STUDIES ON AMERICAN PARAGONIMIASIS VI. ANTIBODY RESPONSE IN THREE DOMESTIC CATS INFECTED WITH *PARAGONIMUS KELLICOTTI.**

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Abstract

Sera obtained approximately every 10 days from three domestic cats exposed to varied numbers of metacercariae of *Paragonimus kellicotti* were examined for complement fixing and precipitating antibody for a period of 150 days after exposure. The responses obtained are shown and discussed.

Introduction

Serological tests have been developed for the clinical diagnosis of human paragonimiasis infections (Sadun *et al.,* 1959), but there have been few attempts to determine the type of antibody response throughout the course of human or animal infections. Sadun *et al.* (1959) have studied the complement fixing antibody response in cats experimentally infected with *Paragonimus westermani.* These authors first detected complement fixing antibody 30 days following the inoculation of a single dose of metacercariae and remained positive for at least 130 days. In their work, however, there was no description of the antibody response between 30 and 130 days, or of possible fluctuations in antibody titers.

Several recent attempts have been made to characterize the antigens from adult *Paragonimus westermani* and *P. kellicotti* obtained from naturally and experimentally infected cats (Yogore, Jr., Lewert, and Madraso, 1965; and Seed, Sogandares, and Mills, 1966). These reports are in close agreement that adult worms have a minimum of 5 precipitating antigens as shown by agar-diffusion techniques. As in the previous work on complement fixing antibody, however, no attempt was made to follow the type of antibody response throughout the entire infection. It is considered conceivable that failure to determine the antibody response throughout an infection might possibly produce misleading results. It is also believed possible that the quantity and specificity of precipitating antibody molecules might vary throughout the infection.

This work describes our observation of sera obtained from infected cats approximately every 10 days throughout the course of 150-day infections.

Materials and Methods

Three domestic cats (5 to 10 lbs body wgt.) were exposed by pipetting, *per os,* varied numbers of metacercariae of *P. kellicotti.* The cats were partially sedated by an intraperitoneal injection (1 m. 4% solution/5 lbs body wgt.) of thiamylal sodium (Surital®, Parke, Davis and Co.) and bled by heart puncture. The cats were bled prior to exposure to *P. kellicotti* and at approximately 10 day intervals until they were killed. Sera were stored at −30°C until needed. The adult worms were harvested from cats, their uteri removed, washed several times in normal saline, and then stored at −30°C until needed. The cats were necropsied for helminthic infections of the intestine. These cats were maintained in

*This work was initiated under sponsorship of a research grant (AI 03386-06 TMP) from the National Institutes of Health and was directly supported by research grants GB 3036 and GB 5235 from the National Science Foundation.

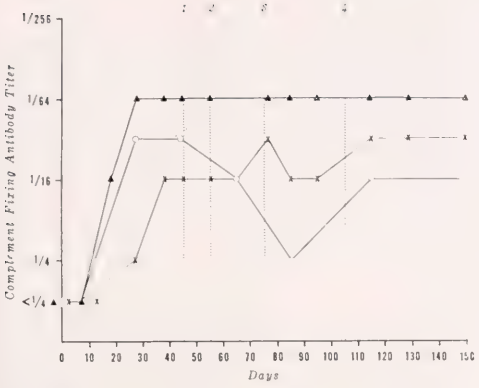

Figure 1. The time course of complement fixing antibody in cats infected with *Paragonimus kellicotti.* 1. A chronic cough first observed in cats CFS-20 and 21. 2. Eggs of *P. kellicotti* first observed in fecal samples from cat CFS-21. 3. Eggs of *P. kellicotti* first observed in fecal samples from cat CFS-20. 4. CFS-18 fed a second metacercaria. X cat 18; ○ cat 20; ▲ cat 21.

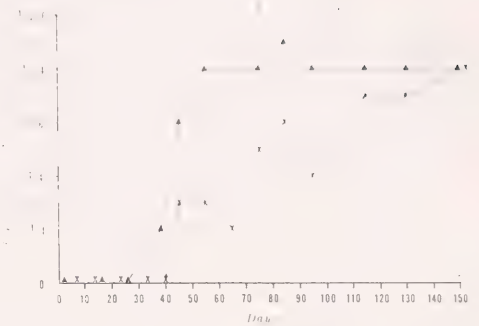

Figure 2. The time course of precipitating antibody in cats infected with *Paragonimus kellicotti.* See Figure 1 for explanation of numbers 1 to 4. X cat 18; ▲ cat 21.

relatively good health throughout the infection as shown by continuous weight gains.

Stool samples were obtained weekly from the infected cats, and were examined by Ritchie's (1948) ether-formalin concentration techniques for eggs of *P. kellicotti.*

Paragonimus extracts were prepared as previously described (Seed, Sogandares, and Mills, 1966). Approximately 20 adult worms were suspended in 8 ml of distilled water and homogenized with the aid of a hand glass homogenizer. The homogenate was then centrifuged at 12,000 × G for 10 min in a refrigerated International Centrifuge. The sediment was discarded and the supernatant was used as the antigen. All antigens were stored at −30°C until needed.

Protein was assayed by the method of Lowry *et al.* (1951). All antigen suspensions were made to contain 4.0 to 5.0 mgs protein per ml. In a previous publication (Seed, Sogandares, and Mills, 1966) we stated that our suspensions contained 0.4 to 0.45 mg protein per ml. This was an error in the placement of a decimal point in the manuscript and text and should have read 4.0 to 4.5 mg protein per ml.

Serological tests:

1) Agar diffusion tests were performed as described by Ouchterlony (1958). The agar used consisted of a 0.5% distilled water solution of Oxoid Ionagar, No. 2 to which was added glycine (0.5%) and merthiolate (0.1%) (Thimerosal®, Eli Lilly and Co.). The agar was then adjusted to pH 7.0 with 0.5M NaOH. 20 ml of agar were poured into each petri plate and wells were made with a No. 5 cork borer. The distance between the centers of adjacent wells was 14.0 mm. The wells were filled with approximately 0.1 ml of antigen or antibody. The plates were kept at room temperature and the results recorded at 24, 48 and 72 hours.

Precipitating antibody was titrated in agar diffusion plates using twofold dilutions. The end-point was taken as the last dilution at which a visible precipitin line was found.

2) The complement fixation test was performed by a modification of the Kolmer method for syphilis (Kolmer, Spaulding, and Robinson, 1951). A ¼ dilution of antiserum was the lowest dilution tested, due to the anti-complementary activity of some cat antisera. Glycerinated anti-sheep hemolysin, lyophilized guinea pig complement, and 2% sheep red blood cells, were all obtained from the Colorado Serum Company, Denver.

Results

The presence of both complement fixing and precipitating antibody was detected in sera of infected cats, confirming previous results. A minimum of 6 precipitating bands was found in agar diffusion plates indicating the presence of at least six antigens in extracts of adult *P. kellicotti.* This is one

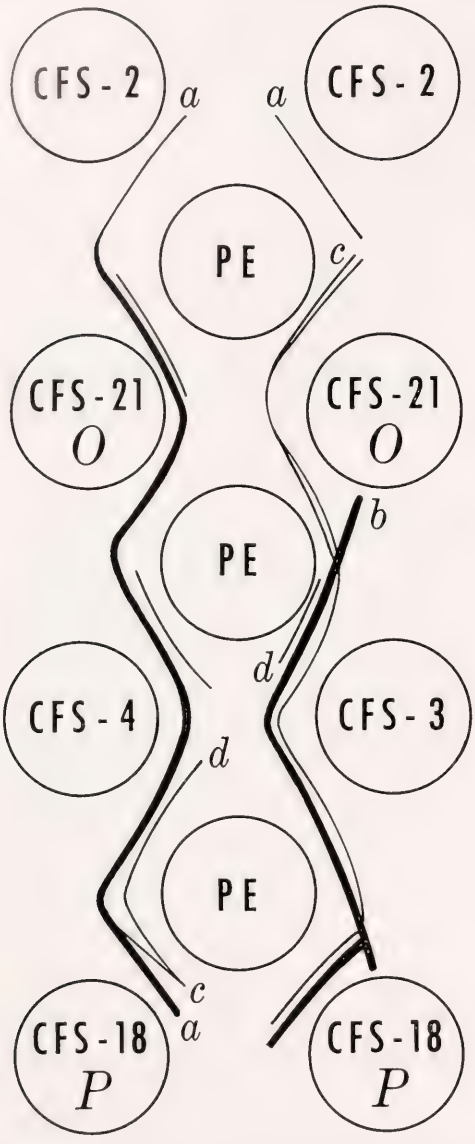

Figure 3. Gel diffusion patterns produced by infected cat serum and *Paragonimus kellicotti* extracts. The *Paragonimus* extract (PE) contained 4.0 mg protein per well; 0.1 ml antigen or antibody was used per well. Sera CFS-2, 3 and 4 have been described previously (Seed Sogandares and Mills, 1966). The plates were read at 24 and 48 hrs and a final reading was made at 72 hrs.

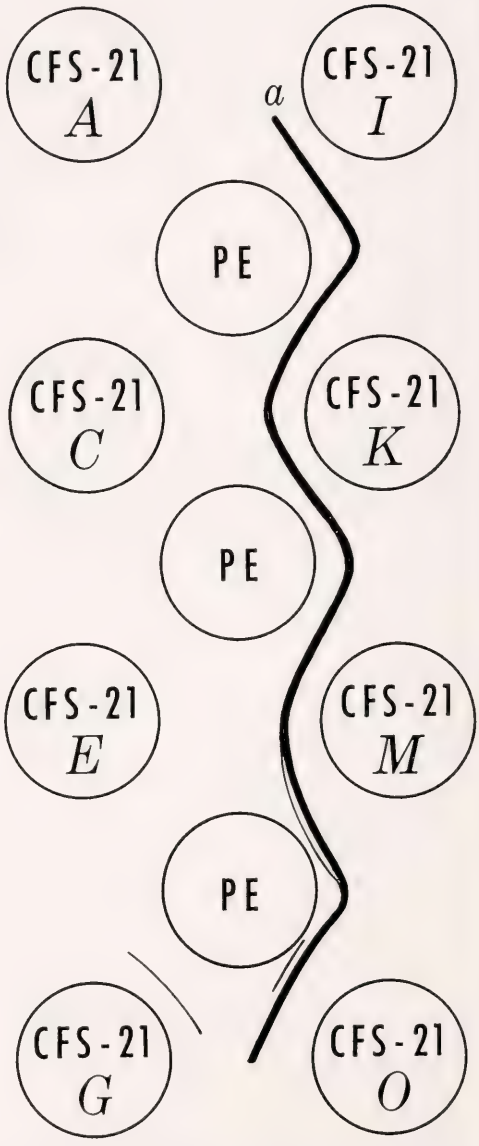

Figure 4. Gel diffusion patterns produced by sera, from an infected cat, taken at varied time intervals and tested against *Paragonimus kellicotti* extracts. The methods and materials were identical to that described for Figure 4. The time in days at which serum was obtained: CFS-21A (0), CFS-21C (8), CFS-21E (27), CFS-21G (43), CFS-21I (65), CFS-21K (85), CFS-21M (115), and CFS-21O (150).

Table 1
Data on infected cats from which sera were obtained.[1]

Cat number	Number of metacercariae fed	*Paragonimus* recovered at autopsy	Other helminth infections[4]	Patency of *P. kellicotti*	Maximum precipitation titer	Maximum complement fixation titer
CFS-18	2[2]	2[3]	unidentified nematode	none	1/64	1/32
CFS-20	42	36[5]	*T. cati* *D. caninum*	patent	–	1/32
CFS-21	21	21[5]	*T. cati* *D. caninum*	patent	1/128	1/64

[1] All cats were infected for 150 days.
[2] Fed one metacercaria 8 March 1966, and another 21 June 1966.
[3] Unencysted migrating preadults recovered at autopsy.
[4] Determined by fecal examinations and by autopsy.
[5] Adults only recovered.

more antigen than reported previously (Seed, Sogandares, and Mills, 1966). These results are in agreement with those of Tada (1967) who observed a maximum of six precipitin bands in serum from a rat infected with *Paragonimus miyazakii*. The detection of an additional band is presumably due to the greater length of time allowed for pattern development prior to the final recording of our results (48 versus 72 hours). As previously shown, the precipitating antibody response appears to vary in infected cats. Serum CFS-3 contained at least one unique antibody specificity not found in CFS-18 and 21 (Figure 3). Serum CFS-20 contained no detectable precipitating antibody, although the largest number of adult worms was recovered from this cat (Table 1). Figure 4 suggests that although the intensity and position of the precipitating bands in agar diffusion plates changes during the early stages of the infection, the same bands are found throughout the infection. This suggests, assuming our methods are sensitive enough, that the cat does not experience different antigenic stimuli from the adult worms during the course of the infection.

Figures 1 and 2 show the precipitating and complement fixing antibody titers throughout the course of a *Paragonimus* infection of cats. Both the precipitating and complement fixing antibody curves are similar in that once antibody is detected there is a sharp rise in titer and then a plateau is attained. The antibody titers are then maintained at a high level throughout the course of the infection. One obvious difference between the precipitating and complement fixing antibody curves is that complement fixing antibody is detected considerably earlier in the infection. Cat (CFS-20) contained no detectable precipitating antibody by the agar diffusion test but significant complement fixing antibody titers were found.

Figures 1 and 2 also show that there is no obvious correlation between the rise and titer of complement fixing and precipitating antibody, and egg production or clinical symptoms (chronic cough). Cat (CFS-18), which never showed the presence of eggs in its stool, contained definite precipitating and complement fixing antibody. The complement fixing antibody was detected considerably earlier than the passage of eggs or a noticeable cough.

Conclusions

It is apparent from these data that both complement fixing and precipitating antibodies arise relatively early (17 and 37 days) during *P. kellicotti* infections of the cat. The antibody titer then reaches a plateau and remains high throughout the infection. This is to be expected since the adult worms would presumably release antigen(s) throughout the entire infection. Our results are similar to those reported by Sadun *et al.* (1959) for *P. westermani* infections in the cat using the complement fixation test, and also So (1959) who investigated the precipitating antibody response in rabbits and dogs infected with *P. ohirai*.

Tada (1967), however, reported a decrease in the number of precipitating anti-

bodies in sera from rats with long-term *P. miyazakii* infections. He suggested that the cyst surrounding the adult worms might impede the passage of excretory substances and, therefore, account for the reduced antibody response.

It was of interest that cat (CFS-20) which had the highest worm burden failed to show detectable precipitating antibody. One additional cat, (CFS-2), which was reported upon previously and had the next highest worm burden (30 adults recovered), also showed an unexpectedly low precipitating antibody titer. Numerous suggestions can be made to explain the low level or absence of detectable precipitating antibody in heavily infected cats. However, the most interesting possibilities to us are the phenomena of immune paralysis or the absorption and removal of circulating antibody by the large number of adult worms and their antigens. Both explanations might readily account for the data obtained, and are amenable to further experimentation.

Sogandares (1966) indicated that *P. kellicotti* infections became patent only in cats whose lung cysts contained two or more worms. Cat CFS-18 had been fed a single metacercaria, followed by a second 106 days later; the infection never became patent even 44 days after feeding of the second metacercaria, and two unencysted migrating preadults were recovered after 150 days. Patency usually results 30 to 44 days, past exposure to metacercariae, when two or more worms locate in a single lung cyst. In view of Sogandares' (1966) findings and in our recovery of two unencysted immature worms from cat CFS-18, in whose sera both complement fixing and precipitating antibodies could be demonstrated, it is strongly suggested that some complement fixing and precipitating antibodies may be produced in the absence of eggs passing through the host lung tissues. Since the sera from cat CFS-18 had essentially the same precipitin patterns in agar diffusion plates as sera from other cats containing adult worms, our findings also suggest that preadults produce antigens in common with the adult worms. This is in agreement with the work of Tada (1967) who found precipitating antibody to adult worms in infected rats prior to the encystment of the adults in the lung.

The detection of complement fixing antibody considerably earlier than the detection of precipitating antibody, as well as the detection of complement fixing antibody in cat (CFS-20) which failed to produce significant levels of precipitating antibody, might be due to the greater sensitivity of the complement fixation test. The possibility should also be considered that the complement fixing antigen(s) and the precipitating antigens are not identical. The early rise in complement fixing antibody might prove the complement fixation test valuable as a possible diagnostic tool especially early in infection prior to patency. Sadun *et al.* (1959) previously suggested a similar use of the complement fixation test based on their observations of *P. westermani* infections in cats.

Acknowledgments

The authors are indebted to Sue Carlysle Dike for technical assistance, and to Mrs. E. Peter Volpe for execution of the figures.

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December 23, 1968