

# Zoonotic Infections in Military Scout and Tracker Dogs in Vietnam

A. D. ALEXANDER, L. N. BINN, B. ELISBERG, P. HUSTED, D. L. HUXSOLL,  
J. D. MARSHALL, JR., C. F. NEEDY, AND A. D. WHITE

*Divisions of Veterinary Medicine, Communicable Disease, and Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20012, Military Dog Veterinary Service, Lackland Air Force Base, Texas 78236, and U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland 21701*

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Significant levels of antibodies indicative of a variety of zoonotic infections were demonstrated in sera collected from 64 U.S. military scout and tracker dogs after service in the Republic of Vietnam (RVN). Scrub typhus antibodies were found in 45% of the specimens, melioidosis in 19%, group B arbovirus in 49%, and leptospirosis in 62%. Only 38% of the seropositive reactions for leptospirosis could be related to overseas infections. Tests were conducted on paired sera obtained from 32 of the dogs before service in RVN. Significant increases in titer were demonstrated with scrub typhus, melioidosis, leptospirosis, plague, group B arbovirus, and *Rickettsia canada* antigens. After 2 to 6 months of service in RVN, 8 of 19 dogs developed antibody titers to at least one of four zoonotic diseases. Only 3 of 45 dogs with 7 or more months of service failed to develop antibodies to one or more of the agents. The serological findings pose questions on the potential epidemiological importance and veterinary significance of scrub typhus, group B arbovirus, leptospirosis, and melioidosis infections in dogs.

Scout and tracker dogs have been used extensively in infantry operations by U.S. armed forces in the Republic of Vietnam (RVN) (26). In operational areas military dogs and troops may be exposed to the same environmental hazards of infection with various zoonoses. Both man and dog are known to be susceptible to natural infection with causative agents of leptospirosis (1, 25), melioidosis (32, 33), Japanese B encephalitis and other arboviruses (31), and plague (30). The etiological agents of these diseases, except plague, have proved to be significant causes of febrile illness among troops in RVN. Moreover, they were responsible along with scrub typhus and chikungunya fever for a major proportion of illnesses which were presented as undiagnosed fevers (10-12, 21, 29). We therefore posed questions as to whether a military dog was infected with any of these agents and if it could serve as an indicator for the presence of zoonotic agents of real or potential military medical significance. Serological evidence of diverse zoonotic infections acquired by military dogs in RVN is presented.

## MATERIALS AND METHODS

**Source of sera.** Sera were derived from 64 healthy military dogs which had been returned from RVN to

Lackland Air Force Base, Texas, in May 1971 for reassignment. None of the dogs had a history of disease while in RVN. They were predominantly males of the German shepherd breed and were originally recruited in the U.S. All had been immunized against rabies, canine distemper, infectious canine hepatitis, and leptospirosis (*Leptospira interrogans; canicola* and *icterohemorrhagiae* serotypes). In RVN they had served as scout or tracker dogs for periods ranging from 2 to 36 months. Approximately 70% of the dogs had more than 6 months of overseas service. Sera derived from 32 of the 64 dogs prior to their overseas service also were available for comparative tests. These samples were obtained routinely for a surveillance program for canine viral infections (6). Sera from 20 of the 32 dogs were obtained within a month prior to overseas shipment. Sera from the remainder were obtained from 1 month to 1 year before service in RVN. The activity of military dogs and animal husbandry practices for these dogs in RVN were described by Nims et al. (26).

**Serological tests.** Dogs were tested for rickettsial antibodies by an indirect fluorescent-antibody test (IF). The test was based upon the procedure used to demonstrate rickettsial antibody in human sera (14). Canine sera diluted 1:40 were first screen-tested with a pooled antigen comprised of a typhus group, a spotted fever group, scrub typhus, Q fever rickettsiae, and a rabbit anti-dog 7S globulin fluorescein conjugate (C. F. Needy et al., unpublished data). Positive sera were then tested at dilutions of 1:160 and 1:640 for

reactions with individual scrub typhus, spotted fever group, typhus group, Q fever, and *Rickettsia canada* (23) antigens. Reactions at a dilution of 1:160 and greater were considered to be significant.

The indirect hemagglutination (IHA) test was used to detect melioidosis antibodies (2). Reactions at 1:80 or greater dilutions of serum were considered to be significant. This criterion was selected on the basis of previous IHA serological findings on sera from 100 normal dogs in the U.S. At this titer nonspecific IHA reactions occurred in 1 to 2% of the samples.

Arbovirus antibodies were detected by means of the hemagglutination-inhibition (HI) technique by using goose erythrocytes (9). Chikungunya virus, African strain 168, was used as the source of antigen to detect group A arbovirus antibodies. The antigen was prepared by the method of Eckels et al. (13). Tests for antibodies for group B arbovirus were done with antigen prepared from Japanese B virus strain 1311 by the method of Clarke and Casals (9). Prior to tests, all sera were treated with acetone and adsorbed with goose erythrocytes. Eight units of antigen were used in each test. Titers of 1:20 and greater were considered to be significant.

The microscopy agglutination technique with 18 different serotype antigens was used to detect leptospiral antibodies (3). Significant titers were 1:25 and greater.

Tests for plague antibody were carried out by means of an IHA technique with a fraction I plague antigen (8). Reactions at serum dilutions of 1:8 and greater were considered to be significant.

## RESULTS

The prevalence of diverse antibodies in 64 scout and tracker dogs is shown (Table 1). High rates of serologically positive sera were found in tests for scrub typhus (45%), melioidosis (19%), group B arbovirus infection (49%), and lepto-

spirois (62%). The results of repeat tests with paired sera obtained from 32 dogs before and after service in RVN are shown (Table 2). In this group 16 dogs which were seropositive for scrub typhus had no detectable rickettsial antibody prior to shipment overseas. Significant antibody conversions for melioidosis were demonstrated in eight of nine seropositive dogs. One dog had an exceptionally high nonspecific reaction of 1:80 with the preservice serum. Fifteen of the 32 dogs with paired sera had significant levels of antibody for group B arbovirus. Only one of the 15 seropositive dogs had Group B arbovirus antibody prior to service in Vietnam.

Significant antibody titers were demonstrated in single dogs for spotted fever group rickettsiae, *R. canada*, chikungunya virus, and plague. The plague and *R. canada* antibodies were detected only in sera collected after service in RVN. The geographic origin of the chikungunya and spotted fever group antibodies could not be determined because preservice specimens were not available from these animals.

Leptospiral antibodies were found in 15 of 32 dogs before service in RVN and in 19 dogs in this group after their return to the U. S. Significant titer conversions were demonstrated in five dogs, two to serotype *bataviae*, two to serotype *borincana*, and one to serotype *grippotyphosa*. A comparison of the leptospiral agglutinins in 15 of 32 preservice serum samples and 40 of 64 post-service sera is shown (Table 3). Fourteen of the 15 preservice seropositive dogs had agglutinins for *canicola* or *icterohaemorrhagiae*, or both serotypes, whereas only 62% of the seropositives in postservice specimens had similar reactions.

TABLE 1. Prevalence of antibodies in 64 scout and tracker dogs in the Republic of Vietnam

Antibody	Test <sup>a</sup>	Positive reaction		Distribution of titers and no. of dogs
		No.	Per cent	
Rickettsia <sup>b</sup> .....	IF	30	47	
Scrub typhus.....	IF	29	45	1:160-10, 1:640-19
Spotted fever.....	IF	1	<2	1:160
<i>R. canada</i> <sup>c</sup> .....	IF	1	<2	1:160
Melioidosis.....	IHA	12	19	1:80-8, 1:160-3, 1:320-1
Group B arbovirus.....	HI	31	49	1:20-13, 1:40-7, 1:80-4, 1:160-5, 1:320 or >-2
Group A arbovirus.....	HI	1	<2	1:20
Leptospirosis.....	MA	40	62	1:25-25, 1:100-9, 1:400-5, 1:1600-1
Plague.....	IHA	1	<2	1:128

<sup>a</sup> Abbreviations: IF, immunofluorescent; IHA, indirect hemagglutination; HI, hemagglutination inhibition; MA, microscopic agglutination.

<sup>b</sup> IF test with pooled typhus, spotted fever group, scrub typhus, and Q fever antigens. Tests of screen test positive sera with typhus group and Q fever antigens were negative.

<sup>c</sup> Serum also had scrub typhus antibody titer 1:640.

TABLE 2. Serological findings in 32 military scout dogs before and after service in Republic of Vietnam

Antibody	No. positive		Titer changes <sup>a</sup>	
	Pre-service	Post-service	No.	Per cent
Scrub typhus	0	16	16	50
Spotted fever	0	0	0	
<i>Rickettsia canada</i>	0	1	1	3
Melioidosis	1 <sup>b</sup>	9	8	25
Group B arbovirus	1	15	14	44
Group A arbovirus	0	0	0	
Leptospirosis	15	19	5	16
Plague	0	1	1	3

<sup>a</sup> Criteria: rickettsia, from <1:40 to 1:160 or >; melioidosis, from <1:40 to 1:80 or >; arbovirus, from <1:10 to 1:20 or >; leptospirosis, from <1:25 to 1:100 or >; plague, from <1:4 to 1:32.

<sup>b</sup> Reaction considered to be nonspecific.

TABLE 3. Distribution of predominant leptospiral agglutinins in scout and tracker dogs before and after service in Republic of Vietnam<sup>a</sup>

Type of antibody	Positive reaction preservice (32 dogs)		Positive reaction postservice (64 dogs)	
	No. <sup>b</sup>	Per cent	No. <sup>c</sup>	Per cent
<i>canicola-ictero</i> <sup>d</sup>	14	93	25	62
<i>grippu-djasiman</i> <sup>d</sup>	1	7	4	38
<i>alexi-pyrogenes</i> <sup>d</sup>			3	
<i>autumn-pomona</i> <sup>d</sup>			1	
<i>bataviae</i>			3	
<i>borincana</i>			3	
<i>tarassovi</i>			1	
Total Dogs positive (%)	15	100	40	100
	47		62	

<sup>a</sup> Abbreviations: ictero., icterohemorrhagiae; grippo., grippotyphosa; autumn., autumnalis.

<sup>b</sup> Distribution of titers and number of dogs: 1:25-7, 1:100-6, 1:400-2.

<sup>c</sup> Distribution of titers and number of dogs: 1:25-25, 1:100-9, 1:400-5, 1:1600-1.

<sup>d</sup> Predominant titers to one or more antigenically related serotypes.

The reactions in the remaining seropositive sera were elicited against diverse serotypes which are not commonly found in U.S. dogs.

The distribution of antibodies for scrub typhus, melioidosis, group B arbovirus infection, and leptospirosis in the 64 dogs, according to length

of service in RVN, is shown (Table 4). Each of these antibodies was demonstrable in one to five of 19 dogs after 2 to 6 months of service. The prevalence rates in dogs for each agent after 6 months to 2 years of station in RVN ranged from 25 to 62%. High antibody prevalence rates for scrub typhus and group B arbovirus infection were shown in dogs with 2 to 3 years of overseas service. This same group, however, had an apparent decreased seropositive reactor rate for leptospirosis and melioidosis which may reflect limitations in the sample size. These data were examined again to determine the number of kinds of antibody found in dogs with regard to their length of service in RVN. After 2 to 6 months in RVN, 8 of 19 dogs developed antibody titers to at least one of four zoonotic agents. Only 3 of 45 dogs with 7 or more months of service failed to develop antibodies to one or more agents. In the group of 64 dogs, 23 had antibody titers for one agent, 20 for two, six for three and one for all four disease agents.

DISCUSSION

Antibodies found after service in RVN in the serum of dogs that had been raised and trained in the U.S. could reasonably be due to infections incurred in RVN when the etiological agents or other serologically related microorganisms are not known to be present in the U.S. This supposition was affirmed by demonstrating serological conversion from negative to positive when sera obtained before and after service in RVN were tested.

TABLE 4. Distribution of antibodies in 64 scout and tracker dogs by length of service in Republic of Vietnam

Antibody	No. positive/total dogs within monthly groupings				
	2-6	7-12	13-24	25-36	Total
Scrub typhus	5/19	9/18	9/16	6/11	23/64
Melioidosis <sup>a</sup>	1/19	5/18	4/16	1/11	11/64
Group B arbovirus <sup>a</sup>	2/19	10/18	10/16	8/11	30/64
Leptospirosis <sup>b</sup>	2/19	5/18	5/16	2/11	14/64

<sup>a</sup> One dog which had serological titer for melioidosis and one dog which was seropositive for group B arbovirus prior to service in the Republic of Vietnam were not included among positives.

<sup>b</sup> Dogs with seropositive reactions to *canicola* or *icterohaemorrhagiae* and one dog with a low titer (1:25) to *grippotyphosa* and *djasiman* serotypes in pre- and postservice sera were not included among positives.

The high incidence of infection in dogs with *R. tsutsugamushi* was unexpected. To our knowledge there have been no definitive studies on natural or experimental scrub typhus infections in dogs. Blake et al. (7) in an extensive review of scrub typhus casually mentioned early findings by Japanese workers that "dogs inoculated with infectious material were thought to be less susceptible than monkeys." Dogs are known hosts for vector chiggers (17). It is likely that they were infected in RVN by natural exposure. The regular treatment of the dogs with insecticides effectively controlled flea infestation but did not afford protection against mites and ticks. Limiting exposure to the arthropod vector may have been responsible for the failure to find serological evidence of murine typhus which is known to be prevalent in Vietnam. Although human cases of tick typhus have occurred in RVN, little is known about the prevalence or distribution of the spotted fever group rickettsiae. Any conclusion about the finding of *R. canada* antibody in the serum of one dog after service in RVN would be unwarranted at this time.

The serological evidence for the occurrence of subclinical canine melioidosis was consistent with the results of previous serological surveys carried out in RVN at the U.S. Army 9th Medical Laboratory in Saigon in 1967, (D. L. Huxsoll, unpublished data) and again in 1970 (R. Howarth, unpublished data). Recently, severe clinical forms of melioidosis have been recognized in military dogs in RVN (33).

The antibody detected with the Japanese B virus antigen could have resulted from exposure prior to overseas service to a group B arbovirus, such as the agent of St. Louis encephalitis which occurs in the U.S. and which can infect dogs (16, 22). The tests with paired sera from 32 dogs revealed that only one had preexisting antibody, indicating the occurrence of infection in the U.S., and that 14 other dogs had contracted group B arbovirus infections in RVN. Group B arbovirus HI antibody detected after return to the U.S. resulted from infections with either Japanese B encephalitis virus or other group B arboviruses which occur in RVN (10). Inapparent infections in dogs with Japanese B encephalitis virus are known to occur in Japan (18, 27) and Malaysia (28). Because dengue is found principally in urban regions the risk of exposure in operational areas would be minimal. It is likely that Japanese B encephalitis virus was the source of the group B arbovirus antibody. However, possible exposure to tick-borne members of the group cannot be excluded. Other cultural or

serological studies would be required to establish the identity of the causative group B agent(s).

Group A arbovirus HI antibody was found in only one postservice specimen. The source of the infection and its cause could not be ascertained. Chikungunya infections in man in RVN occur principally in urban areas, and exposure to the virus in the operational areas of the dogs would be markedly less. The susceptibility of dogs to this virus is unknown.

To interpret the results of tests for leptospiral antibody, it was necessary to consider the influence of natural infections contracted in the U.S. and vaccine prophylaxis. In past serological surveys of unvaccinated German shepherd dogs in the U.S. by Alexander et al. (1) and Thomas and Evans (34), leptospiral antibodies were demonstrated in 12.8% of 838 and 12.4% of 1,161 dogs, respectively. The reactions were preponderantly against *canicola* and *icterohaemorrhagiae* serotypes. Examination of the distribution of types of leptospiral antibody responses in 15 dogs which were positive before they went to RVN revealed that 93% of the reactions were against *canicola* and *icterohaemorrhagiae* serotypes. In contrast, in 40 dogs which had leptospiral antibodies after service in RVN, approximately 38% of the seropositive reactions occurred with antigens of nine other serotypes which commonly cause human or canine leptospirosis in Southeast Asia (5, 15, 35). These reactions were most likely the result of infections contracted in RVN and occurred in approximately 22% of all the dogs examined. The demonstration of antibody titer conversion to *bataviae*, *grippytyphosa*, or *borincana* serotypes with paired specimens served to corroborate the significance of the distinctive reactions with these serotypes in single post-service sera. Because of the current vaccine prophylaxis in military dogs, it was not possible to evaluate the frequency of infections with *canicola* or *icterohaemorrhagiae* serotypes in RVN. In South and North Vietnam, leptospiral infections in dogs have been demonstrated previously but have not been extensively studied (15, 35). Clinical manifestations of leptospirosis in military dogs in RVN have rarely been recognized (19).

Plague antibody was demonstrated only in 1 of 64 military dogs. On the other hand, Rust et al. (30) reported that 7 to 14% of native dogs in Vietnam have plague antibodies. Unlike the flea-infested indigenous dog which forages for food, the military dog is well provisioned and is routinely treated with insecticides. These factors may have figured significantly in limiting the exposure hazards of military dogs to plague.

The findings in this study direct attention to the

potential usefulness of dogs to identify geographic areas where man might contract scrub typhus, Japanese B encephalitis or other group B arbovirus diseases, melioidosis, and leptospirosis. Heretofore, the dog has been advantageously used as a sensitive indicator to locate plague foci (4, 20, 30). With rare exception, little is known about the importance of these infections as causes of febrile disease or morbidity in dogs. Dogs infected with leptospirosis can be the cause of sporadic cases or outbreaks of the disease in man (24, 25). Because animals infected with the other rickettsial, viral, and bacterial agents may constitute potential reservoirs, the epidemiological and public health importance of these zoonotic canine infections should be investigated.

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