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Rickettsia conorii isolated from *Rhipicephalus sanguineus* introduced into Switzerland on a pet dog

O. Péter [1], W. Burgdorfer [1], A. Aeschlimann [2], and P. Chatelanat [3]

[1] Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Epidemiology Branch, Rocky Mountain Laboratories, Hamilton, Montana 59840, USA
[2] Zoological Institute, University of Neuchâtel, 2000 Neuchâtel, Switzerland
[3] 33 Moillebeau, 1209 Geneva, Switzerland

Abstract. A tick/rickettsial survey in a household near Geneva, Switzerland, revealed that 30 (40%) of 75 nymphs and adults of the brown dog tick, *Rhipicephalus sanguineus*, were infected with a rickettsial agent biologically and antigenically indistinguishable from *R. conorii*, the causative agent of boutonneuse fever. Introduced in 1976 from either southern France or Italy by the family's pet dog, the tick infestation had steadily increased until 1981 when control measures were initiated. During 1980 and 1981, four persons associated with the household's pet dog contracted a febrile illness diagnosed as boutonneuse fever.

Introduction

Boutonneuse fever – the tick-borne typhus fever of northern Africa and of the Mediterranean regions – is caused by *Rickettsia conorii*, a rickettsial agent transmitted by the brown dog tick, *Rhipicephalus sanguineus*. The disease has also been reported in the Netherlands, Germany and Switzerland, in persons exposed to *R. sanguineus* while traveling or vacationing in areas where boutonneuse fever and its tick vector are prevalent (Baumgartner et al. 1966; Weyer 1976).

In 1980, boutonneuse fever was diagnosed in one person living near Geneva, Switzerland. It appears that the patient was bitten by an infected *R. sanguineus* that had been introduced in 1976 from either southern France or Italy by a pet dog. Three additional persons associated with the same dog developed similar clinical manifestations in 1981 [1]. By that time, the household maintaining the dog was heavily tick-infested and control measures were initiated. Prior to these, we had the opportunity to collect several hundred ticks from the household; some were examined for rickettsial

Offpint requests to: W. Burgdorfer

[1] A detailed account on the clinical histories of the four patients will be published elsewhere

agents. This paper deals with the isolation from *R. sanguineus* of a rickettsial agent indistinguishable from *R. conorii*.

Material and methods

Collection and examination of ticks

Investigation of the patient's household yielded a large number of engorged nymphs and flat adults in all the rooms, but especially in those regularly frequented by the family dog. Ticks were collected by forceps from the cracks and crevices of the stucco walls. At the laboratory they were examined by the hemolymph test (Burgdorfer 1970). Rickettsiae-positive ticks were fed on rabbits. After they became engorged and dropped off, they were dissected individually and smears were prepared from hypodermis and Malpighian tubules. These were then stained by the Giménez method (Giménez 1964) or were treated with a fluorescein isothiocyanate-labeled immune serum against *Rickettsia rickettsii* – a conjugate broadly reactive for all spotted fever group rickettsiae.

Isolation and identification of rickettsiae

For the isolation of the rickettsia, the tissues of one engorged infected female tick were triturated in 3 ml brain heart infusion broth (BHI), and 0.25–0.50 ml of the suspension was injected i.p. into each of two male guinea pigs (Hartley strain) and four male meadow voles (*Microtus pennsylvanicus*). The temperature of the guinea pigs was recorded daily for 12 days. On the second day of fever (≧40° C), 5 ml heparinized blood was taken by heart puncture and stored at −20° C. Two voles were killed on days 5 and 6 after inoculation. Again blood was drawn from each animal and stored at −20° C. In addition, smears were prepared from scrapings of the peritoneum and tunica vaginalis and were stained by Giménez or with FITC-labeled antibodies against *R. rickettsii*. The remaining two voles were bled by retro-orbital procedure on days 6 and 13. On day 21 they were exsanguinated and their sera used for serologic evaluation by microagglutination (MA) (Fiset et al. 1969) and microimmunofluorescence (MIF) tests (Philip et al. 1978).

For culturing, the frozen blood samples of guinea pigs and voles were thawed, diluted with 2 parts BHI and inoculated into monolayers of chicken embryo fibroblasts. When rickettsial growth was established, the isolate, referred to as GE-1, was transferred to Vero- and L-cells following the procedure of Cory et al. (1974). For serological evaluation, an antigen was prepared according to Ormsbee et al. (1978).

For identification of the GE-1 isolate, mice were inoculated i.p. with 0.25 ml of a 10^3 suspension (w/v) of tissue culture-grown rickettsiae. After 5 and 21 days, they were bled by retro-orbital procedure, and their sera were evaluated by MA for antibodies to : *R. conorii* (Simko), *R. rhipicephali* (3-7-♀6), *R. slovaca* (B) and Swiss agent (C-5-P♀15), and by MIF also to *R. rickettsii* (Wachsmuth) and *R. sibirica* (No. 246).

To compare immune responses elicited by GE-1 with those by the boutonneuse fever agent, *R. conorii*, we inoculated mice and guinea pigs with tissue culture-grown *R. conorii* (Simko). Their sera were evaluated by MA and MIF. In addition, we evaluated the serum of the first patient who in 1980 became ill with suspected boutonneuse fever.

Results

Thirty (40%) of 75 nymphal and adult *R. sanguineus* were hemolymph test positive for intracellular pleomorphic, oval to rod-shaped rickettsiae (Fig. 1). Microscopic examination of tick tissues revealed the presence of the microorganisms in all the tissues, with particular intense infections in those of the female genital system.

Inoculation of infected tick suspensions into male guinea pigs caused fever that started on day 3 or 4 and lasted 3 to 4 days. Pronounced scrotal

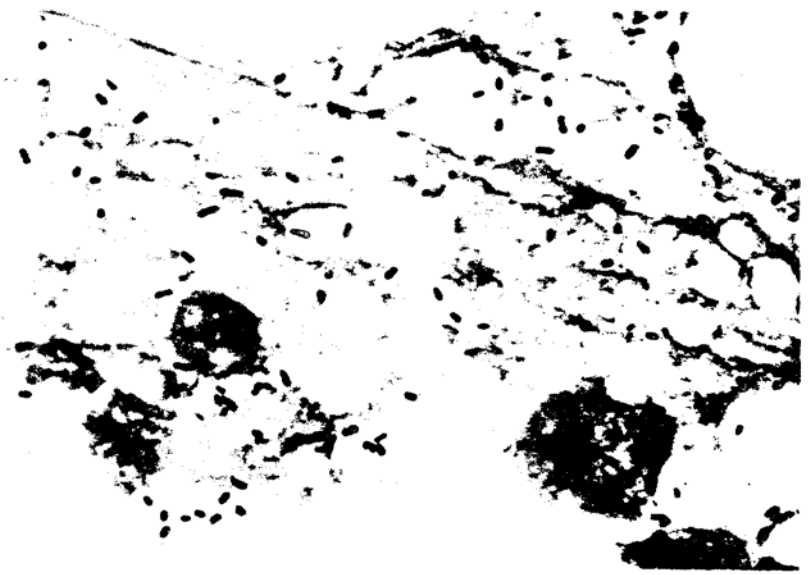

Fig. 1. *R. conorii* in the hypodermis of an infected *R. sanguineus* (Giménez stain, × 1850)

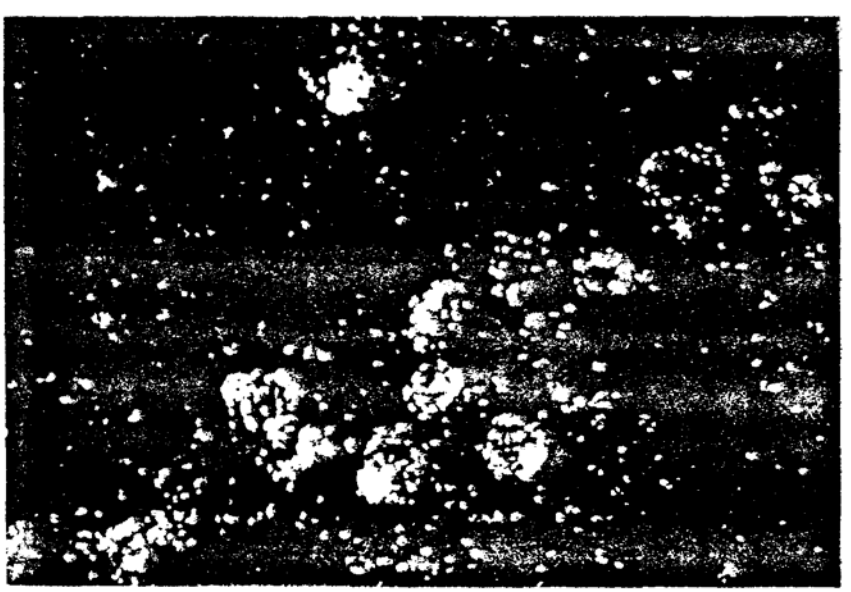

Fig. 2. Massive rickettsial growth in the tunica vaginalis of a meadow vole (fluorescent antibody stain, × 700)

swelling occurred without necrosis. Meadow voles, on the other hand, developed scrotal swelling with necrosis and exhibited massive rickettsial infections in their peritoneum and tunica vaginalis, often to the extent that cells were packed with rickettsiae (Fig. 2).

Table 1. Identification of the GE-1 isolate by microagglutination (MA) test

Etiologic agent	Immune sera		Antigens				
			GE-1	*R. conorii* (Simko)	*R. rhipicephali* (3-7-♀6)	*R. slovaca* (B)	Swiss agent (C-5-P♀15)
GE-1	Voles	6[a]	1024[b]	256	4	32	0
		13	2048	512	16	128	0
		21	4096	2048	64	256	0
	Guinea pig	21	512	64	32	32	0
	Mice	5	2048	128	0	32	0
		21	256	64	0	16	0
R. conorii (Simko)	Mice	5	2048	128	4	32	0
		21	256	64	0	16	0
	Guinea pig	21	128	64	ND	ND	ND
R. sanguineus rickettsia (GE-1)	Human G.C.	1,5 years	256	32	64	64	32

[a] Number of days after inoculation [b] Reciprocal titers
ND: Not done

Table 2. Identification of the GE-1 isolate by indirect microimmunofluorescence (MIF) test

Etiologic agent	Immune sera		Antigens						
			1	2	3	4	5	6	7
GE-1	Mice	21[a]	640[b]	640	40	160	160	80	80
	Voles	21	20,480	10,240	640	640	2,560	2,560	160
	Guinea pig	21	1,280	1,280	160	320	320	320	160
R. conorii (Simko)	Mice	21	1,280	2,560	40	160	320	160	20
	Guinea pig	21	320	640	40	80	80	80	40
R. sanguineus (GE-1)	Human G.C.	1,5 years	160	160	120	120	120	120	80

1 GE-1; *2 R. conorii* (Simko); *3 R. rhipicephali* (3–7–♀6); *4 R. rickettsii* (Wachsmuth); *5 R. sibirica* (No 246); *6 R. slovaca* (B); *7* Swiss agent (C–5–P♀15)

[a] Number of days after inoculation [b] Reciprocal titers

Rickettsiae were isolated in chicken embryo fibroblasts, from the blood of voles taken on day 5 after inoculation. In the original culture, rickettsial growth was moderate; it became massive in subpassages and could also be established into Vero- and L-cells.

Identification of the GS-1 isolate by MA and indirect MIF is summarized in Tables 1 and 2. Accordingly, mice, voles, and guinea pigs immunized with this rickettsia reacted with all the spotted fever group antigens, but the highest titers were against the boutonneuse fever agent, *R. conorii*. Simi-

larily, mice and guinea pigs immunized against *R. conorii* showed antibodies against the other spotted fever group antigens.

Also included in the tables is the result of serologic evaluation of the serum from the first patient who was thought to have boutonneuse fever in 1980. Although taken more than one year after onset of the illness, the serum still had antibodies to GE-1.

Discussion

It appears that the tick focus near Geneva had been initiated in 1976 from France or Italy through the tick-infested pet dog. No doubt some of the original imported ticks were infected with *R. conorii*, because four persons contracted a febrile illness, that was diagnosed clinically as boutonneuse fever. Our investigations confirmed the presence of *R. sanguineus* and revealed a large percentage (40%) of them to be infected with a rickettsia indistinguishable from *R. conorii*, the agent of boutonneuse fever. In laboratory animals, such as guinea pigs and meadow voles, the GE-1 isolate produced infections similar to those produced by *R. conorii* and elicited immune responses characteristic of it.

Probably the brown dog tick, *R. sanguineus*, is brought into Switzerland far more often than is reported (Aeschlimann and Büttiker 1975). Vacationers to and from Mediterranean countries, where this species of tick occurs abundantly, are often accompanied by their pet animals, particularly dogs, that are mainly responsible for transferring the ticks from one locality to another. Although not indigenous to Switzerland, *R. sanguineus* in the presence of its blood source, may survive and develop especially indoors (Aeschlimann and Büttiker 1975). This is also documented in West Berlin, for instance, where the tick was introduced in 1968 from the Mediterranean region and where it now occurs in several autochthonous foci (Hoffman 1981). Because *R. sanguineus* is not only a vector but also a reservoir by virtue of transovarial passage of *R. conorii* to the progeny of an infected female tick (Blanc and Caminopetros 1932), a focus of infection, once established, may persist for many years.

Although transmission of *R. conorii* to man usually occurs through the bite of an infected tick, infection may also result from contamination of the conjunctiva with infectious tick material (Pieri 1933). This has been reported for persons who rub their eyes after having deticked dogs. The mode of transmission to the person referred to in this paper is not known but very likely was by tick bite because clinical history includes the development of a typical tache noire in the left groin.

References

Aeschlimann A, Büttiker W (1975) Importation de Tiques en Suisse (*Acarina: Ixodoidea*). Bull Soc Ent Suisse 48:69–75

Baumgartner R, Buchler U, Savary A (1966) Eine kleine Epidemie von Fièvre boutonneuse in Basel. Schweiz Med Wschr 96:398–401

Blanc G, Caminopetros J (1932) Etudes épidémiologiques et expérimentales sur la fièvre boutonneuse, faites à l'Institut Pasteur d'Athènes. Arch Inst Pasteur Tunis 20:343–394

Burgdorfer W (1970) Hemolymph test. A technique for detection of rickettsiae in ticks. Amer J Trop Med Hyg 19:1010–1014

Cory J, Yunker CE, Ormsbee RA, Peacock M, Meibos H, Tallent G (1974) Plaque assay of rickettsiae in a mammalian cell line. Appl Microbiol 27:1157–1161

Fiset P, Ormsbee RA, Silberman P, Peacock M, Spielman SH, (1969) A microagglutination technique for detection and measurement of rickettsial antibodies. Acta virol 13:60–66

Giménez DF (1964) Staining rickettsiae in yolk-sack cultures. Stain Technol 39:135–140

Hoffman G (1981) Die braune Hundezecke (*Rhipicephalus sanguineus* L.) in Berlin (West). Bundesgesundheitsbl. 24:41–50, 153–163

Ormsbee R, Peacock M, Philip R, Casper E, Plorde J, Gabre-Kidan T, Wright L (1978) Antigenic relationships between the typhus and spotted fever groups of rickettsiae. Amer J Epidemiol 198:53–59

Philip RN, Casper EA, Burgdorfer W, Gerloff RK, Hughes LE, Bell EJ (1978) Serologic typing of rickettsiae of the spotted fever group by microimmunofluorescence. J Immunol 121:1961–1968

Pieri J (1933) La fiévre exanthématique du littoral méditerraneen ou fièvre boutonneuse. Doin G. et Cie, Paris

Weyer F (1976) Fleckfieber und Tourismus, Bundesgesundhbl 19:313–321