because of the obscurities outlined above, and for other reasons already referred to in the Introduction, it is evident that the isolation of *R. burneti* from this Kentish sheep tick does not necessarily imply that the tick plays any part in transmitting infection among these animals.

SUMMARY

1. *Rickettsia burneti* has been isolated from the tick *Haemaphysalis punctata* collected from sheep in an endemic area of Q fever in south-east England.

2. Isolation from one batch may have been due to contamination from the wool of sheep excreting *R. burneti*, but evidence is presented that isolation from another batch was due to the presence of the rickettsia in the tick tissues.

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REFERENCES

- BERGE, T. O. & LENNETTE, E. H. (1953). *Amer. J. Hyg.* **5**, 125.
DERRICK, E. H. (1953.) *Med. J. Aust.* **1**, 245.
MALLOCH, R. A. & STOKER, M. G. P. (1952). *J. Hyg., Camb.*, **50**, 502.
MARMION, B. P. (1954). *Trans. R. Soc. trop. Med. Hyg.* **48**, 197.
MARMION, B. P., STEWART, J., RICHMOND, P., BARBER, H. & STOKER, M. G. P. (1954). *Lancet* **i**, 1288.
MARMION, B. P., STOKER, M. G. P., MCCOY, J. H., MALLOCH, R. A. & MOORE, B. (1953). *Lancet*, **i**, 503.
NUTTALL, G. H. F. & WARBURTON, C. (1915). A Monograph of the Ixodoidea, Part III, p. 384. Cambridge University Press.
RANSOM, S. E. & HUEBNER, R. J. (1951). *Amer. J. Hyg.* **53**, 110.
STOKER, M. G. P., BROWN, R. D., KETT, F. J. L., COLLINGS, P. C. & MARMION, B. P. (1955). *J. Hyg., Camb.*, **53**, 313.
WEYER, VON F. (1953). *Z. Tropenmed u. Parasit.* **4**, 344.

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