BioOne COMPLETE

Serum Protein Changes in Immune and Nonimmune Pigeons Infected with Various Strains of Trichomonas Gallinae *

Authors: KOCAN, RICHARD M., and HERMAN, CARLTON M.

Source: Journal of Wildlife Diseases, 6(1) : 43-47

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-6.1.43

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Serum Protein Changes in Immune and Nonimmune Pigeons Infected with Various Strains of *Trichomonas Gallinae* *

RICHARD M. KOCAN and CARLTON M. HERMAN

Patuxent Wildlife Research Center

Laurel, Maryland 20810

Received September 4, 1969

Abstract

Serum protein changes were studied in immune and nonimmune pigeons infected with three different strains of *Trichomonas gallinae*. Strain I (nonvirulent) produced no change in the relative concentration of serum components. Strains II (oral canker) and III (Jones' Barn) produced decreases in albumin and alpha globulins, and increases in beta and gamma globulins between the 7th and 20th days post infection. Birds infected with strain II began to return to normal by the 20th day, while all those infected with strain III were dead between 10 and 14 days post infection.

Two serum protein patterns resulted from infection of immune birds with the Jones' Barn strain. One showed no change in relative protein concentrations and no tissue invasion by the parasite while the other was similar to that seen in nonimmune birds infected with a strain producing oral canker. These also showed evidence of tissue invasion by the parasite.

It was concluded that tissue invasion was necessary to evoke a quantitative change in serum protein concentrations.

Introduction

Trichomonads are primarily invaders of hollow organs and are reportedly unaffected by humoral antibody.[3,4] *Trichomonas gallinae* however, has several strains which invade tissue and should stimulate some protective humoral response by the host. Since recovery from infection by any strain of *T. gallinae* confers immunity to trichomoniasis on the host,[9] this study was undertaken to determine what, if any, serum protein changes occurred following infection by each strain type in previously uninfected (nonimmune) and immune pigeons, in hopes of better understanding the cross immunity among the various strains.

*Part of a paper given at the 1969 Annual Wildlife Disease Conference, June 16-19, at the National Animal Disease Laboratory, Ames, Iowa.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Materials and Methods

All pigeons used for primary infections were 4 to 12 months old and obtained from the *T. gallinae*-free loft at the Patuxent Wildlife Research Center. Three birds were used for each trichomonad strain tested. Those birds used for reinfection were survivors of the primary infections, serving as their own controls, or wild caught adult birds found to be carrying the organism. Infections were initiated by direct mechanical transfer of organisms from carriers to experimental birds.

To eliminate the organisms from the birds prior to reinfection, drinking water was treated with 1 mg/ml Enheptin® for 10 days. Microscopic and cultural[1] examination following treatment confirmed the loss of trichomonads. Reinfections were initiated between 2 and 4 weeks following treatment.

Serum was obtained from 1 ml blood samples collected immediately prior to infection and on days 7, 10, 15, and 20 following infection.

Electrophoretic fractionation of serum was accomplished using Gelman high resolution buffer and Sepraphore III® support strips at 400V for 55 minutes. The strips were stained with Ponceau S, each band (albumin, alpha, beta, gamma) was eluted in 0.1 N NaOH, and O.D. readings taken on a Spectronic 20 at 540 mu. Alpha 1 and 2 were treated as one band due to difficulty in getting consistently clear separations of these.

Three strain types of *T. gallinae* were used for comparison. The nonvirulent strain (I) was isolated from a feral pigeon squab and produced no gross lesions in nonimmune pigeons. The oral canker strain (II), isolated from a mourning dove, produced cankers in the mouth of nonimmune pigeons. These first appeared on the 7th day post infection and subsided by the 12th to 14th day. The Jones' Barn strain (III), courtesy of Dr. R. Stabler, always produced liver lesions and was 100% fatal for nonimmune birds. Because of its high degree of predictability only strain III was used for reinfections.

All birds not being used for reinfection were necropsied at the time of death or on the 20th day post infection, and cultures of liver, lung and crop were made in Diamond's medium.[1] This served to detect trichomonads in the tissues even when lesions were not grossly visible.

Results

Primary Infections (nonimmune birds):

Each of the three strain types produced a different serum protein pattern in nonimmune pigeons. (Figure 1a, b, c). No change was noted during infection with strain I; while an obvious decrease in albumin and increase in beta globulin occurred with strains II and III. Less dramatic changes occurred in the alpha band, which decreased two fold and the gamma band, which doubled between days 7 and 15 post infection. The changes accompanying strain II began to return toward normal by the 20th day post infection, while those accompanying infection with strain III persisted until the death of the birds between 10 and 14 days post infection.

Necropsy: No lesions could be found grossly in pigeons infected with strains I and II on the 20th day post infection, nor could organisms be cultured from anywhere except inside the throat and crop. Strain III pigeons were found to harbor organisms in their liver, lungs and crop, while liver lesions were consistently present with frequent metastases to surrounding organs. None of the strain III birds survived beyond 15 days.

Table I shows the total protein values for nonimmune pigeons infected with the three strains of *T. gallinae*. There was

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

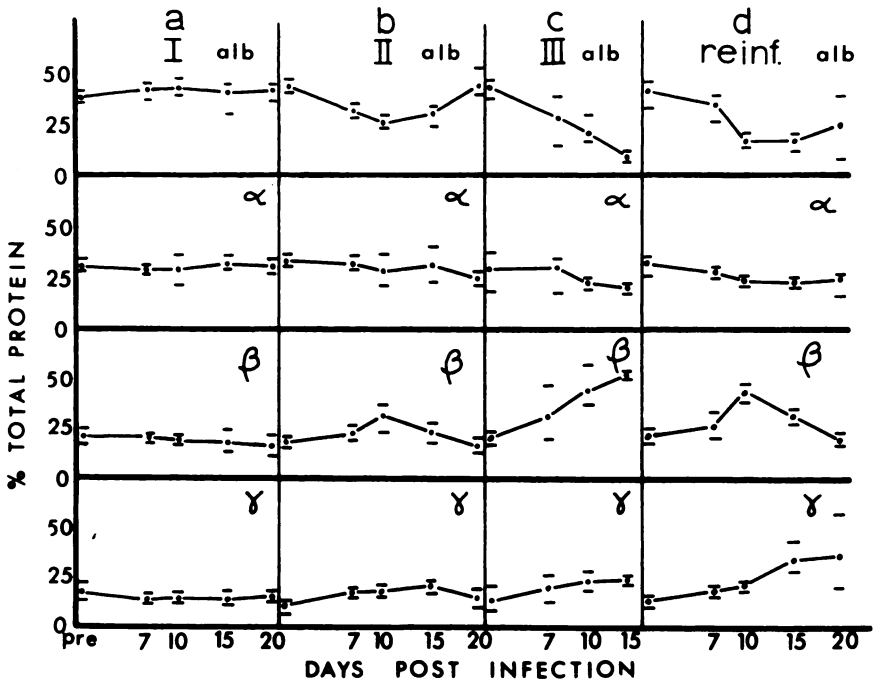


FIGURE 1. *Changes in serum protein fractions, expressed as a percent of total protein, accompanying infection of immune and nonimmune pigeons with 3 different strains of* T. gallinae. *(a, b, c — nonimmune birds). (a) Strain I: nonvirulent; (b) Strain II: oral canker; (c) Strain III: Jones' Barn — fatal; (d) Strain III: Immune pigeons with tissue invasion.*

apparently no detectable change during infection by the nonvirulent strain I while strains II and III produced an increase in total protein on the 10th day post infection, corresponding to the maximum beta globulin level.

Reinfections (immune birds):

Two patterns resulted from infection of immune pigeons with the Jones' Barn strain. One pattern was identical to that resulting from infection of a nonimmune bird with a nonvirulent strain — no change in serum protein distribution. This occurred in one strain I survivor and two wild adults with unknown history. The second pattern was similar to that obtained after infection of a nonimmune bird with an oral canker producing strain (Figure 1d). One bird that

TABLE I. *Total protein changes in nonimmune pigeons infected with three strains of* T. gallinae.

Day Sample taken	Mean total protein-mg. %	Strain
Pre	2.40	nonvirulent
10	2.38	I
15	2.37	
20	2.42	
Pre	2.31	oral canker
10	2.93	II
15	2.67	
20	2.53	
Pre	2.47	Jones' Barn
7	2.45	(fatal)
10	2.99	III

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

recovered from strain I, one from strain II, and a wild adult composed this group. The return toward normal varied more in this group than in the strain II infected pigeons.

Necropsy; Three pigeons were negative for internal lesions and tissue organisms other than those in the throat. Three others had one or two small granular nodules in their livers which appeared to be healing lesions and cultured positive for *T. gallinae*. Both liver and lung tissue cultured positive in two of these birds while only liver was positive for trichomonads in the third.

The three pigeons with no lesions and negative organ cultures were those which showed no change in relative serum protein distribution. The three having evidence of internal lesions and organs positive for the parasite were those whose patterns were similar to the strain II infected pigeons.

Discussion

Tissue invasion by *T. gallinae* appears to be necessary in both immune and nonimmune pigeons before a quantitative humoral response will occur. Since the changes include increases in beta and gamma globulins, it is tentatively assumed that there is an increase in antibody to *T. gallinae* or its products. The rapid increases in beta globulins followed by its replacement with gamma globulin is reminiscent of most antibody responses whether due to infection or immunization. Whether or not this antibody is protective remains to be proven. Since weak or nonprotective antibody is frequently produced in response to parasite products released into the host, it will also be necessary to determine if the antibody is directed toward the parasite or its products.

The rapid increase in beta globulin followed by its rapid decline, and the increase in total protein occurring simultaneously may be the result of normal antibody production or it may represent the production of C-reactive protein. Since this substance can be found in association with beta globulin, and is new protein produced as a result of tissue necrosis, it may well be the cause of the beta globulin increase.[5] Further evidence in support of C-reactive protein is that the beta increase immediately followed the first sign of lesions (strain II, III and reinfections), and subsided immediately after they disappeared (strain II and reinfections), while it persisted in those birds dying with severe tissue necrosis (Figure 1c). Since total protein increased at the same time beta globulin increased, there would be no change in the shape of the curves if total protein rather than % total protein for each band was plotted. However, there would be a greater increase in the beta globulin curve on day ten.

The obvious decrease in albumin and depression in alpha globulin levels occurring simultaneously with the beta and gamma globulin rise is probably the result of normal physiological equalization of intravascular colloid osmotic pressure.[2]

Due to the difficulty in obtaining large numbers of *T. gallinae*-free pigeons the number of birds in each group was not large enough for accurate statistical analysis, but there appeared to be a greater response to tissue invasion in reinfected birds than in birds infected for the first time. Both beta and gamma levels increased approximately 50% more in the reinfected group where tissue invasion occurred than in the strain II infected group. This is probably the result of an anamnestic response to reinfection. It is interesting to note that in this group of birds some were sufficiently resistant to inhibit any tissue invasion, while others allowed invasion to occur even though they were able to survive the infection and ultimately clear the parasite from the internal organs.

Since two different strains were used, the value of direct comparison of reinfections is questionable. It is unfortunate,

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

that reinfection by strain II produces no lesions and therefore no humoral response, and primary infection by strain III produces 100% mortality, thus precluding any reinfections. Obviously humoral anamnestic responses cannot be measured for either of these strains unless a titering system is developed which measures actual antibody rather than total globulin production.

Literature Cited

1. DIAMOND, L. S. 1957. The establishment of various trichomonads of animals and man in axenic cultures. J. Parasitol. 43: 488-90.
2. GUYTON, A. C. 1961. *Medical Physiology*. W. B. Saunders Co., Philadephia and London. 58 pp.
3. KERR, W. R. and M. ROBERTSON. 1946. Experimental infections in virgin heifers with *Trichomonas foetus* in vaccinated and unvaccinated animals. J. comp. Path. 56: 101-13.
4. ————. 1947. A study of the re-exposure to *Tr. foetus* of animals already exposed to the infection as virgin heifers, with some observations on the localization of antibody in the genital tract. J. comp. Path. 57: 301-13.
5. RAFFEL, S. 1961. *Immunity*. Appleton-Century-Crofts, Inc., New York. 70 pp.
6. STABLER, R. M. 1948. Protection in pigeons against virulent *Trichomonas gallinae* acquired by infections with milder strains. J. Parasitol. 34: 150-3.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use