Serologic Response of Turkeys to an Agent Associated with Infectious Enteritis (Bluecomb)

C. A. CARSON, S. A. NAQI, AND C. F. HALL

Departments of Veterinary Microbiology and Veterinary Public Health, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843

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The serologic response of turkey poults to an infectious enteritis (bluecomb disease) agent was analyzed. Serum samples collected from poults on post-inoculation days 3, 7, 14, and 21 were compared to serum from normal poults by electrophoresis and immunoelectrophoresis. Albumin concentrations were decreased whereas alpha and gamma globulins were increased during the disease. Qualitative and quantitative changes occurred in all migration zones.

Infectious enteritis (bluecomb disease) is a highly infectious disease of turkeys which causes severe production losses in certain parts of the United States and Canada. The disease is characterized by a sudden onset, dehydration, and diarrhea. Although turkeys of all ages can be infected, extensive outbreaks are more common in young brooding poults. Bacteria (12) as well as viruses (3, 4, 14) have been incriminated as etiologic agents, but definitive evidence for a single causative agent has not been presented.

Recovered turkeys may become carriers of the bluecomb agent and develop lifelong resistance to a second attack of bluecomb (8). However, the nature of such resistance is not known. This investigation was designed to study the serum protein response during the acute and subacute phases of the disease.

MATERIALS AND METHODS

Experimental turkeys. Turkey poults which were 1 day old and known to be free from Salmonella pullorum, Salmonella gallinarum, and Mycoplasma gallisepticum infections were obtained from a commercial hatchery.

One group of 100 poults was used for inoculation and subsequent study of serum proteins by electrophoresis. Thirty poults were used as uninoculated controls. Infected and control poults were kept in separate brooder batteries and fed a standard turkey ration.

A second group of 50 poults was used for the study of serum protein changes by immunoelectrophoresis. Ten of these poults remained as uninoculated controls. Inoculated poults were kept on cane litter in a brooder house, and controls were kept in separate brooder batteries.

Inoculum. Poults were inoculated with the Minnesota bluecomb agent (obtained from B. S. Pomeroy and C. T. Larsen, University of Minnesota) which has been maintained by serial passage in poults at Texas A&M University. The inoculum was prepared as a 20% suspension of intestinal material from infected birds in phosphate-buffered saline. The suspension was centrifuged at 4,000 x g and filtered through 450 and 220 nm filters (Millipore Corp., Bedford, Mass.). Poults received 0.5 ml of the inoculum by mouth.

Collection of serum for electrophoresis. Due to the high mortality in poults infected with the agent used in this study, only half of the infected birds remained alive for blood collection. Since electrophoresis can be done with a small quantity of serum, data for individual birds were obtainable. Blood was collected from poults by decapitation. Fifteen infected poults were bled on day 3 postinoculation (PI), 15 were bled on day 7 PI, 7 were bled on day 14 PI, and 10 were bled on day 21 PI. Seven control poults were bled on days 3, 7, 14, and 21 PI. Serum was separated from individual samples and stored at -45 C.

Preparation of hyperimmune serum. Since a small quantity of blood is obtainable from individual poults it was necessary to pool blood samples from five poults to produce antigen used for production of each hyperimmune serum. Five separate serum pools collected from infected poults 3, 7, 14, and 21 days PI and from control poults 14 days PI were used to prepare aluminum potassium sulfate (alum-precipitated) antigens (9) for production of hyperimmune serum in rabbits.

Electrophoresis. Sera from normal poults and those collected from poults 3, 7, 14, and 21 days PI were subjected to cellulose acetate electrophoresis (model R-100 Microzone Electrophoresis System, Beckman Instruments Inc., Fullerton, Calif.). Procedures for electrophoresis were as recommended in electrophoresis operating manuals (Methods Manual for model R-100 Microzone Electrophoresis System and model R-100 Cell Instruction Manual, Beckman Instruments Inc.) and used in this study.
Instruments Inc.). Densitometer (Beckman R-100 Microzone Densitometer, Beckman Instruments Inc.) tracings of electrophoresis strips were obtained by the procedure outlined in a standard operating manual (Densitometer Manual, Beckman Instruments Inc.).

Immunoelectrophoresis. The micromethod introduced by Scheidegger (19) and outlined in an operating manual (LKB 6800A Immunoelecetrophoresis Equipment Operating Manual, LKB-Producer AB, P. O. Box 76, Stockholm-Bromme 1, Sweden) was used. Electrophoresis separations were made at ambient temperature, using a current of 375 V and 7 to 9 mA per tray of six slides.

Serum samples collected from normal and infected poults 3, 7, 14, and 21 days PI were separated electrophoretically and compared by reaction with sera from rabbits inoculated with each of the respective alum-precipitated antigens. Test sera (5 uliters) were placed in the wells, and hyperimmune serum (250 uliters) was placed in the troughs.

RESULTS

Electrophoresis. Mean serum protein concentrations for control and infected poults appear in Table 1. Three days PI total serum protein values in infected poults were significantly below control values, but this difference no longer existed on day 7 PI. Albumin values were reduced on day 3 PI but were elevated above control values on day 21 PI. Total globulin values were decreased on day 3 and elevated on day 14 PI. Alpha globulin values (percent of total serum protein) were elevated above control values on days 3 and 14 PI, beta globulin values remained essentially unchanged, and gamma globulin values were elevated on day 14 but decreased on day 21 PI.

Immunoelectrophoresis. Additional alpha (Fig. 1, arrow A), beta (Fig. 1, arrows B and D) and immunoglobulin M (IgM) (Fig. 1, arrow F) fractions were present in acute serum when compared with immunoelectrophoretic patterns produced by normal serum. A beta globulin (Fig. 1, arrow C) and the immunoglobulin G (IgG) (Fig. 1, arrow E) fraction were also increased in the acute serum. In the pattern where serum collected 14 days PI was used, the IgM (Fig. 2, arrow A) and IgG fractions (Fig. 2, arrow C) were unchanged with respect to patterns in which turkey serum collected 3 days PI was used. The immunoglobulin A (IgA) fraction, not observed in acute or normal serum, was evident 14 days PI (Fig. 2, arrow B).

Twenty-one days after inoculation, the IgA and IgM arcs were no longer visible, but the IgG arc extended further toward the cathode and was more pronounced than in the control serum.

DISCUSSION

The results of these electrophoretic and immunoelectrophoretic analyses indicate that significant protein changes occurred in the sera of infected poults. A sudden sharp drop in the albumin value followed infection. In spite of the greatly decreased intestinal absorption of nutrients that may follow injury to gastrointestinal cells and reduced feed and water intake in affected birds, synthesis of serum globulins occurred in response to infection. This is indicated by the change in the proportion of globulin fractions as shown in Table 1.

Turkey serum proteins closely followed the patterns of mobility of fractions definitively identified in human serum and sera of other animal species (1, 2, 6, 13, 15). Therefore, the designations of alpha globulin, beta globulin, IgM, IgA, and IgG used in this report were based upon visual comparison with immunoelectrophoretic patterns described for human serum proteins and serum proteins of other animal species.

Immunoelectrophoretic patterns were analyzed for the presence and absence of various arcs of precipitation when sera from infected poults were compared to sera from controls. Variations in concentration of the serum protein fractions were estimated by the intensity of the precipitin arcs and their proximity to the trough (5). The significance of such protein changes was not ascertained in this study because the antigens associated with the bluecomb agent are not known. However, it is suggested that these changes could have been induced by the bluecomb-associated agent. When bluecomb-specific antigens are isolated and identified, the significance of related serologic variations may be re-evaluated.

The initial immunoglobulin response to the disease process involved the formation of IgM. The subsequent immunoglobulin response seemed to be limited to the formation of IgA. Either IgA was absent in the normal and 3 day PI serum or it was present in such low concentration that a precipitation arc was not detectable. The presence of IgA in poultry serum has been questioned (7). In other species it is a short-lived fraction which is not commonly elevated in response to disease.

The importance of circulating immunoglobulins in systemic disease is well documented. In enteric infections such as bluecomb, immunoglobulins present in the gastrointestinal secretions may be important. Such immunoglobulins may be the sole determinants of immunity in such diseases. It has been shown (7) that the
# Table 1. Comparison and evaluation of mean serum protein values of control turkeys and bluecomb-infected turkeys

| Constituent                        | Serum protein value at day PI |
|------------------------------------|-----------------------------|
|                                    | 3  | 7  | 14 | 21 |
| **Total serum protein (TSP) (gm/100 ml)** |     |     |    |    |
| Control turkeys                    | 2.8570 | 2.5125 | 2.7571 | 3.1429 |
| SEM                               | .38 | .35 | .38 | .38 |
| Infected turkeys                   | 2.0625 | 2.2600 | 3.0143 | 3.4700 |
| SEM                               | .25 | .26 | .38 | .32 |
| Control vs infected a              | * | ‡ | ‡ | ‡ |
| **Albumin (g/100 ml)**             |     |     |    |    |
| Control turkeys                    | 1.2857 | 1.100 | 1.4714 | 1.3429 |
| SEM                               | .38 | .35 | .38 | .38 |
| Infected turkeys                   | .8600 | .9100 | 1.2700 | 1.5700 |
| SEM                               | .25 | .26 | .38 | .32 |
| Control vs infected a              | * | ‡ | ‡ | * |
| **Total globulin (g/100 ml)**      |     |     |    |    |
| Control turkeys                    | 1.5714 | 1.4125 | 1.2857 | 1.8000 |
| SEM                               | .38 | .35 | .38 | .38 |
| Infected turkeys                   | 1.2060 | 1.2870 | 1.7429 | 1.9000 |
| SEM                               | .25 | .26 | .38 | .32 |
| Control vs infected a              | * | ‡ | ‡ | * |
| **Albumin/globulin**               |     |     |    |    |
| Control turkeys                    | 0.8300 | 0.8400 | 1.1514 | 0.7500 |
| SEM                               | .38 | .35 | .38 | .38 |
| Infected turkeys                   | 0.7094 | 0.6867 | 0.7257 | 0.8310 |
| SEM                               | .25 | .26 | .38 | .32 |
| Control vs infected a              | ‡ | ‡ | * | ‡ |
| **Albumin (% of TSP)**             |     |     |    |    |
| Control turkeys                    | 45.5143 | 44.1250 | 53.1571 | 42.2143 |
| SEM                               | .38 | .35 | .38 | .38 |
| Infected turkeys                   | 41.4875 | 40.3933 | 41.8857 | 44.8500 |
| SEM                               | .25 | .26 | .38 | .32 |
| Control vs infected a              | * | ‡ | * | ‡ |
| **α-Globulin (% of TSP)**          |     |     |    |    |
| Control turkeys                    | 17.1143 | 23.2375 | 20.5429 | 23.6429 |
| SEM                               | .38 | .35 | .38 | .38 |
| Infected turkeys                   | 25.4813 | 25.0133 | 25.6143 | 24.5800 |
| SEM                               | .25 | .26 | .38 | .32 |
| Control vs infected a              | * | ‡ | * | ‡ |
| **β-Globulin (% of TSP)**          |     |     |    |    |
| Control turkeys                    | 30.2871 | 26.8125 | 22.5286 | 22.4249 |
| SEM                               | .38 | .35 | .38 | .38 |
| Infected turkeys                   | 28.4750 | 26.5567 | 23.3143 | 24.9100 |
| SEM                               | .25 | .26 | .38 | .32 |
| Control vs infected a              | ‡ | ‡ | ‡ | ‡ |
| **γ-Globulin (% of TSP)**          |     |     |    |    |
| Control turkeys                    | 7.1290 | 5.8380 | 3.7710 | 11.7290 |
| SEM                               | .38 | .35 | .38 | .38 |
| Infected turkeys                   | 3.5750 | 8.0630 | 9.1570 | 5.7070 |
| SEM                               | .25 | .26 | .38 | .32 |
| Control vs infected a              | ‡ | ‡ | * | * |

* Standard error of the mean.
* Symbols: *, significant $P < 0.05$; ‡, not significant.

The mucosal lining of the gastrointestinal tract and the upper respiratory tract secrete an immunoglobulin (IgA) which is structurally and probably functionally different from serum IgA. Although the role of circulating IgA in protection against enteric infection is unknown, it is recognized that enteric disease (gastroenteritis, ileocolitis, and exudative enteropathy) persists in agammaglobulinemic human patients in whom systemic infections have been alleviated.
by parenteral gamma globulin administration (11). Elevation in the levels of circulating IgA and IgM in the acute phase of infectious enteritis of turkeys and the subsequent disappearance of these immunoglobulins suggest that they are not important in the long-term immunity which characterizes this disease.

Histopathologic findings in bluecomb disease indicate that the morphological responses to the infection include inflammatory cellular reactions in the lamina propria of the alimentary tract. These responses are characterized by infiltration of heterophils, lymphocytes, and reticuloendothelial cells in the lamina propria. Such a cellular response in the alimentary tract is significant since the lamina propria contains the special secretory antibody system. These findings suggest that the antigens asso-

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**FIG. 1.** Immunoelectrophoresis of serum collected 3 days PI with the bluecomb agent (top well) and serum collected from normal control poult (bottom well). Trough (2 mm wide) contains hyperimmune serum from rabbit injected with alum-precipitated antigen prepared from serum used in the top well. Arrow A indicates additional alpha globulin arc; B indicates additional beta globulin; C indicates increase in quantity of beta globulin; D indicates additional beta globulin arc; E indicates increase in quantity of IgG; F indicates IgM.

**FIG. 2.** Immunoelectrophoresis of serum collected 14 days PI with the bluecomb agent (bottom well) and serum collected from normal control poult (top well). Trough (2 mm wide) contains hyperimmune serum from rabbit injected with alum-precipitated antigen prepared from serum collected 14 days PI. Arrows A and C indicate IgM and IgG, respectively, which were unchanged with respect to serum collected 3 days PI; arrow B indicates the IgA arc, which was not observed in acute or normal serum.
associated with the bluecomb agent stimulate the secretory antibody system of the alimentary tract. Since recovered birds may become permanent carriers of the disease agent, it can be speculated that the agent continues to remain in the alimentary tract and may stimulate continuous antibody production in recovered birds. In the case of infectious enteritis in turkeys, circulating IgG, which remained elevated 21 days PI, may also be important in the total defense mechanism.

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