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IMMUNOLOGY

Immune System Dysfunction During Exposure to Poult Enteritis and Mortality Syndrome Agents[1]

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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)

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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 hematological 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 10^5 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

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TABLE 1. Body weights of poults exposed to Poult Enteritis and Mortality Syndrome (PEMS) agent(s)

	Days post-PEMS exposure[1]				
Group	1	6	9	16	23
	(g)				
PEMS	82.4	100.4[a]	107.8[a]	211[a]	321.2[a]
Control	83.1	142[b]	199.7[b]	357.1[b]	481.3[b]

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]The data are the mean body weights (grams) from six randomly selected female poults per group on each indicated day.

(Barnes and Guy, 1995). Based on these observations, and without the identification of a single PEMS causative agent, it is logical to assume that any one of the virus(es), either alone or in combination with the possible bacterial involvement, may interact to cause this syndrome.

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 microheamagglutination technique as previously described (Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994; Lepage *et al*., 1996). The titers were expressed as the $\log_2$ 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 of age (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[1] exposure	Bursa		Thymus[2]		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	0.04[a]	0.11[b]	0.07[a]	0.09[b]
14	0.10[a]	0.16[b]	0.01[a]	0.09[b]	0.07[a]	0.09[b]
17	0.11[a]	0.20[b]	0.007[a]	0.08[b]	0.08[a]	0.10[b]

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

[1]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.

[2]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 levels of IgM were lower (but not significantly so) in PEMS-exposed poults, whereas IgG levels exhibited a slight but significant reduction when compared with the unexposed poults only at 3 d post-PEMS exposure (data not shown). In Trial 2 (Table 5), poults in the PEMS group had significantly reduced total and IgM anti-SRBC antibodies at 4 and 8 d post-SRBC injection ($P \leq 0.05$). Although anti-SRBC IgG levels did not differ between the PEMS-exposed and unexposed groups, by Day 11 post-SRBC injection poults in both groups had comparable total, IgM, and IgG antibody titers (Table 5).

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

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

Days post-PEMS[1] exposure	Bursa		Thymus[2]		Spleen	
	PEMS	Control	PEMS	Control	PEMS	Control
0	0.06	0.06	0.07	0.08	0.1	0.11
6	0.07[a]	0.12[b]	0.05[a]	0.14[b]	0.16[a]	0.20[b]
9	0.08[a]	0.16[b]	0.04[a]	0.21[b]	0.13[a]	0.30[b]
16	0.2[a]	0.36[b]	0.12[a]	0.36[b]	0.26[a]	0.48[b]
23	0.31[a]	0.50[b]	0.21[a]	0.35[b]	0.35[a]	0.57[b]

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

[1]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.

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

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

Post-SRBC	PEMS	Control
(d)	(mean/$\log_2$)	
3	3.4[b]	4.4[a]
5	9.1	10.1
9	7.3	7.7
12	6.0	6.0

[a,b]Means within a row with no common superscript differ significantly ($P \leq 0.05$).

[1]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.

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 the poults in each trial were exposed to PEMS agent(s) via different infected seeder poults rather than controlled injection with a defined agent. The changes in lymphocyte populations in these organs have not been determined yet. Preliminary immunohistochemistry observations suggest lymphoid depletion and fewer surface immunoglobulin-positive B-lymphocytes in the bursas from PEMS-exposed poults than in the bursa of unexposed controls (unpublished observation). These findings support previously reported observations in turkey spiking mortality by Brown (1992), who noted necrosis of the bursa similar to that seen in Infectious Bursal Disease in chickens, thymic atrophy, and a reduction in cell-mediated response to PHA-P.

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, $CD4^+$, $CD8^+$ 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[1]

	Days post-SRBC challenge								
	4 d			8 d			11 d		
Group	Total	MES	MER	Total	MES	MER	Total	MES	MER
	Antibody types ($\log_2$)								
Control	5.5[b]	5.1[b]	0.4	6.5[b]	5.9[b]	0.6	3.9	3.4	0.5
PEMS	3.8[a]	3.0[a]	0.8	4.7[a]	3.6[a]	1.1	3.2	2.4	0.8

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]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.

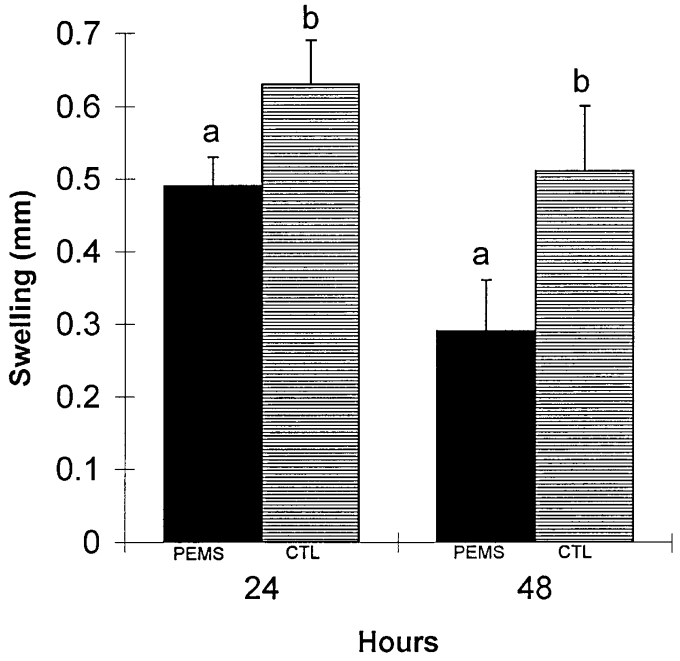

FIGURE 1. The response of Poult Enteritis and Mortality Syndrome (PEMS) and control (CTL) poults to phytohemagglutinin-P injection. The bars represent the mean PHA-P-mediated swelling above the saline-injected control toes in 10 poults per group injected at 3 wk of age (2 wk post-PEMS exposure). The letters indicate significant ($P \leq 0.05$) differences within two treatment groups at the given times.

repertoire 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.

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