IMMUNOLOGY

Immune System Dysfunction During Exposure to Poult Enteritis and Mortality Syndrome Agents

M. A. QURESHI, F. W. EDENS, and G. B. HAVENSTEIN

Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27695-7608

ABSTRACT Poult Enteritis and Mortality Syndrome (PEMS) is a condition of yet undefined etiology. Affected flocks may exhibit 100% morbidity with mortality up to 50% or more between 2 to 4 wk of age. The current study reports the immune status of poults experimentally infected with PEMS agent(s) in various trials. When compared with the unchallenged controls, PEMS-infected poults had significant atrophy of the bursa (up to 2-fold), thymus (up to 11-fold), and spleen (up to 2-fold) ($P \leq 0.05$). When challenged with SRBC, PEMS-infected poults had 1 to 2 log$_2$ lower anti-SRBC antibody titers than the controls ($P \leq 0.05$). Responsiveness to a mitogenic lectin, phytohemagglutinin-P, was reduced significantly in PEMS poults ($P \leq 0.05$). These data show that the immune system of the poults is compromised significantly during PEMS infection in terms of lymphoid organ integrity and humoral and cell-mediated immunity. These findings imply, therefore, that immune dysfunction may contribute to the mortality observed during PEMS outbreaks.

(Key words: Poult Enteritis and Mortality Syndrome, immune dysfunction, poult)

1997 Poultry Science 76:564–569

INTRODUCTION

Poult Enteritis and Mortality Syndrome (PEMS) is a newly identified disease condition of turkey poults. The disease is acute and infectious with a rapid onset (Barnes and Guy, 1995; Barnes et al., 1996). The affected poults exhibit signs of feed refusal, vocalization, enteritis, diarrhea, decreased growth, high mortality, and flock unevenness. The mortality typically ranges from 1 to 5%/d for 3 to 7 d between 11 and 28 d of age, followed by severe stunting in the survivors. The morbidity appears to be near 100%, whereas mortality can easily exceed 50% or more. Barnes et al. (1996) have described two clinical forms of PEMS; the most severe is called Spiking Mortality of Turkeys (SMT), whereas the milder form has been named Excess Mortality of Turkeys (EMT). Therefore, PEMS incorporates both SMT and EMT as these two clinical entities are now known to be the same disease (Barnes et al., 1996).

The etiological agent(s) for PEMS are currently not known. Several studies have suggested metabolic disorders (such as altered pancreatic function and carbohydrate assimilation) as a possible cause of poor weight gain and perhaps early poult mortality (Phelps et al., 1987a; Donaldson and Christensen, 1994). In these reports hematomal changes, such as decreased leukocyte counts, were also found to be correlated with early poult mortality (Phelps et al., 1987b). However, the physiological aberrations alone can not explain the infectious nature of this problem.

Several viruses have been found to be associated with acute diarrheal diseases affecting young turkeys (Reynolds et al., 1987; Thouvenelle et al., 1995). Currently, efforts to isolate the etiological agent(s) have resulted in the identification of several potential viral and bacterial etiological agents from PEMS-affected poults. The viral candidates include enteropathogenic viruses (coronaviruses, birnaviruses, entero-like viruses, rotavirus, especially type D, and adenoviruses), bacteria (Salmonella, Escherichia coli, Campylobacter, Bacteroides, and Clostridia), and protozoa (Cryptosporidia and Cochlosoma) (Barnes and Guy, 1995; Barnes et al., 1996). Attempts to reproduce this disease experimentally with a single agent have, to this date, been unsuccessful. However, infection with a combination of viruses was reported to cause high mortality (Barnes and Guy, 1995).

Recently two “atypical” E. coli strains have also been isolated from PEMS-affected poults (F. W. Edens, unpublished data). These agents have been shown to cause many of the signs (vocalization, severe enteritis, mortality, and depressed growth in survivors) when given by gavage at a very low dose, i.e., approximately 10$^8$ bacteria per bird at 1 or 6 d of age. With the available leads so far, it is clear that the PEMS condition is highly infectious, affected houses are difficult to disinfect, and fecal material or bird-to-bird contact appears to be the primary source of PEMS transmission.
The objective of the current investigation was to develop an immune profile of poults during PEMS exposure. The immunological assessment included the quantification of lymphoid organ integrity and humoral and cell-mediated immunity.

MATERIALS AND METHODS

Animals

Poults (all female) were obtained from commercial sources at day of hatch. They were housed on the floor in the North Carolina State University Dearstyne Avian Research Center (NCSU DARC) in isolation rooms using pine shavings litter. A turkey starter diet (North Carolina Agricultural Research Service) and water were available for ad libitum consumption following placement. No vaccination was employed.

PEMS Exposure

Healthy poults representing 10% of the groups to be challenged with the PEMS etiological agent(s) of 5 to 6 d of age were transported from the NCSU DARC to the NCSU College of Veterinary Medicine (CVM). At the CVM, PEMS is passed from one set of poults to another, and our seeder-poults were co-housed with a group of poults exhibiting clinical signs of PEMS. After approximately 12 h, the exposed poults were returned to the DARC and were housed with their unexposed pen mates. The experimental group was then designated as PEMS-exposed. As compared with the poults in the isolated control (unexposed) group, at 2 to 3 d postexposure, the PEMS-exposed poults started exhibiting clinical signs associated with the PEMS, i.e., vocalization, severe diarrhea, dehydration, decreased feed consumption, and high mortality. This challenge protocol was used in all trials.

Lymphoid Organ Integrity

Poults in the exposed and unexposed groups were weighed prior to euthanasia at various stages of post-PEMS exposure. The bursa of Fabricius, thymus (all thymic lobes from left side of the neck of each poult), and spleen were removed and weighed. The organ weights were measured to the nearest milligram and were expressed as the percentage of body weight.

Antibody Response

At 3 wk (14 d postexposure, Trial 1) and 2 wk (7 d postexposure, Trial 2) of age, poults in the exposed and unexposed groups were given a single 1-mL intravenous injection of a 7% saline suspension of SRBC. Blood samples were drawn at various times post-SRBC injection, and the collected serum was heat inactivated at 56 C for 30 min and stored at -20 C until tested for anti-SRBC antibody levels. The antibody titers in terms of total, mercaptoethanol-resistant (MER, presumably IgG) and sensitive (MES, presumably IgM) were quantified using a microhemagglutination technique as previously described (Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994; Lepage et al., 1996). The titers were expressed as the log2 of the reciprocal of the last dilution in which visible agglutination was observed.

Cell-Mediated Immunity

The in vivo lymphoproliferation was quantified by injecting phytohemagglutinin-P (PHA-P) into the poults of both groups at 3 wk (2 wk post-PEMS exposure) as previously described (Kidd et al., 1994). The toe web between the third and fourth digits of the left foot was injected with 100 μg of PHA-P dissolved in 100 μL of sterile saline. The right foot was injected in an identical manner to that of left foot with 100 μL of saline to serve as a control. The toe webs were measured with a constant tension caliper before injection and at 24 and 48 h after PHA-P injection. The data were expressed as the PHA-P-mediated minus the saline-injected control swelling (millimeter) in both treatment groups.

Statistical Considerations

All data were analyzed using the General Linear Model procedure of SAS® (SAS Institute, 1985), and the treatment means were separated using Duncan’s multiple range test.
TABLE 2. Lymphoid organ weights from female poults during Poult Enteritis and Mortality Syndrome (PEMS) infection (Trial 1)

| Days post-PEMS exposure | Bursa | Thymus | Spleen |
|-------------------------|-------|--------|--------|
|                         | PEMS  | Control| PEMS   | Control| PEMS  | Control|
| 3                       | 0.13  | 0.14   | 0.06   | 0.09   | 0.05  | 0.05   |
| 5                       | 0.16  | 0.13   | 0.08   | 0.13   | 0.07  | 0.06   |
| 7                       | 0.15  | 0.19   | 0.10   | 0.11   | 0.09  | 0.08   |
| 10                      | 0.17  | 0.18   | 0.04a  | 0.11b  | 0.07a | 0.09b  |
| 14                      | 0.10a | 0.16b  | 0.01a  | 0.09b  | 0.07a | 0.09b  |
| 17                      | 0.11a | 0.20b  | 0.007a | 0.10b  | 0.08a | 0.10b  |

The means within a row for a given organ with no common superscript differ significantly ($P \leq 0.05$).

Poults were exposed to PEMS agent(s) at 4 d of age. At each of the days post-PEMS exposure, poults from each group were euthanatized and organs collected. The data are the means of percentage organ weights relative to body weight.

All thymic lobes from left side of the neck were collected from each poult.

RESULTS

The effects of PEMS infection on body weights of poults observed in a representative trial are presented in Table 1. The data show that the onset of growth suppression in PEMS poults is extremely rapid. The PEMS-exposed poults had significantly reduced body weights ($P \leq 0.05$) as compared with the weights of the controls from 6 d postexposure up to Day 23, when the last body weights were taken.

Lymphoid organ weight data from two separate trials are provided in Tables 2 and 3, respectively. Bursal, thymic, and splenic atrophy were observed in both trials soon after PEMS exposure when compared with the unexposed poults. This suppression in lymphoid organ growth approached statistical significance when bursa exhibited a 1.2- to 2.3-fold reduction, thymus exhibited a 1.7- to 11-fold reduction, and spleen exhibited 1.2- to 2-fold reduction in weight over the unexposed controls in both trials. Data for the production of antibodies against SRBC are given in Tables 4 and 5 from two separate trials. In Trial 1 (Table 4), the poults in the PEMS group had a one log decrease, comparable to control poults in total anti-SRBC antibody levels ($P \leq 0.05$) by 3 d post-SRBC injection. The antibody levels in the PEMS group continued to be lower (but not significantly) at 5 and 9 d postinjection, but by Day 12 both groups had comparable anti-SRBC antibody levels.

The response of poults to PHA-P injection in Trial 1 is presented in Figure 1. Measured at 24 and 48 h post-PHA-P injection, skin swelling was significantly less in PEMS-exposed poults ($P \leq 0.05$) than in their unexposed controls. Similar suppression was observed in Trial 2, in which poults in PEMS group showed a 1.5-fold reduction in skin thickness in comparison with their unexposed controls at 24 h ($P \leq 0.05$) but not at 48 h post-PHA-P challenge (data not shown).

DISCUSSION

The causative agent(s) of PEMS is still not known. The fact that healthy poults can become infected when housed overnight with poults showing clinical signs of illness provides supporting evidence.

TABLE 3. Lymphoid organ weights from female poults during Poult Enteritis and Mortality Syndrome (PEMS) infection, Trial 2

| Days post-PEMS exposure | Bursa | Thymus | Spleen |
|-------------------------|-------|--------|--------|
|                         | PEMS  | Control| PEMS   | Control| PEMS  | Control|
| 0                       | 0.06  | 0.06   | 0.07   | 0.08   | 0.1   | 0.11   |
| 6                       | 0.07a | 0.12b  | 0.05a  | 0.14b  | 0.16a | 0.20a  |
| 9                       | 0.08a | 0.16b  | 0.04a  | 0.21b  | 0.13a | 0.30a  |
| 16                      | 0.2a  | 0.36b  | 0.12a  | 0.36b  | 0.26a | 0.48a  |
| 23                      | 0.51a | 0.50b  | 0.21a  | 0.35b  | 0.35a | 0.57a  |

The means for a given organ within a row with no common superscript differ significantly ($P \leq 0.05$).

Poults were exposed to PEMS agents at 4 d of age. Organs from poults from each group were collected on the day prior to exposure and days thereafter as indicated. The data are the means of percentage organ weights relative to body weights.

All thymic lobes from the left side of the neck were collected from each poult.

Downloaded from https://academic.oup.com/ps/article-abstract/76/4/564/1528109 by guest on 27 July 2018
suggests that the syndrome is extremely infectious, causing nearly 100% morbidity, which is characterized by growth retardation and severe diarrhea.

In the current study, the immune status of the poults experimentally exposed to PEMS agent(s) and housed in isolation rooms was examined in comparison to the unexposed controls housed in similar isolation rooms. The immune assessment was carried out by utilizing assays described in the avian immune assessment panel (Dietert et al., 1994). These included 1) the bursa, thymus, and spleen weight to body weight ratio as a measure of lymphoid organ integrity, 2) antibody response against SRBC as a measure of humoral immunity, and 3) PHA-P toe web assay as a measure of cell-mediated immunity. The PEMS-exposed poults exhibited significant suppression in all of these immunological end points when compared with the unexposed controls. The lymphoid organ data indicate that the growth of both primary and secondary lymphoid organs was suppressed significantly. Thymic atrophy started earlier than bursal atrophy in PEMS-exposed poults and was of a greater magnitude (fold decrease) than for the bursa and spleen. These atrophic changes in lymphoid organs started earlier in Trial 2 than in Trial 1 post-PEMS exposure. Such variation may be due to a possible uneven PEMS exposure as discussed earlier. A central feature of the humoral immune response requires an organism to possess a vast

### TABLE 4. Anti-sheep red blood cells antibody response of female poults exposed to Poult Enteritis and Mortality Syndrome (PEMS) agent(s), Trial 1

| Post-SRBC (d) | PEMS | Control |
|--------------|------|---------|
| 3            | 3.4<sup>b</sup> | 4.4<sup>a</sup> |
| 5            | 9.1 | 10.1 |
| 9            | 7.3 | 7.7 |
| 12           | 6.0 | 6.0 |

<sup>a,b</sup>Means within a row with no common superscript differ significantly (P ≤ 0.05).

<sup>1</sup>Poults were exposed to PEMS agent(s) at 5 d of age. Ten poults per group were injected intravenously with a 7% saline suspension of SRBC in 1-mL volume per bird at 3 wk of age for antibody response.

Because the bursa (Glick et al., 1956; Paramithiotis and Ratcliffe, 1994) and thymus (Arstila et al., 1994) serve as the primary organs of lymphopoiesis, alterations in the development of these organs in response to a possible lymphotropic agent(s) will result in altered immunological functions associated with B and T lymphocytes. This indeed, was found to be the case. When antibody response against SRBC was quantified, poults in the PEMS group were clearly suppressed. Within the first 3 to 4 d post-SRBC injection, PEMS poults had 1 to 2 log lower antibody levels. This suppression persisted for the entire 7 d period post-SRBC injection. In both antibody response trials, the observed decline in antibody levels was comparable between the PEMS-exposed and unexposed poults at the terminal stage of the primary anti-SRBC antibody response. By this age, PEMS exposure induces significant bursal, thymic, and splenic atrophy. However, what is not yet known is the integrity of lymphoid components (e.g., lymphocyte numbers, CD4<sup>+</sup>, CD8<sup>+</sup> cells) during the progression of lymphoid organ atrophy and disease. Studies are currently ongoing that would help in establishing any correlation between the lymphoid cell numbers:subpopulation ratios and the observed slower induction of primary antibody response in the PEMS-affected poults. Furthermore, antibody levels around Day 8 to 9 after SRBC injection in PEMS-exposed poults were lower than the unexposed poults numerically in Trial 1 (Table 4) and statistically in Trial 2 (Table 5). This variation may also be due to an uneven PEMS exposure as discussed earlier. A central feature of the humoral immune response requires an organism to possess a vast

### TABLE 5. Anti-sheep red blood cells antibody response of female poults exposed to Poult Enteritis and Mortality Syndrome (PEMS) agent(s), Trial 2

| Group | Total | MES | MER | Total | MES | MER | Total | MES | MER |
|-------|-------|-----|-----|-------|-----|-----|-------|-----|-----|
| Control | 5.5<sup>b</sup> | 5.1<sup>b</sup> | 0.4 | 6.5<sup>b</sup> | 5.9<sup>b</sup> | 0.6 | 3.9 | 3.4 | 0.5 |
| PEMS   | 3.8<sup>a</sup> | 3.0<sup>a</sup> | 0.8 | 4.7<sup>a</sup> | 3.6<sup>a</sup> | 1.1 | 3.2 | 2.4 | 0.8 |

<sup>a,b</sup>Means within a column with no common superscript differ significantly (P ≤ 0.05).

<sup>1</sup>Poults were exposed to PEMS agent(s) at 5 d of age. Fifteen poults were injected intravenously with a 7% saline suspension of SRBC in 1-mL volume per bird at 2 wk of age.
The response of Poult Enteritis and Mortality Syndrome (PEMS) and control (CTL) poults to phytohemagglutinin-P injection. The bars represent the mean PHA-P-mediated swelling above the saline-injected control toes in 10 poults per group injected at 3 wk of age (2 wk post-PEMS exposure). The letters indicate significant ($P \leq 0.05$) differences within two treatment groups at the given times.

reertoire of antibodies to protect itself against foreign pathogens. The findings of our current study imply that during PEMS exposure, poults cannot mount an effective primary antibody response as needed to fight bacterial or viral infections.

When lectin PHA-P is injected intradermally into animals, the response primarily involves stimulation of T cell division with minimal effects on B cells (Tizard, 1994); therefore, lymphoproliferation in response to PHA-P is considered a good *in vivo* measure of T lymphocyte function. In this study, PEMS poults exhibited reduced swelling in response to PHA-P injection, suggesting a suppression in lymphoproliferative ability as compared with the unexposed poults. It is well documented that avian cytotoxic T lymphocytes (CD8+) are key players in killing virus-infected cells (Schat, 1994). Furthermore, T-helper cells (CD4+) are crucial in expanding the B lymphocyte mediated antibody repertoire by producing cytokines with B lymphocyte proliferation potential (Arstila *et al.*, 1994). The data from the current study clearly show an alteration in T lymphocyte response in PEMS poults, thereby implying a possible alteration in immune protection mechanisms involving T lymphocytes.

In conclusion, the findings of the current study suggest that PEMS agent(s) induce an immunosuppressive condition in poults. One can compare this condition with the previously known infectious bursal disease and reovirus-induced immunosuppressive disorder in chickens. Both of these viruses are known to cause bursal atrophy, and humoral and cell-mediated immunosuppression (Sharma *et al.*, 1994). Similarly, chicken anemia virus infection has been shown to cause atrophy and hypocellularity in chick thymus (Bounous *et al.*, 1995). It is not clear whether mortality observed in poults is a direct result of infection with PEMS agent(s) or that the infection results in an immune dysfunction, which then leads to enhanced invasiveness and secondary infections with viral or bacterial agents resulting in death. Nevertheless, immune dysfunction seems to be a strong correlate with the pathogenesis of PEMS disease in poults.

**REFERENCES**

Arstila, T. P., O. Vainio, and O. Lassila, 1994. Central role of CD4+ T cells in avian immune response. Poultry Sci. 73: 1019–1026.

Barnes, H. J., and J. S. Guy, 1995. Spiking mortality of turkeys (SMT) and related disorders—an update. Pages 16–21 in: Proceedings 19th Annual North Carolina Turkey Industry Days Conference. Raleigh, NC.

Barnes, H. J., J. S. Guy, T. P. Brown, and F. W. Edens, 1996. Poult enteritis mortality syndrome (“spiking mortality of turkeys”) and related disorders—an update. Pages 1–8 in: NCSU Quarterly Update to Poultry PEMS Task Force, April. North Carolina State University, Raleigh, NC.

Bounous, D. I., M. A. Goodwi, R. L. Brooks, Jr., C. M. Laminichane, R. P. Campagnoli, J. Brown, and D. B. Snyder, 1995. Immunosuppression and intracellular calcium signaling in splenocytes from chicks infected with chicken anemia virus, CL-1 isolate. Avian Dis. 39:135–140.

Brown, T. P., 1992. Acute enteritis as a cause of spiking mortality in turkey poults. Pages 20–29 in: Proceedings Elanco Turkey Technology Seminar, May, Nashville, TN.

Dietert, R. R., K. A. Golemboski, and R. E. Austic, 1994. Environment-immune interactions. Poultry Sci. 73: 1062–1076.

Donaldson, W. E., and V. L. Christensen, 1994. Dietary carbohydrate effects on some plasma organic acids and aspects of glucose metabolism in turkey poults. Comp. Biochem. Physiol. 109A:423–430.

Glick, B., T. S. Chang, and R. G. Jaap, 1956. The bursa of Fabricius and antibody production in the domestic fowl. Poultry Sci. 35:224–226.

Kidd, M. T., M. A. Qureshi, P. R. Ferket, and L. N. Thomas, 1994. Dietary Zinc-methionine enhances mononuclear-phagocytic function in young turkeys. Biol. Trace Element Res. 42:217–229.

Lepage, K. T., S. E. Bloom, and R. L. Taylor, Jr., 1996. Antibody response to sheep red blood cells in a major histocompatibility (B) complex aneuploid line of chickens. Poultry Sci. 75:346–350.

Paramithiotis, E., and M.J.H. Ratcliffe, 1994. Survivors of bursal B cell production and emigration. Poultry Sci. 73: 991–997.

Phelps, P. V., F. W. Edens, and V. L. Christensen, 1987a. The post hatch physiology of turkey poult. I. Growth and development. Comp. Biochem. Physiol. 86A:739–743.
Phelps, P. V., F. W. Edens, and V. L. Christensen, 1987b. The post hatch physiology of the turkey poult. II. Hematology. Comp. Biochem. Physiol. 86A:745–750.

Qureshi, M. A., and G. B. Havenstein, 1994. A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed “typical” 1957 and 1991 broiler diets. Poultry Sci. 73:1805–1812.

Reynolds, D. L., Y. M. Saif, and K. W. Theil, 1987. A survey of enteric viruses of turkey poults. Avian Dis. 31:89–98.

SAS Institute, 1985. SAS® User’s Guide: Statistics. Version 5 Edition. SAS Institute Inc., Cary, NC.

Schat, K. A., 1994. Cell-mediated immune effector functions in chickens. Poultry Sci. 73:1077–1081.

Sharma, J. M., K. Karaca, and T. Pertile, 1994. Virus-induced immunosuppression in chickens. Poultry Sci. 73:1082–1086.

Thouvenelle, M. L., J. S. Haynes, D. L. Reynolds, 1995. Astrovirus infection in hatchling turkeys. Histologic, morphometric, and ultrastructural findings. Avian Dis. 39:328–336.

Tizard, I., 1994. Immunology: An Introduction. 4th ed. Saunders, New York, NY.

Yamamoto, Y., and B. Glick, 1982. A comparison of the immune response between two lines of chickens selected for differences in the weight of the bursa of Fabricius. Poultry Sci. 61:2129–2132.