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Case Series

Microbiology Section

Four Cases of Laboratory Acquired Q Fever: Lessons from the Past and a Brief Review of Literature

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ABSTRACT

The aim of this communication is to highlight the dangers of Q fever outbreaks in laboratories attempting isolation of *Coxiella burnetii* (*C. burnetii*) and other rickettsiae without Bio Safety Level (BSL)-3 facilities. This series is reported for the benefit of Microbiologists who evince interest in Rickettsial research. Four cases of Laboratory-acquired Q fever is presented among the laboratory personnel in a tertiary care teaching Hospital in Karnataka in 1977, which has not been reported yet. Four persons (one Lecturer, one animal technician and two M.Sc. students) who assisted in blood collection from slaughtered domestic livestock from butcheries and/or participated in the isolation of *C. burnetii* by guinea pig inoculation became seropositive for Q fever. The presence of antibodies to *C. burnetii* was demonstrated by two specific serological tests, Micro Agglutination Test (MAT) and Complement Fixation Test (CFT) for Q fever. They responded well to treatment with oxytetracycline, the drug of choice. It should be known that even microscopic demonstration of *C. burnetii* and other rickettsiae in the ectoparasites is hazardous, due to liberation of infective aerosol in the process. A safe commercial Q fever vaccine for laboratory and farm workers is presently available in Australia alone. Therefore, preventive measures like use of BSL-3 facility in the laboratory as well as animal house, wearing N95 face masks and protective clothing (PPE) are some of the essential precautions to follow so as to prevent laboratory acquired Q fever infection, which is an important occupational hazard.

Keywords: *Coxiella burnetii*, Laboratory acquired infection, Microagglutination test, Phase I and Phase II, Rickettsioses, Zoonosis

INTRODUCTION

Laboratory infection is a common feature of Q fever as well as with all other rickettsioses. Some early Rickettsiologists who discovered and worked with the rickettsiae, later succumbed to the infection. The first case of laboratory-acquired Q fever in Rocky Mountain laboratory, Montana, USA was reported by Dyer RE in 1938 [1]. Johnson JE and Kadull PK analysed 50 cases of laboratory-acquired Q fever between 1950 and 1965 at Army Biological Laboratories, Maryland, USA [2]. They reviewed similar infections during 1938-1953 in USA, Australia, Romania, Germany, Spain, France, Czechoslovakia and England. Pike RM analysed 3921 cases of Laboratory associated infections, (with 2465 from USA) and concluded that 15% of them were due to Rickettsiae [3]. Hall CJ et al., reported laboratory outbreak of Q fever in Bristol University (UK), involving 28 persons [4]. The source was traced to the surgical removal of aborted twin foetuses from a sheep in that University facility. The infection could be latent, moderate to severe or sometimes even fatal to both man and animals. The Largest Q fever outbreak due to the spread of infective aerosols generated by aborting dairy goats in Netherlands was in 2009 and as a precautionary measure 51,820 goat were culled [5]. During 2007 and 2009, 3523 human cases were reported [6].

Authors past experience of laboratory acquired Q fever in four staff of Microbiology Laboratory in a tertiary care teaching Hospital in Karnataka in 1977 is shared in this series. Their exposure to *C. burnetii* was confirmed by specific serological tests. The main focus is to caution Microbiologists in the developing countries, who without realising the danger of potential hazard, might attempt rickettsial isolation in laboratories without proper BSL-3 facilities.

Methodology and Procedure

These cases were reported during November 1975 to December 1977 at the Department of Microbiology, when BSL-2/BSL-3 containment facilities were unknown. Facilities for the yolk sac cultivation of *C. burnetii* and other rickettsiae were available in two National Laboratories only viz., Virus Research Centre, Poona (Now National Institute of Virology) and Indian Veterinary Research Institute, Izatnagar, UP), India.

Demonstrating the natural occurrence of *C. burnetii* in domestic animals and their ectoparasites:

Spleen samples were collected from the slaughter houses from different mammals, portions of which were homogenised to isolate *C. burnetii* as per standard procedures. Spleens of 21 sheep, 19 goats, 10 cattle, three buffaloes, one each of dog, bat, spleens and brains of 94 rats, two bandicoots, 21 reptiles and 1720 ectoparasites were examined for the presence of *C. burnetii*, by the guineapig inoculation method. The viscera were pooled first (sheep-5 pools; goat-5; cattle-2; buffaloe-1; dog-1; rats-18; bandicoots-2, bat-1, reptiles-21, fowls-10 and ectoparasites-21 pools) before guineapig inoculation. A 10 per cent w/v emulsion of the spleen/brain in sucrose phosphate glutamic acid solution containing 1000 IU of penicillin and 250 µg of streptomycin per mL was obtained. After ensuring their sterility for bacteria and fungi, these were inoculated intraperitonealy into seronegative male guineapigs weighing about 400-500 g [7].

Apart from mammals, 34 reptiles belonging to three different groups, viz., skink, snakes and house-lizards were investigated for evidence of Coxiellosis as described above [8]. Rats were involved in the study because natural occurrence of *C. burnetii* in bandicoots has been reported [7].

Male guineapigs negative for *C. burnetii* antibodies were used. Pooled viscera from 230 animals (66 pools) and 1,720 ectoparasites (21 pools) were inoculated intraperitoneally and observed for 30 days with daily recording of rectal temperature. Blood was collected for serological tests from guineapigs having fever of ≥40°C for three consecutive days. These animals were sacrificed and impression smears were made from their spleen, and stained with Giemsa. Spleen tissue was homogenised and inoculated into other set of

male guineapigs. (Blind passage), and observed for 15 days with daily temperature recordings and blood was collected on day 16 for serological tests.

Blood for serology was collected on days 15, 30 and 45 from guineapigs which remained apyrexial for 30 days post inoculation.

The male guineapigs set aside for the Q fever research in the Microbiology laboratory were first examined for the presence of antibodies to *C. burnetii* by Phase I and Phase II by MAT and to Phase II CFT. Only seronegative guineapigs were used for *C. burnetii* isolation attempts. Seroconversion showing evidence of the presence of antibodies to *C. burnetii* was considered as a proof of the presence of *C. burnetii* in the viscera of animals. If guineapigs inoculated with ectoparasites/spleens of livestock show seroconversion, the natural occurrence of *C. burnetii* in those livestock/ectoparasites was confirmed [7-11].

Serological Tests

Fiset P et al's., MAT was performed with the coloured antigen of *C. burnetii* (Henzerling strain) received from World Health Organisation (WHO) Collaborating Centre for Rickettsial Reference and Research, Czechoslovakia. Nine Mile antigen of *C. burnetii* Phase II, received from Rocky Mountain Laboratory, USA was used in CFT [12]. Prior to the commencement of this research work, blood was collected from four persons associated with animal handling (to test for baseline antibody titre) and another 11 staff for control. (Teaching 4 and non teaching 7). During August 1977 to March 1978 blood samples were collected at intervals of six to eight weeks from these 15 members of Microbiology department to search for Q fever antibodies. Any participant if found positive for *C. burnetii* antibody, was subjected to additional screening for typhus group rickettsiae, spotted fever group rickettsiae (Antigen courtesy: Dr. R.A. Ormsbee, Rocky Mountain Laboratory, USA, and Dr. J. Urvolgyi and Dr. J. Kazar, WHO collaborating centre for Rickettsial Reference and Research, Czechoslovakia) and *Brucella* organisms (Brucella antigen for tube agglutination test was purchased from IVRI, Izatnagar, UP).

During August 1977 to March 1978 blood samples collected at an interval of six to eight weeks of 15 members of Microbiology department were examined for Q fever antibodies. These 15 comprised of four members of our team and the remaining are Microbiology Faculty and staff. Out of the 15, four members were tested seropositive for Q fever and the rest 11 were tested seronegative.

Case Description

A total of 151 mammals belonging to eight different species were selected in this study, grouped into 35 pools and examined for the presence or natural occurrence of *C. burnetii*, by the guineapig inoculation method. Based on the seroconversion in the guineapigs, goat, sheep, dog and rats were found to harbour Q fever rickettsia in their tissues.

The break-up was as follows: (i) Three pools of spleen and brain emulsions made from 16 rats; (ii) two pools of spleen emulsions from 12 sheep; (iii) One pool of goat spleen obtained from two goats; and (iv) a single spleen of dog. Among the reptiles, one rat snake (*Ptyas mucosus*) and a skink (*Mabuya carinata*) harboured *C. burnetii*, as inferred by the seroconversion of guineapigs inoculated with the tissue emulsions of these two reptiles. *C. burnetii* could not be isolated from the fowls. In view of the potential hazard, no attempt was made to isolate *C. burnetii* by way of inoculating the blood/viscera of seroconverted guineapigs in the yolk sacs of chick embryos. Giemsa stained viscera of antibody positive guineapigs were all negative for *C. burnetii*.

This surveillance was initiated because there was some suspicion with some of the members of the team had fever/malaise/myalgia. These two symptoms by themselves do not call for Q fever testing, but since the staffs were involved in handling animals for a few months to more than a year, the possibility of acquiring Q fever was suspected and wanted to rule it out. During the course of the research, based on clinical symptoms but positive serology, four persons in the laboratory probably contracted Q fever. Brief details are given in [Table/Fig-1]. Case-2 was an active member of our team and hence was subjected to the serological tests to make sure if he had any subclinical infection. In fact, according to Whelan J et al., 60% of Q fever cases are asymptomatic [5]. Eleven subjects included in this study were seronegative for Q fever in MAT and CFT and had normal LFT results.

Liver involvement is common in Q fever (Dupont HL et al.,) [13]. However, all these four persons had no elevation of liver enzymes

Details	Case-1	Case-2	Case-3	Case-4
Age (years)/Sex	23/M	22/M	21/M	28/M
Occupation	M.Sc. Student	M.Sc. Student	Animal house technician, Microbiology Department	Lecturer
Chief complaint	Sudden onset of high fever, on and off over the past five days with chills, excessive tiredness, loss of appetite and body pain	Body pain, exhaustion, reduced appetite. Loss of weight-5 kg in the last three months	Loss of appetite and mild body pain	Loss of weight of 5 to 6 kg, over a period of six months. Severe back pain, tiredness and mild loss of appetite
History of presenting illness	Abrupt onset of fever of five days duration (100-102°F) fatigue, cough	No febrile episode. Excessive fatigue	No febrile episode. Fatigue and anorexia	No febrile episode. Feeling of exhaustion
Past medical history	No significant past medical history	No significant past medical history	No significant past medical history	Had viral Hepatitis A infection six years earlier, and fully recovered
Clinical features	Fever, fatigue, malaise, myalgia, dry non productive cough	Malaise, myalgia	Malaise	Myalgia and malaise
Investigations	Chest X-ray: No Active Disease (NAD) Blood culture: Negative Widal test: Negative Blood smear -Negative for Malarial parasite and microfilaria Liver function tests: (Bilirubin, SGPT, SGOT and AP) Normal Q fever serology: Positive, 53 days post febrile episode. Phase-I and Phase II *C. burnetii* agglutinin titre-8 and Phase II CFT titre-128* [Table/Fig-2] Serology for Typhus fever group, spotted fever group and Brucellosis: Negative	Investigation for Q fever was done, since he assisted in the collection of animal sera of livestock from various slaughter houses. Chest X ray: NAD Liver function tests: (Bilirubin, SGPT, SGOT and AP) Normal. Serology for Q fever was positive. Phase-I and Phase II *C. burnetii* agglutinin titre-8 and 16, respectively. Phase II CFT titre-64 [Table/Fig-2]** Serology for typhus fever group, spotted fever group and Brucellosis: Negative	Though he was asymptomatic, he was involved in assisting the guinea pig inoculation for rickettsial isolation experiments. Used to clean the inoculation cabinets and animal cages. Chest X ray: NAD Liver function tests: (Bilirubin, SGPT, SGOT and AP.) Normal. Q fever serology positive. Phase-I and Phase II *C. burnetii* agglutinin titre-8 and 2, respectively. Phase II CFT titre-32 [Table/Fig-2]*** Serology for Typhus fever group, Spotted fever group and Brucellosis: Negative	Had contact with livestock for four years in collecting blood samples of animals from slaughter houses. Carried out rickettsial isolation attempts from several animals and the ectoparasites. Chest X-ray: NAD Liver Function tests: (Bilirubin, SGPT, SGOT and AP) Normal. Q fever serology positive. Phase-I and Phase II *C. burnetii* agglutinin titre-8 **** each for both Phases. Phase II CFT Negative [Table/Fig-2] serology for typhus fever group, spotted fever group and Brucellosis: Negative

Diagnosis	Initial diagnosis-? viral fever Subsequent diagnosis: Acute Q fever	Suspected case of Q fever	Asymptomatic Q fever	Asymptomatic Q fever
Treatment	Initial treatment: Analgesics and antipyretics Subsequent treatment course of oxytetracycline (500 mg- 6 hourly×30 days)	Course of oxytetracycline (500 mg- 6 hourly×30 days)	Course of oxytetracycline (500 mg- 6 hourly×30 days)	Course of oxytetracycline (500 mg- 6 hourly×30 days)

[Table/Fig-1]: Brief details about four cases of laboratory acquired Q fever.
*High CFT titre of 128 was seen in case-1; **Case-2 moderately high titre; ***Case-3 moderate MAT titre and Moderately high CFT titre. ****Low, but significant MAT Titre of 8 and negative CFT was observed in case-4

(Bilirubin, SGPT, SGOT and ALP). Additionally, sera from all four cases were found negative for antibodies to typhus group and spotted fever group rickettsiae and *Brucella* organisms. Serological response of four cases of laboratory acquired Q fever is given in [Table/Fig-2].

S. No.	Days of blood collection	Reciprocal of antibody titres in			LFT (Bilirubin, SGOT, SGPT and ALP)
		Ph. I MAT	Ph. II MAT	Ph. II CFT	
1.	Day 1	-	-	-	Normal
	Day 69	8	8	128	
	Day 90	2	2	64	
	Day 102; Day 162; Day 182	2	2	32	
2	Day 1	2	-	16	Normal
	Day 27	8	16	64	
	Day 73	4	2	32	
	Day 110	2	2	32	
	Day 164; Day 209	2	2	16	
3	Day 1	-	-	-	Normal
	Day 74	8	-	32	
	Day 97	2	2	16	
	Day 127	2	2	8	
	Day 164; Day 183	-	-	-	
4	Day 1; Day 20; Day 47	-	-	-	Normal
	Day 88	8	8	-	
	Day 110; Day 130; Day 173; Day 200	-	-	-	
5-15 (controls)	Day 1 Day 15-Day 28	-	-	-	Normal

[Table/Fig-2]: Serological response of four cases of laboratory acquired Q fever.
Significant MAT titre Phase I/Phase II: ≥1:1; Significant CFT titre Phase II: ≥1:8
Case-1: Shows initial seroconversion with high titres in MAT and CFT and decline later
Case-2: Fourfold increase in titres between 1st and 2nd samples, followed by declining titres
Case-3: Shows seroconversion followed by a fall in MAT and CFT titres
Case-4: Only seroconversion observed between the 1st and 2nd sample

DISCUSSION

The Q fever has two clinical presentations: Acute Q fever and chronic Q fever. According to Roest HI et al., about 60% of Q fever infections are asymptomatic, although develop antibodies to *C. burnetii* [6]. Symptoms vary from fever, headache, myalgia, malaise, anorexia, flu-like illness, self-limiting atypical pneumonia (30-50%), non productive cough, chest pain etc. All four cases belonged to this category, with mild symptoms.

Only a small percentage of 1% to 2% of acute cases progress to chronic Q fever over a period of several years. Endocarditis and chronic granulomatous hepatitis are the common manifestations of chronic Q fever, and the former condition needs prolonged antibiotic therapy for 18 months and may require valve replacement [14-16]. Abortion in pregnant women, although rare, has been reported from India [17]. Tetracycline has been the drug of choice for all rickettsial infections including Q fever and still remains so in the form of doxycycline. Trimethoprim-Sulphamethoxazole is an alternate drug, when doxycycline cannot be administered. The first case of Human Q fever was reported by Kalra SL in 1954 in an American Physician, by inoculating his acute phase blood into guinea pig and mice and diagnosing by rising antibody titre in successive blood samples [18]. A single case of acute Q fever Pneumonia was reported by Panjwani A et al., [19]. The first molecular evidence of acute *C. burnetii* pneumonia was provided by Raj Gangoliya S et al., followed by Pradeep J et al., [20-22]. Among the two patients of Raj Gangoliya S et al., with atypical pneumonia, one was a 64-year-old male, a rancher, with a 5-day history of fever, dry cough, shortness of breath and hepatosplenomegaly; echocardiography revealed features suggestive of endocarditis. The second patient was a 72-year-old farmer with fever and breathlessness for six days with hepatosplenomegaly; a chest X-ray showed bilateral lower zone consolidation [22]. The present study on acute fever cases with pneumonia has revealed that most patients had common clinical features such as myalgia, chills and rigour abdominal pain, nausea, vomiting and hepatomegaly. Molecular diagnosis for Q fever or the gold standard Immunofluorescence assay were not known in those days and serodiagnosis was confined to specific CFT and MAT available then [21-24]. The presence of Phase I agglutinin in the early serum sample of case 4 is unusual. However, similar responses have also been observed in other 3 cases of laboratory infection.

Isolation of *C. burnetii* from four cases was not attempted due to two reasons:

1. There were no prolonged fever or lung signs in these four persons.
2. Oxytetracycline was administered within a week or two after found seropositive.

Microbiology department in the tertiary care teaching hospital in Karnataka was cramped up for space and different sections were not cordoned off. Hence, chances of acquiring infection were equal for all the individuals of this laboratory, in the event of the infective aerosol escaping out of the Rickettsiology section. A large-scale infection of the laboratory personnel did not occur because of the possible precautions taken: Animal cages were disinfected daily. The inoculation hood fitted with UV lamp was switched on an hour before and after the experiment. Hood as well as the cabin was sterilised with formalin vapour periodically. Thus, only those who handled infected guineapigs and/or collected livestock blood during slaughtering, contracted the infection. *C. burnetii* may be excreted by the guineapigs in their urine and faeces. Follow-up of the four cases beyond six to seven months could not be achieved since three out of four cases left the institution. *C. burnetii* antigens have been completely used up which was provided by two International Rickettsial Research and Reference Laboratories in USA and Czechoslovakia. There were no commercial kits available then for serodiagnosis of coxiellosis/Q fever. Isolation of *C. burnetii* by inoculation in the yolk sacs of developing chick embryos could not be attempted in view of the hazard to laboratory workers due to the non availability of special isolation laboratory in that institution.

CONCLUSION(S)

Globally, Rickettsial research is gaining importance over the past two decades, with emphasis on Scrub typhus, Tick typhus (Spotted fever) and Q fever. Even demonstrating rickettsial antigens in ectoparasites by Immunofluorescence microscopy is potentially dangerous due to generation of infective aerosols. Caging of the inoculated laboratory animals must receive adequate care and attention, preferably Level 3 containment facility. Protective masks (preferably N 95) and PPE clothing must be worn by workers engaged in rickettsial work. Proper sterile precautions must be observed by those involved in Q fever research, since *C. burnetii*

is a potential threat for bioterrorism. Safe and effective commercial Q fever vaccine is presently available only in Australia.

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