

# Antibodies against rickettsia in humans and potential vector ticks from Dhofar, Oman

\*Idris M A<sup>1,2</sup>, Ruppel A<sup>2</sup>, Petney T<sup>3</sup>

## الأجسام المضادة للريكتسيات والقراد الناقل لها بمحافظة ظفار

م. إدريس ، أ. روبل ، ت. بتي

**الملخص:** الريكتسيات هي عصيات بكتيرية واسعة الانتشار تصيب الإنسان والحيوان. تنتقل هذه الجرثومات بواسطة أنواع مختلفة من المفصليات من بينها القراد مسببة أمراضاً مثل الحمى البقاع (التيفوس) ، حمى جبال الروكي البقاع والحمى القرابية. لقد تم اكتشاف الإصابة بهذه الجرثومات في منطقة الشرق الأدنى في كل من مصر ، سوريا ، باكستان ، إثيوبيا والصومال. حسب علمنا لا توجد معلومات عن عدوى الريكتسيات بين الأفراد في شبه الجزيرة العربية بما فيها سلطنة عمان. لقد تم في هذه البحث مسح مصلى للأجسام المضادة عدوى الريكتسيات بفحص 347 مصلاً تم جمعها من تلاميذ المدارس (بسبح ، طوى إعتبر ، ضلكوت و رخيوت) ومراجعي العيادة الخارجية بمدينة الحق ومزارعين بصلالة بمحافظة ظفار. وقد تمت دراسة الأجسام المضادة بطريقة التناق المناعي أثبت البحث أن 59% من الأمصال تحتوي أجساماً موجبة للإصابة بالريكتسيات. أيضاً تم جمع ودراسة 707 قرادة من 102 حيواناً (جمال ، أبقار وماعز) من محافظة ظفار. ودراسة وتصنيف القراد وجد أنه ينتمي لأنواع الحلميات المتغيرة الشكل، والقراد الزجاجي العين بأنواعه والقراد ذو الرأس المروحي. وجميع هذه الأنواع من القراد يمكنها نقل العدوى مما يدل على إمكانية انتشار الإصابة بالريكتسيات بتلك المنطقة.

**ABSTRACT:** *Objective* – To determine the extent of rickettsial infections prevalence of potential vector ticks in the rural population of Dhofar, Oman. *Method* – Human sera (n = 347) were obtained from six rural localities (school children, farmers, outpatients) in Dhofar, Sultanate of Oman. Sera were tested by immunofluorescence for the presence of antibodies reacting with *Rickettsia conorii* antigen. *Results* – More than half the samples (59%) gave positive reactions (titres of at least 1:64). Ticks (n=707) were collected from cattle, camels and goats (n=102) and included *Amblyomma variegatum*, *Hyalomma a. anatolicum*, *H. dromedarii*, *H. rufipes* and *Rhipicephalus spp.*, all of which can potentially transmit rickettsiae to humans. *Conclusion* – The results suggest that rickettsial infections are common among the rural population of Dhofar.

**KEY WORDS:** Rickettsia, antibodies, immunofluorescence, vector ticks, cattle, camels, goats, Dhofar, Oman

Rickettsial infections of man are widely distributed.<sup>1</sup> They cause several forms of disease including spotted fevers, which are transmitted by ticks. In the near east, the presence of spotted fever rickettsiae as pathogens of man is well documented for Israel and Egypt<sup>2-5</sup> and for Somali refugees.<sup>6</sup> However, no information is available for much of the Arabian Peninsula, including the Sultanate of Oman. The potential for the disease to occur in this area is high as a variety of tick species, which can transmit rickettsiae, has been recorded in Yemen<sup>7</sup>, Saudi Arabia<sup>8</sup> and Oman.<sup>9</sup>

The present study was carried out in order to obtain an estimate of the prevalence of antibodies against *Rickettsia conorii* in the Dhofar Province, which covers the southern part of the Sultanate of Oman. A detailed account of the environmental geography of Dhofar region has been given by Sale.<sup>10</sup> Except for the

regional capital Salalah, the population has a predominantly rural lifestyle where people live in close proximity to their animals. Ticks were therefore collected from domestic stock in this region to investigate the presence of potential vectors of spotted fever rickettsiae.

### METHOD

The study localities were illustrated by Idris<sup>11</sup> except for Shab Al-Saeb, Rakhyut, Tawiattair and Sudh all within 30 to 160 km from Salalah, the capital of Dhofar. Blood was collected from volunteer school children (aged 8-16 years) in four localities in the Dhofar Province (Sudh, Dhalqut, Tawiattair and Rakhyut), from an outpatient clinic in Madinat Al-Haq and from workers at the Royal Farm in Salalah.

Sera were transported frozen to Heidelberg where they were tested by the indirect fluorescent antibody

<sup>1</sup>Department of Microbiology and Immunology, Sultan Qaboos University, P.O.Box: 35, Postal Code: 123, Muscat, Sultanate of Oman;

<sup>2</sup>Department of Tropical Hygiene and Public Health and <sup>3</sup>Department of Parasitology, University of Heidelberg, Im Neuenheimer, Feld 324, D-69120 Heidelberg, Germany

test (IFA) using *Rickettsia conorii*-Spot IF (bioMérieux Deutschland GmbH, Nürtingen, Germany). Principally, the serum is placed on a *Rickettsia conorii*-Spot IF antigen fixed slide. Antibodies fixed to this antigen are revealed by a fluorescein labelled anti-human globulin. A positive reaction is indicated by fluorescence of *R. conorii* on the slide, visible under an ultraviolet (UV) microscope.

Sera were diluted in two-fold series from 1:16 to 1:256 and applied to the slides. These were incubated in a moist chamber for 30 minutes at 37°C, washed twice for five minutes each time in Tris (0.01M)-buffered (pH 7.2) saline (PBS) with 0.05% Tween 20, dipped in distilled water and drained. They were then incubated as above in a moist chamber with fluorescein (FITC)-conjugated goat antibodies against human IgG (Behringwerke, Marburg, Germany) diluted 1:100 in PBS with 0.01% Evans Blue. Slides were washed as above and coverslips mounted with Fluoprep (bioMérieux). The results were read in a fluorescence microscope at  $\times 400$  magnification. According to the manufacturer, only fluorescent reactions with patient serum dilutions of at least 1:40 can be considered positive. In this study, fluorescent reactions obtained at dilutions of 1:64 or higher were taken as a positive reading. Confirmatory determinations were done for some random samples of sera and yielded identical results.

Ticks were collected from livestock at Dhalqut, Madinat Al-Haq, Tawiattair and Shab Al-Saeab areas through the cooperation from the animal's owners and veterinary practioners. In addition, the skins of freshly slaughtered camels, cows and goats were searched for ticks at Salalah municipality slaughter house, where animals are brought from various localities in Dhofar. Care was taken to obtain tick specimens from several sites including the ears, groin and anal area. Ticks from each animal were stored separate and preserved in 70% ethanol. Ticks were determined with respect to species, development stage and sex.

#### RESULTS AND DISCUSSION

Table 1 shows that between 42% and 66% of human sera from each locality gave a positive reaction. The results obtained from children were not detectably different from those obtained from adults. The data show that more than half (59%) of those individuals tested had antibodies against *R. conorii*. This suggests that rickettsial infections are common among the rural population of Dhofar. The situation appears similar to that in the Nile river delta of Egypt, where up to 96%, 81% and 37% of the school age children were found

seropositive for *R. burnetii*, *R. typhi* and *R. conorii*, respectively.<sup>4</sup>

TABLE 1.

*Prevalence of antibodies against Rickettsia conorii among individuals from six localities in Dhofar, Oman*

Population	Locality	<i>Rickettsia Conorii</i> - Spot I.F	
		No. of sera tested	No. of sera with positive reaction*
School children	Sudh	64	31 (48)
	Dhalqut	71	47 (66)
	Tawiattair	54	34 (63)
	Rakhyut	67	41 (61)
Outpatients	Madinat Al-Haq	60	39 (65)
Farm workers	Salalah	31	13 (42)
Total		347	205 (59)

In order to search for potential vectors for *R. conorii*, the ticks, collected from 102 animals (cows, camels and goats), were screened for species with a known ability to transmit this pathogen (Table 2). A total of 707 adult ticks were identified, which might contribute to the natural epidemiological cycle of *R. conorii* on the following basis: (i) All these tick species use domestic stock as regular hosts for one or more stages in their life history and are, therefore, likely to come into contact with humans. (ii) *Amblyomma variegatum* and *Hyalomma rufipes* are known vectors of *R. conorii*;<sup>12,13</sup> immature stages of *A. variegatum* frequently, and adults of *H. rufipes* occasionally, attack humans.<sup>9,14</sup> (iii) *H. anatolicum ssp.* is a known vector for *R. conorii*, and *H. dromedarii* was reported to be (one out of 70 specimens) infected with spotted fever group rickettsiae.<sup>5</sup> Both *H. anatolicum ssp* and *H. dromedarii* attack humans.<sup>14</sup>

The genus *Rhipicephalus* includes *R. sanguineus*, one of the main vectors of *R. conorii*. This species has often been confused with *R. turanicus* which is known to attack humans, but the status of *R. turanicus* as a vector for *R. conorii*, is uncertain<sup>15</sup> and needs to be elucidated.<sup>16,17</sup> *Rickettsia massiliae*, a species closely related to *R. conorii*, has also been isolated from *R. turanicus*.<sup>18</sup> In addition to *R. turanicus*, *R. camicasi* belongs

TABLE 2

*Ticks collected from domestic stock and implicated in the transmission of Rickettsia in Dhofar, Oman*

Host (numbers sampled)	Tick species (numbers collected)					Total
	<i>Amblyomma variegatum</i>	<i>Hyalomma anatolicum</i>	<i>Hyalomma dromedarii</i>	<i>Hyalomma rufipes</i>	<i>Rhipicephalus spp.</i>	
Cattle (77)	35	68	42	8	16	169
Camels (21)	32	7	463	15	0	517
Goats (4)	0	0	0	0	21	21
Total	67	75	505	23	37	707

to the *R. sanguineus* group of species and both are morphologically similar: it is difficult to distinguish their males and their engorged females are almost indistinguishable<sup>19</sup> from each other. The involvement of *R. camicasi* in disease epidemiology is unknown.<sup>16</sup> Both *R. turanicus* and *R. camicasi* are found in Oman.<sup>9</sup> In view of the problems encountered in identifying vectors of *R. conorii* in the *R. sanguineus* group, the identification of *Rhipicephalus* to species level was not attempted.

This report confirms the earlier records of tick species in Oman by Hoogstraal.<sup>9</sup> It amply demonstrates the presence of ticks on domestic stock with the potential to transmit spotted fever group rickettsiae to humans. This, together with the rural life style of the local population,<sup>20,21</sup> is compatible with a high prevalence of seropositivity in humans. However, the following three points deserve further investigation:

*First:* whereas the serological data demonstrate a substantial prevalence of rickettsial infections, the cut-off titre set in immunofluorescence determines the sensitivity and specificity of the results as was suggested by comparative Western blotting performed with specific protein antigen.<sup>22</sup> Thus, the true prevalence of *R. conorii* infections might be lower than the percentages of seropositivity.

*Second:* according to the manufacturer, the test antigen is not specific for *R. conorii* and the exact taxonomic status of the rickettsiae present in Dhofar remains to be determined. *R. conorii* is antigenically diverse<sup>23</sup> and, in addition to *R. conorii*, several new spotted fever group rickettsial strains have recently been described from ticks including *A. variegatum* and *R. turanicus* from the Mediterranean and Africa.<sup>24-26</sup> We cannot also exclude the possibility that *R. typhi* or *R.*

*proوازekii*, which are not transmitted by ticks, might occur in Dhofar and cross-react in the serologic test.

*Third:* it cannot be deduced from the mere presence of antibodies, whether infections with rickettsiae represent a significant source of disease in Dhofar, as serology also detects asymptomatic cases of spotted fever as well as persisting antibodies.<sup>27-29</sup> Observation of the possible clinical spectrum of the disease should clarify this point.

#### CONCLUSION

To our knowledge, this report is the first serological study of rickettsial infections in the Oman and, together with the tick survey, demonstrates that transmission of such infection is possible and does occur. Quantitative epidemiological aspects require further studies. The population of Dhofar has appreciable seroprevalence rates for brucellosis<sup>20</sup> and toxoplasmosis,<sup>21</sup> which are also transmitted from domestic animals to humans. However, the recently improved living conditions and health services in Dhofar should significantly reduce the prevalence of these pathogens, and the diseases transmitted from livestock to humans in this region should reduce correspondingly.

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