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POIKILOTHERMS AS RESERVOIRS OF Q-FEVER *(Coxiella burnetii)* IN UTTAR PRADESH[1,2]

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Abstract: Water snakes *(Natrix natrix)*, rat snakes *(Ptyas korros)*, cobras *(Naja naja)*, pythons *(Python molurus)*, tortoises *(Kachuga sp.)*, plankton fish *(Cirrhina mrigala)*, frogs *(Rana tigrina)*, toads *(Bufo sp.)* and monitors *(Varanus indicus)* were screened for evidence of Q-fever infection by the capillary agglutination test on sera to detect antibodies and/or by attempts to demonstrate *Coxiella burnetii* in spleen and liver samples. Sero-reactors were observed among water and rat snakes, pythons and tortoises. The organism was isolated from the spleen and liver of the monitor, tortoise and python.

INTRODUCTION

A wide variety of animals including man, domesticated and wild mammals and birds have been incriminated as reservoirs of Q-fever.[1,4,5,6] However, there does not seem to be any information on record regarding Q-fever among poikilotherms. The purpose of this communication is to report the evidence of Q-fever infection in reptiles, amphibians and fish.

MATERIALS AND METHODS

The animals were caught from the Tarai belt of Uttar Pradesh near Pantnagar from October, 1976 to March, 1978. An open fish pond near the University dairy farm was the main source of fish, water snakes and tortoise.

The animals were immobilized by ether anaesthesia and their sera were collected by cardiac puncture within 2 to 8 hr of capture.

Sera were tested for antibodies against *C. burnetii* by the capillary agglutination test (CAT).[7] The phase I CAT antigen,[7] was obtained from the Center for Disease Control, Atlanta, Georgia USA. This antigen was prepared from infected chick embryo yolk sacs. Prior to testing, sera were inactivated at 56 C for 30 min. The CAT procedure has been described earlier.[9] Positive and negative serum controls were included with each test for comparison. Samples giving a CAT titre of 1:8 or more were considered positive.

To demonstrate *C. burnetii*, a 10% suspension of spleen plus liver from each animal, or pooled specimens from 2 to 6 animals within a species, was prepared in phosphate buffered saline (PBS). The samples were treated with penicillin at 1,000 i.u./ml. Samples found to be sterile on blood agar were inoculated either into chick embryos via the yolk sac (YS) method[3] or in 2 to 5 sero-negative mice to obtain evidence of sero-conversion[2] and to demonstrate the presence of thermostable rickettsiae. For the latter, a 10% suspension of spleen and liver, obtained from mice infected 1 to 4 weeks previously, was prepared in PBS, treated at 56 C for 30 min, and then passed in chick

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embryos by the YS method for testing viability. Inoculated and control chick embryos were examined on 8th day post-inoculation (DPI) by Macchiavello's stain for evidence of rickettsiae in smears of yolk sac membrane (YSM). If the YSM was positive it was further processed in mice as above. A sample which induced 1:16 or more CAT titre in one or more mice at 30 DPI, and gave evidence of heat resistant rickettsiae in spleen and liver, was considered to harbour *C. burnetii.*

RESULTS AND DISCUSSION

C. burnetii agglutinins were detected in 11 of 48 snakes, 2 of 5 pythons, and 2 of 16 tortoises; none of the 15 plankton fish, 66 toads and 7 frogs (Table 1) was positive.

Of the 11 samples processed from 20 snakes, 4 from 7 tortoises, 1 from 2 pythons, 2 from plankton fish and 1 from an Indian monitor, *C. burnetii* was demonstrated in the python, monitor and tortoise. Serum from the monitor could not be tested as this animal was obtained dead.

The evidence of exposure to Q-fever in few species of poikilotherms, based on the demonstration of agglutinins under natural conditions and the recovery of the organism from their visceral organs, suggests that these species may act as reservoirs for *C. burnetii.* Addition of poikilotherms to an already long list of homeotherms complicates the epidemiology of Q-fever. Although no previous data appears to be available on the application and suitability of CAT for poikilotherm serum, in the light of our observations on sero-epidemiology of Q-fever, supported by the recovery of *C. burnetii* from visceral organs, the test, as standardized with mammalian serum,[7] also appears to be suitable for cold-blooded animals.

The source of infection in water snakes and tortoises could be the fish pond, which is situated in the vicinity of a dairy farm[8] from which Q-fever agent has been isolated, or by their diet of rodents and insects. *C. burnetii* infection in rodents in and around Pantnagar has been observed.[9]

Pythons feed on rodents and other mammalian species, many of which are well-known reservoirs of Q-fever. Although a high proportion of the water

TABLE 1. Q-Fever antibodies in poikilotherms.

Animal species	No tested	No positive	CAT titre
*Snakes	48	11	1:8-1:32
Python, Indian *(Python molurus)*	5	2	1:8
Tortoise *(Kachuga sp.)*	16	2	1:8
Fish, Plankton *(Cirrhina mrigala)*	15	Nil	
Toad *(Bufo sp.)*	66	Nil	
Frog *(Rana tigrina)*	7	Nil	
Total	157	15	

*These include water snakes *(Natrix natrix),* rat snakes *(Ptyas korros)* and three cobra *(Naja naja).* All three cobra were negative.

snakes were seropositive, attempts to isolate *C. burnetii* from them was futile. Possibly, in addition to the liver and spleen, intestinal contents and kidneys from a sizeable number of reptiles should be screened for *C. burnetii*. In certain parts of rural India, cattle, buffalo, dogs, sheep, goats and camels have access to ponds usually inhabited by snakes, tortoise and turtles. In addition, contaminated ponds can be a public health hazard as the ponds are used for swimming, irrigation, and bathing of animals. The potability of the water is thus significant in the epidemiology of Q-fever in rural India. Additional species of fish, including carnivorous species should be similarly examined.

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