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COMPARISON OF SEROLOGIC REACTIONS IN EXPERIMENTAL CANINE AND SIMIAN COCCIDIOIDOMYCOSIS

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The work reported here was conducted under Project 4B04-14-004, "Special BW Operations." The expenditure order was 2062. It is a part of a continuing co-operative effort by the Special Operations, Pathology, and Medical Bacteriology Divisions in characterizing the disease coccidioidomycosis. This report was originally submitted as manuscript 5000.

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ABSTRACT

A comparative study of respiratory coccidioidomycosis was made using dogs and monkeys. The serologic findings are presented here. Three groups of monkeys and three groups of dogs were each exposed by the respiratory route to graded doses of approximately 9500, 2500, and 325 dry arthrospores of Coccidioides immitis strain Silveira. Blood for serological tests was drawn from these animals prior to exposure and at 2, 5, 9, 14, 17, and 20 weeks postexposure. Precipitins were detected using agar gel. Sera for c.f. antibodies was pretreated with complement before testing.

Infections in monkeys ranged from lethal to severe, whereas infections produced in dogs were severe to mild. All monkeys in the high-dose group died of pulmonary coccidioidomycosis between Days 11 and 61 postexposure. No dogs died from the disease.

In sera from infected dogs, the highest median precipitin titers occurred at five and nine weeks postexposure. Titers fell after these times in all dose groups. Precipitin titers in the medium- and low-dose groups of monkeys reached their highest median values at 14 weeks postexposure and then declined slightly. Precipitin titers in infected monkeys were consistently higher than those in infected dogs.

Highest median titers for c.f. antibodies occurred at the five-week test period in dogs of all dose groups. After this time period, the median titers in the sera of these dogs declined. In infected monkeys, maximum median titers of c.f. antibodies were observed at nine and 17 weeks postexposure. The median titers forming this plateau were higher than the highest median titers found in sera of infected dogs for c.f. antibodies.

The serologic reactions in the two animal species appeared consistent with the pathologic findings.

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I. INTRODUCTION

Experimental infections with Coccidioides immitis have been reported in monkeys[1-3] and in dogs[4-9] and reference was made to serologic findings in the two species. In experimental infections in monkeys, dermal hypersensitivity preceded the formation of antibodies. Both precipitins and complement fixing (c.f.) antibodies were found. The higher titers of c.f. antibodies correlated with the dose and severity of the disease.

Investigations of similar reactions in infected dogs have not yielded consistent results. Hage and Moulton[6] reported that precipitins were present for only a short time in naturally infected animals. Maddy[8] reported that precipitins tended to persist in disseminated infections. Hugenholtz et al[10] did not detect precipitins and Reed[4] reported only sporadic occurrence in their respective experimental studies in dogs. The long duration of c.f. antibodies and the higher titers of these antibodies in disseminated cases of canine coccidioidomycosis have been reported in natural and experimental infections. Dermal hypersensitivity has not always preceded the formation of c.f. antibodies in experimental infections.

This report compares the precipitin and c.f. antibody reactions occurring in dogs and monkeys receiving similar graded doses of aerosolized arthrospores of C. immitis. A report of the comparative pathogenesis in this investigation has been published.[11] The results of both phases of this comparative study may contribute to a determination of how closely the disease in these animals resembles that in man.

II. MATERIALS AND METHODS

Twenty healthy, mature, mixed-breed dogs* of both sexes (males castrated) weighing between 15 and 25 pounds, and 13 monkeys, Macaca mulatta, of both sexes, weighing five to seven pounds, were used for this investigation. These animals were divided into four groups: high dose (9000 to 10,000 arthrospores), medium dose (2500 to 3000 arthrospores), low dose (300 to 350 arthrospores), and a nonexposed control group. There were five dogs and four monkeys in each dose group, and five dogs and one monkey in the noninfected control group.

Animals in the experimental groups were exposed to an aerosol of arthrospores of dry C. immitis, strain Silveira. The largely uniparticulate arthrospore product was aerosolized in an aerosol chamber by means of compressed air. The monkeys and the dogs from each group were exposed separately. After exposure, monkeys were individually caged and housed in air-conditioned, gas-tight, cabinet systems. The dogs were housed in groups in a similar cabinet system. Individual doses were estimated from the calculated breathing rate of each animal and the concentration of organisms in the air of the chamber at the time of exposure.

Blood was drawn from each animal for precipitin and c.f. tests before exposure and at intervals of 2, 5, 9, 14, 17, and 20 weeks postexposure. Before bleeding, monkeys were tranquilized with one milliliter of piperdine 1 - (1-phenylcyclohexyl) hydrochloride in a concentration of 0.2 microgram per milliliter. Dogs were tranquilized with Sparine hydrochloride,** using one to three milligrams per pound per dog.

Precipitins were detected using agar gel.[12] Sera for c.f. antibodies were pretreated with complement before testing.[13] Details of the methods used in both tests will be reported in another paper.

Blood collected from each animal at each testing was also cultured for presence of C. immitis.

Skin tests were given each animal at the same time blood was obtained. Dogs were tested on the shaved upper surface of the ear. Monkeys were skin-tested on an eye lid. Two coccidioidin products were used; an undiluted, experimentally produced coccidioidin and a 1:10 dilution of a commercial product.***

At 20 weeks postexposure, surviving animals were euthanized with Nembutal**** administered intravenously. A detailed necropsy was performed on each animal. All tissues were fixed in ten per cent buffered formalin, paraffin-mounted, sectioned, Giemsa-stained and examined.

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* In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

** Promozine hydrochloride, gamma-dimethylamine-n-trophylphenothiozine hydrochloride. Wyeth Laboratories, Inc. Philadelphia, Penn.

*** Cutter Laboratories, Berkely 10, California

**** Abbot Laboratories, North Chicago, Ill.

III. RESULTS

Skin test reactions were positive in the majority of challenged animals at five weeks after exposure. At the same time, both precipitins and c.f. antibodies were also detected in these dogs and monkeys.

In sera from infected dogs, the highest median precipitin titers occurred at five and nine weeks postexposure (Figure 1). Titers fell after these time periods in all dose groups, with the most rapid decline occurring in the medium-and low-dose groups. Precipitins disappeared from the sera of dogs in the medium- and low-dose groups at 14 weeks and in the high-dose group at 17 weeks.

Monkeys in the high-dose group all died before the ninth-week testing period. Precipitin titers in the two remaining groups of infected monkeys (low and medium dose) reached their highest median values at 14 weeks postexposure and then declined slightly (Figure 2).

Precipitin titers in infected monkeys were consistently higher than those in infected dogs and persisted at relatively high levels throughout the five-month test period. Also, precipitin titers in sera of both infected dogs and infected monkeys varied, but not necessarily significantly, in direct relation to the dose of arthrospores received.

Complement-fixing antibodies were detected in the sera of infected dogs and infected monkeys at the fifth-week testing period. Highest median titers in infected dogs occurred at five weeks postexposure in all dose groups (Figure 3). After this time period, the median titers in the sera of these dogs fell. In the low- and medium-dose groups, c.f. antibodies disappeared at 14 and 17 weeks, respectively. In infected monkeys, maximum median titers of C.F. antibodies were observed at nine and 17 weeks postexposure (Figure 4). The median titers forming this plateau were higher than the highest median titers found in sera of infected dogs for c.f. antibodies.

No dogs died from coccidioidomycosis. One death did occur in the high-dose group, but was attributable to other causes. In seven of the ten dogs in the medium- and high-dose groups, infection spread from the lungs to involve other tissues. Infection was confined to the lungs in the low-dose group.

All monkeys in the high-dose group died of pulmonary coccidioidomycosis between the Days 11 and 61. No deaths occurred in the medium- and low-dose groups. Disseminated disease was found in five of the eight monkeys in these two dose groups.

C. immitis was not isolated from the blood of any infected animals.

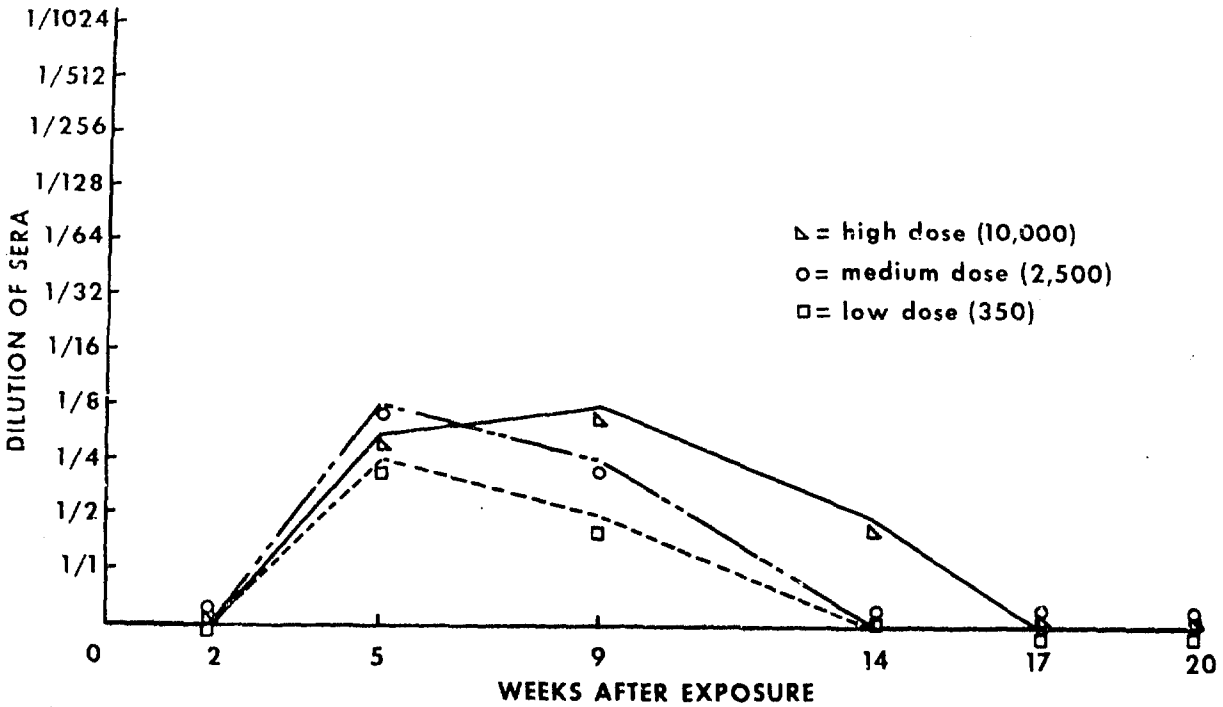

Figure 1. Dog Coccidioidomycosis - Precipitin Titers.

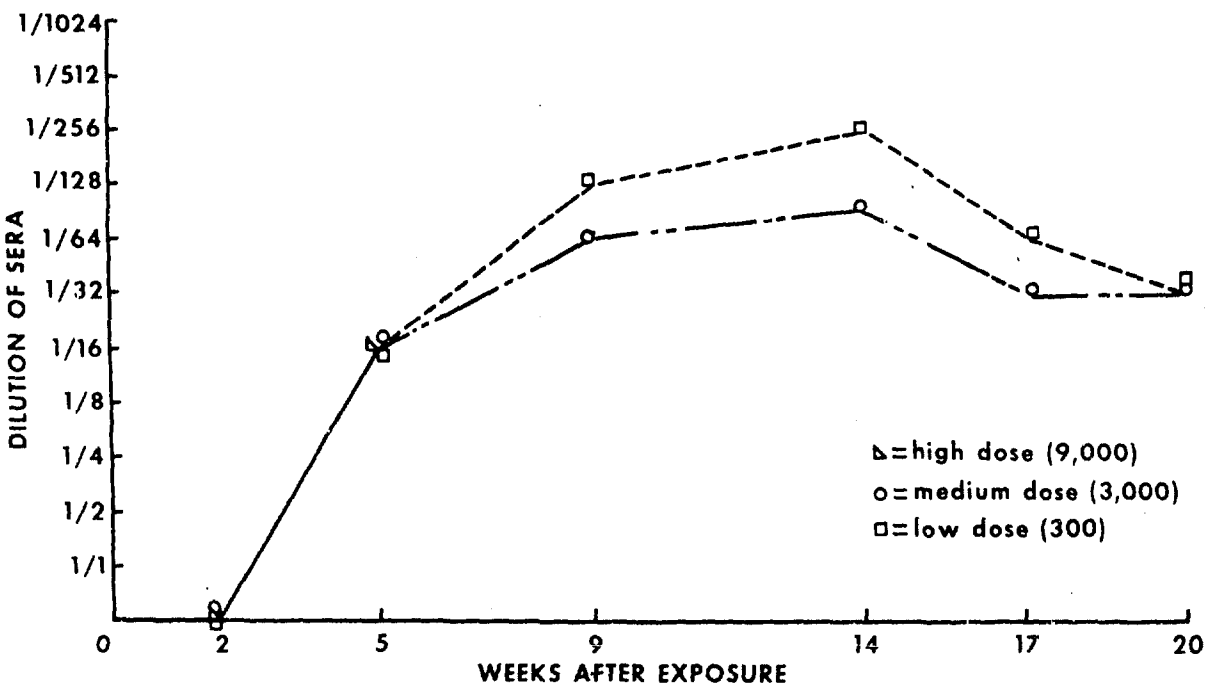

Figure 2. Monkey Coccidioidomycosis - Precipitin Titers.

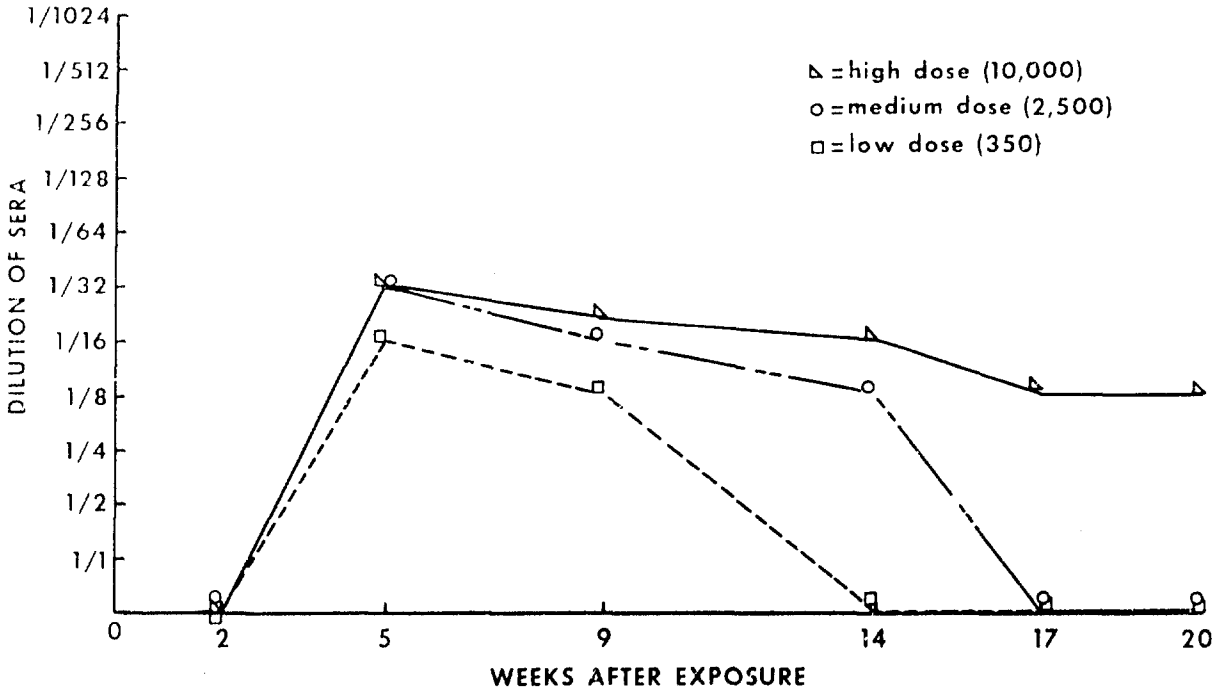

Figure 3. Dog Coccidioidomycosis - Complement Fixation Titers.

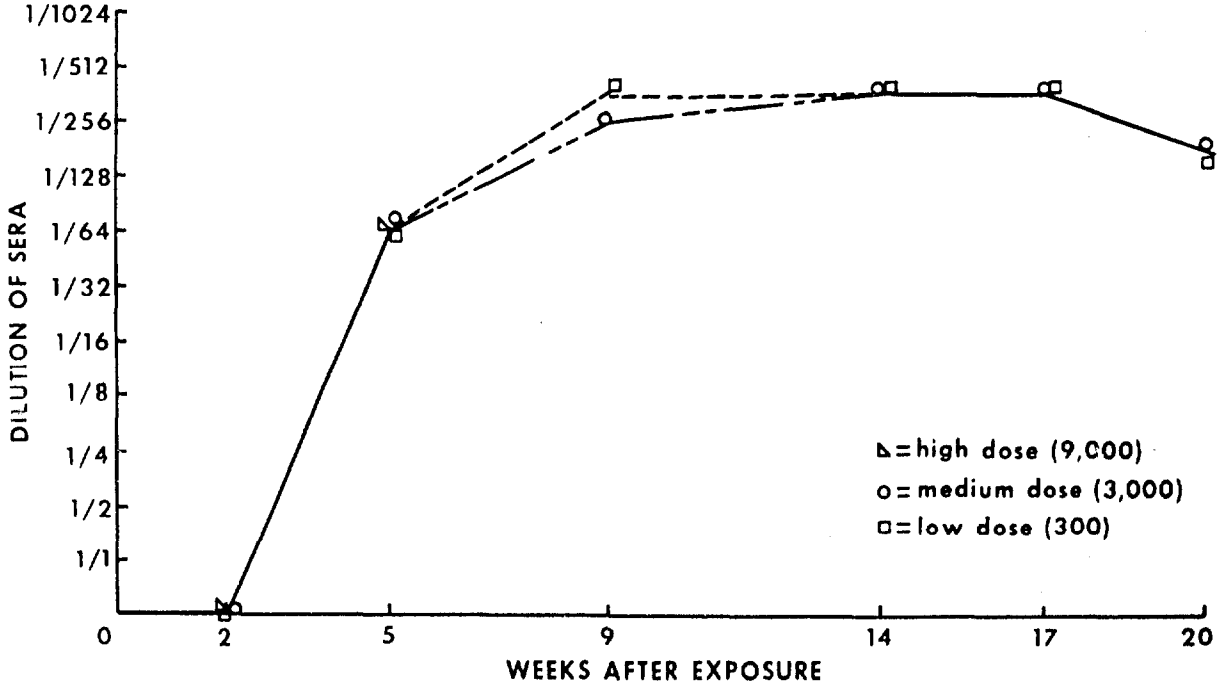

Figure 4. Monkey Coccidioidomycosis - Complement Fixation Titers.

IV. DISCUSSION AND CONCLUSIONS

In comparing the pathologic[11] and serologic responses at similar dose levels, it was found that monkeys reacted more severely than dogs to an infection produced by aerosolized arthrospores of C. immitis. Pathologic study indicated that the experimental infections could be classed as:

(a) Lethal pulmonary, where death appeared to result from primary pulmonary pneumonia,

(b) Severe pulmonary and/or disseminated, where pulmonary involvement was severe and/or where lesions were found in other tissues,

(c) Mild primary pulmonary, where infection appeared less severe and was confined to the lungs.

On the basis of these groupings, all the dog infections were in the last two categories, whereas those in the monkeys were in the first two groups (Figure 5). The one dog death was attributed to causes other than coccidioidomycosis. All the monkeys in the high-dose group died within two months. None of the infections in the surviving monkeys could be considered mild.

The serologic reactions in the two animal species appeared consistent with the pathologic findings. All exposed animals developed both precipitin and c.f. antibodies within five weeks after exposure. However, precipitin and c.f. titers in dogs generally were not high and the antibodies tended to decline in titer or disappear entirely as the period of observation progressed. On the other hand, precipitin and complement-fixation titers in monkeys rose to much higher levels than in dogs and tended to persist throughout the observation period.

The occurrence of precipitin and c.f. antibodies in infected dogs and monkeys can also be compared with the findings of Smith et al[14] in Figures 6 and 7. In these graphs, the per cent positive precipitin and c.f. reactions occurring in dogs and monkeys after exposure are compared with those for humans. The patterns of precipitin formation in dogs was roughly similar to that in man, the positive reactions decreasing after the first month to zero or near zero levels (Figure 6). No drop in precipitin occurrence was observed in monkeys. Precipitins were found in the sera of all surviving monkeys throughout the observation period.

When patterns of occurrence of positive c.f. reactions in sera of the three species are compared, a gradual decrease is noted in dogs (Figure 7). Man remains at a level of approximately 86 per cent reactors and monkeys at 100 per cent reactors for the five-month period. Smith et al[14] have stated that antibodies regress slowly in symptomatic primary infections. The percentage decrease in c.f. antibodies in dogs resulted from their disappearance from the sera of those animals in the medium- and low-dose groups, possibly indicating the very mild nature of infections in those two groups. Man, therefore, may occupy an intermediate position between dogs and monkeys in resistance to this disease.

Animal	Dose Groups	Deaths	PATHOLOGY		
			Lethal Pulmonary Inf.	Severe Pul. &/or Dissemination Infection	Mild Prim. Pulmonary Infection
Dog (5 Animals per Dose)	10,000	1.*		3.	2.
	2,500			4.	1.
	350				5.
Monkey (4 Animals per Dose)	9,000	4.	4.		
	3,000			4.	
	300			4.	

*Nonspecific Death

Figure 5. Pathologic Response Following Aerosol Infection by C. immitis in Dogs and Monkeys.

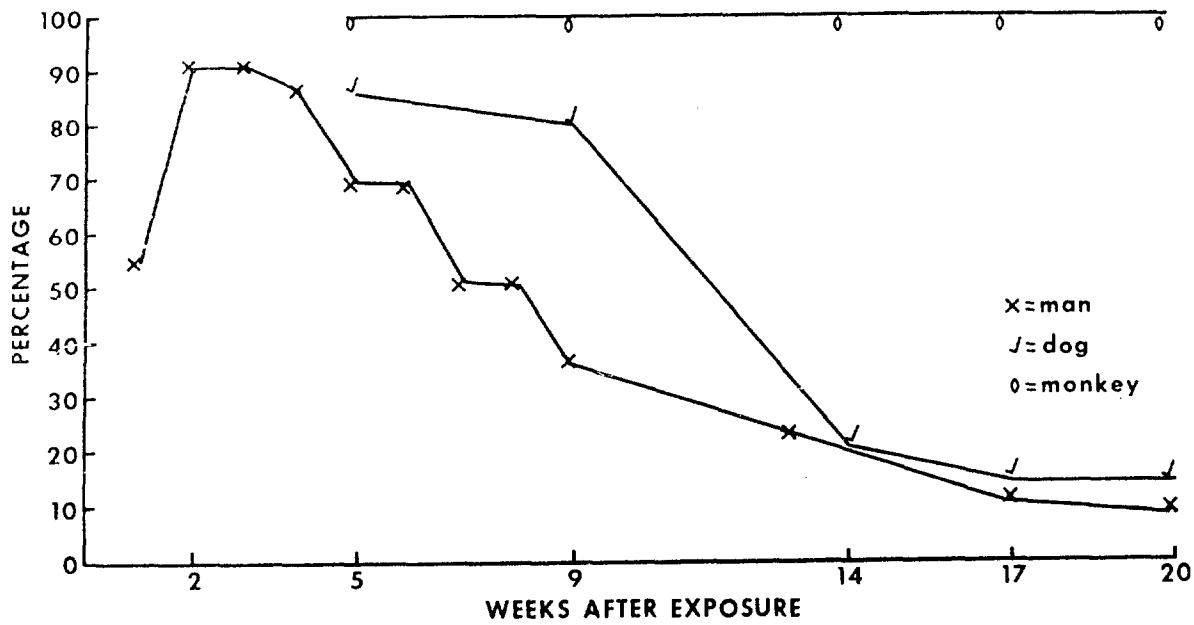

Figure 6. Occurrence of Positive Precipitin Reactions (%) in Dogs and Monkeys Experimentally Infected with C. immitis and with Natural Infections in Man.

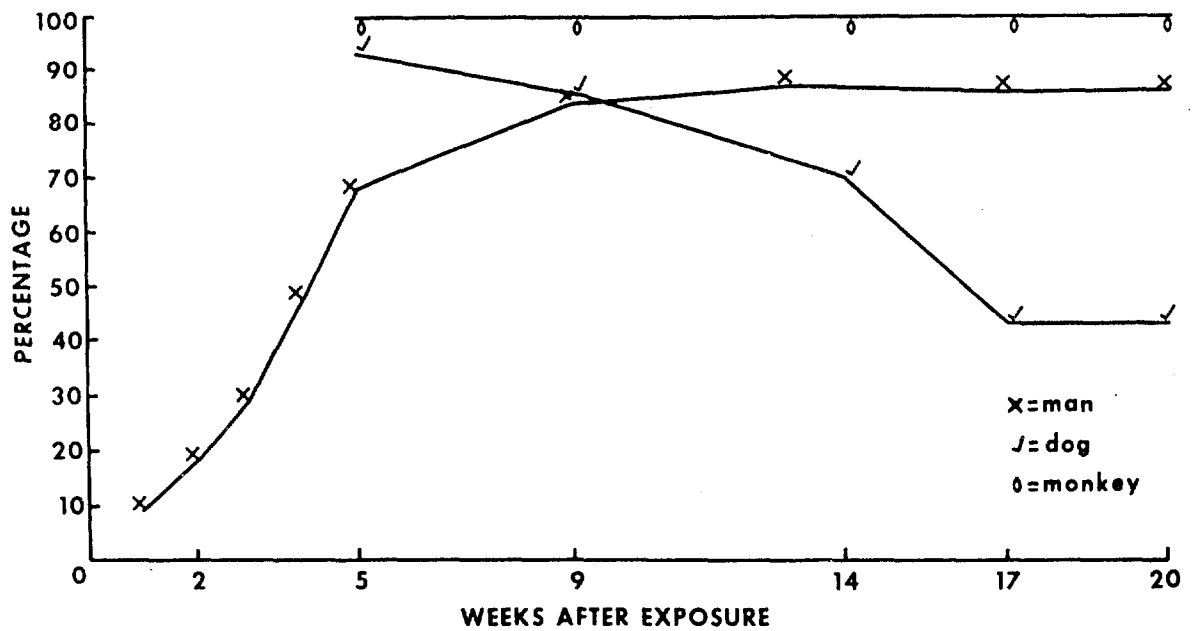

Figure 7. Occurrence of Positive Complement Fixation Reactions (%) in Dogs and Monkeys Experimentally Infected with C. immitis and with Natural Infections in Man.

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