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Experimental reproduction of poult enteritis syndrome: Clinical findings, growth response, and microbiology

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ABSTRACT Poult enteritis syndrome (PES) is an infectious disease of turkey poults characterized by diarrhea, dullness, and depression. Five experiments were conducted to reproduce the disease in turkey poults using intestinal contents of PES-affected birds. In all experiments, poults at 14 d of age were divided into 4 groups and were orally given 2 mL of unfiltered supernatant, filtered supernatant, sediment dissolved in PBS, or PBS alone. Inocula in experiments 1, 3, and 5 consisted of intestinal contents from PES-affected birds of less than 2 wk of age, whereas those in experiments 2 and 4 consisted of intestinal contents from PES-affected birds of 4 to 6 wk of age. Poults in all groups were observed daily for clinical signs. The BW and microbiological criteria in experiments 1, 3, and 5 were evaluated at 5, 10, and 15 d postinoculation, whereas in experiments 2 and 4, these observations were made at 10 and 20 d postinoculation. Rotavirus, astrovirus, and *Salmonella* were present in all 5 inocula. Diarrhea and depression were the major signs in poults given PES material. Significant retardation of growth was observed in poults given any of the 3 PES materials, but this effect was more pronounced in poults given the sediment inoculum. Rotavirus, astrovirus, and *Salmonella* were detected in poults given PES material. In some cases, enterovirus was also detected. No major difference was noticed in experimental reproduction of PES when intestinal contents from different age birds were used as the inoculum.

Key words: poult enteritis syndrome, rotavirus, astrovirus, *Salmonella*

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INTRODUCTION

Poult enteritis syndrome (**PES**) is a disease of young turkeys that has been observed by Minnesota turkey growers and poultry veterinarians in recent years. The syndrome is defined as an infectious, multifactorial disease of young turkeys usually noted between d 1 and 7 wk of age and characterized by diarrhea, depression, and lethargy with pale intestines or ceca with watery contents, or both. At the Minnesota Veterinary Diagnostic Laboratory, 151 cases of PES were reported during 2002 to 2007. Many different agents were detected in these cases including viruses (rotavirus, enterovirus, reovirus, adenovirus), bacteria (*Salmonella* sp., *Escherichia coli*, *Enterococcus*), and protozoa (*Eimeria* sp). Of all the pathogens detected, the proportion of rotavirus and *Salmonella* was greater than any other agent (N. Jindal, unpublished data). This is in contrast to poult enteritis mortality syndrome (**PEMS**), in which coronavirus or attaching and effacing *E. coli* are often detected (Saif et al., 1990; Edens et al., 1997; Guy, 1998; Pakpinyo et al., 2002; Ismail et al., 2003; Teixeira et al., 2007). Recently, Woolcock and Shivaprasad (2008) detected rotavirus-like viruses and small round viruses alone or in combination from poult enteritis cases in California by electron microscopy.

Clinically, PEMS associated with turkey coronavirus has been characterized as excess mortality of turkeys and spiking mortality of turkeys (**SMT**; Barnes and Guy, 2003). The latter was experimentally reproduced by oral inoculation of turkey poults with intestinal contents from SMT-affected birds or by placing 1-d-old poults on litter taken from naturally occurring cases of SMT (Brown et al., 1997; Davis et al., 1997). Perry et al. (1991a) reproduced poult malabsorption syndrome in 1-d-old tom turkeys by placing them on litter known to produce enteric disease in poults. No such studies have been undertaken with PES. To determine whether PES can be experimentally reproduced, we conducted 5 experiments using intestinal contents from PES-affected turkey poults. We conducted 3 experiments with intestinal contents of PES-affected poults of less than 2 wk of age and 2 experiments with intestinal

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contents of PES-affected poults of 4 to 6 wk of age. The aim was to reproduce the PES experimentally and to determine if there was any difference in the severity of disease using intestinal contents from PES-affected birds of different age groups.

MATERIALS AND METHODS

Source of Inoculum

Ten birds showing clinical signs of PES (diarrhea, depression, lethargy) were selected from each of the 5 PES-affected flocks. Birds for experiments 1, 3, and 5 were less than 2 wk of age, whereas those for experiments 2 and 4 were 4 to 6 wk of age. The birds were submitted to the Minnesota Veterinary Diagnostic Laboratory, where they were humanely killed and necropsied. Tissue pools comprising of liver and spleen and other nonenteric visceral organs from these birds were examined for *Salmonella* by enrichment in tetrathionate brilliant green bile-enrichment broth for 24 h at 37°C followed by subcultivation on brilliant green agar for 24 h at 37°C. *Salmonella*-like colonies were picked and confirmed by slide agglutination using polyvalent antiserum against *Salmonella*. After collection of tissues for *Salmonella*, intestinal contents from these birds were collected and pooled for use as inoculum. A part of the pooled intestinal contents, without any processing, was examined for the presence of enteric viruses (rotavirus, astrovirus, enterovirus, and coronavirus) by transmission electron microscopy (**TEM**; Goyal et al., 1987) and for protozoa (*Eimeria* sp.) by the fecal flotation technique (Sloss et al., 1994). The remaining pools were processed further for preparation of experimental inoculum as described below.

Preparation of Inoculum

A 10% suspension of the pooled intestinal contents was prepared in PBS (pH 7.4) followed by homogenization in a stomacher. The homogenate was centrifuged at 784 × *g* for 20 min. The sediment was dissolved in 40 mL of PBS while the supernatant was collected and divided into 2 equal parts. Part 1 was used as an inoculum without any treatment and was named as unfiltered supernatant. Part 2 was filtered through a 0.22-μm filter coated with 4% fetal bovine serum and was named as filtered supernatant. The supernatants and the pellet (sediment) were stored at −20°C until used for experimental inoculation.

Examination of Supernatant and Sediment

Before inoculation, the supernatant and sediment suspension were examined for the presence of viruses and bacteria, respectively. The unfiltered supernatant was examined for the presence of enteric viruses by TEM and reverse transcription-PCR (**RT-PCR**) as described later. The sediment was examined for *Salmonella* following the procedure as described above.

Experimental Design

One-day-old male turkey poults were procured from a local commercial hatchery and were maintained in the isolation building. They were given a starter diet from d 1 until the end of the experiment. Both feed and water were provided ad libitum. At d 14 of age (d 0 of the experiment), 5 poults selected at random were weighed to obtain base line data on BW. All poults were then divided into 4 groups (groups A, B, C, and D). Each of the 4 groups contained 16 poults in experiments 1, 3, and 5, whereas only 10 poults per group were used in experiments 2 and 4 (Table 1). Groups A, B, C, and D were inoculated orally. Group A poults were inoculated with unfiltered supernatant (2 mL/bird). Poults in group B were inoculated with filtered supernatant, group C with sediment dissolved in PBS, and group D with PBS alone (negative control) with the same dose rate as in group A. The birds were followed for 15 d postinoculation (**DPI**) in experiments 1, 3, and 5 and for 20 DPI in experiments 2 and 4. The animal care and experimental protocol were approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Clinical Findings and Growth Response

All poults were observed daily for the development of clinical signs and mortality. To determine the effect of different treatments on BW, 5 randomly selected poults from each group were weighed at 5, 10, and 15 DPI in experiments 1, 3, and 5 and at 10 and 20 DPI in experiments 2 and 4. Growth depression due to PES

Table 1. Experimental groups

	Experiment number	
Parameter	1, 3, and 5	2 and 4
Inoculum from PES[1]-affected poults at age	≤2 wk	4 to 6 wk
Number of birds in each group[2]	16	10
Birds sampled at DPI[3]	5, 10, and 15	10 and 20

[1]PES = poult enteritis syndrome.

[2]The number of groups in each experiment was 4, namely A, B, C, and D.

[3]The number of poults sampled in each group at each interval was 5. DPI = days postinoculation.

was calculated in each experiment by dividing mean BW of poults in treated groups by mean BW of those in the control group at the last day of the experiment. For calculation of overall effect, mean BW of birds in the treated groups (A, B, and C) at the last day of observation (15 DPI in experiments 1, 3, and 5 and 20 DPI in experiments 2, and 4) in all 5 experiments were averaged. This value represented the mean BW of poults due to treatment, which was divided by average of mean BW of poults in the control group (group D) in all 5 experiments. The value (%) so obtained was subtracted from 100 to get percentage of growth depression due to PES. After weighing, these birds were killed with pentobarbital sodium.

Microbiological Examination

Liver and spleen from 5 poults in each group at each interval were pooled and examined for *Salmonella*. Intestinal contents of 5 birds from each group at each interval were pooled and examined for viruses by TEM and for protozoa (*Eimeria* sp.) by fecal flotation. A part of the pool was mixed with PBS (pH 7.4) to make a 10% suspension, which was then homogenized and centrifuged. The supernatant and sediment were examined for the presence of viruses and bacteria, respectively.

RT-PCR for Rotavirus

Total RNA was extracted from 250 μL of the unfiltered supernatants of inocula using Trizol LS reagent (Invitrogen Inc., Carlsbad, CA) following the instructions of the manufacturer. Total RNA was also extracted from unfiltered supernatants of pooled intestinal contents from experimental poults and from a known turkey rotavirus (kindly provided by Y. M. Saif, Wooster, OH) representing a positive control. Extracted RNA was subjected to RT-PCR for the detection of rotavirus using virus-specific primers (NSP4 forward 30: GTGCGGAAAGATGGAGAAC and NSP4 reverse 660: GTTGGGGTACCAGGGATTAA; Pantin-Jackwood et al., 2007). The RT-PCR was performed using Qiagen One-Step RT-PCR kit (Qiagen, Valencia, CA) and the reaction mix consisted of 1× RT-PCR reaction buffer, 320 μ*M* of each deoxynucleoside triphosphate, 0.6 μ*M* of each primer, 2 μL of enzyme blend, and 5 μL of extracted RNA for a total volume of 50 μL. Amplification was carried out in a thermocycler and the steps consisted of reverse transcription at 50°C for 30 min, *Taq* activation at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 1 min and a step of final extension at 72°C for 10 min. The PCR products were visualized on 1.2% agarose gel in Tris-acetate-EDTA buffer. The position of the band (630 bp) on agarose gel confirmed the presence of the virus. The PCR products from some of the positive cases were purified using QIAquick PCR purification kit (Qiagen) and were then sequenced at the Advanced Genomic Analysis Center, University of Minnesota. Sequencing was performed in both directions with the same primers as used in RT-PCR reactions. Sequences obtained were aligned with the existing database using the BLAST search tool available online (http://www.ncbi.nlm.nih.gov).

RT-PCR for Astrovirus

Extracted RNA from inocula, unfiltered supernatants of intestinal contents from experimental birds, and known turkey astrovirus (kindly provided by Y. M. Saif) were subjected to RT-PCR using virus-specific primers from the capsid gene. The primers (forward MKCap19: 5′AGCAGCAGTAGGTGGCAGTG3′ and reverse MKCap8: 5′TCATCATCCTCTCACACTGG3′) reported by Koci et al. (2000a) were used. The reaction mix and reaction conditions were similar to those used for the detection of rotavirus. The PCR products were gel-electrophoresed and the position of the band (849 bp) confirmed the presence of the virus. Purified PCR products from some cases were sequenced and sequences obtained were aligned with the existing database using the BLAST search tool.

RT-PCR for Coronavirus

Extracted RNA from inocula and known turkey coronavirus NC95 Indiana (kindly provided by James Guy, Raleigh, NC) were subjected to RT-PCR for the detection of coronavirus using virus-specific primers from the N gene. The primers (TCVnucleo forward: 5′GGTAGCGGTGTTCCTGA3′, TCVnucleo reverse: 5′CCCTCCTTACCTTTAGT3′) designed by Sellers et al. (2004) were used. The reaction mix consisted of 1× RT-PCR reaction buffer, 320 μ*M* of each deoxynucleoside triphosphate, 0.6 μ*M* of each primer, 2 μL of enzyme blend, and 3 μL of extracted RNA for a total volume of 50 μL. Amplification steps consisted of reverse transcription at 50°C for 30 min, *Taq* activation at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min and a step of final extension at 72°C for 10 min. The PCR products were gel electrophoresed and the position of the band (598 bp) confirmed virus presence. No coronaviruses were detected in inocula and experimental birds so none were sequenced.

Statistical Analysis

Database summaries and plots were used for exploratory data analysis. The outcome of interest, BW, was modeled as a function of treatment group, the DPI at which BW was tested, as well as the interaction between both variables using linear multivariable regression. A GLM was constructed for each of the 5 experiments using the MIXED procedure of SAS version 9.1 (SAS Institute, Cary, NC). A significance level of $P < 0.05$ was used.

Table 2. Body weights of turkey poults inoculated orally with unfiltered supernatant, filtered supernatant, or sediment prepared from intestinal contents of poult enteritis syndrome-affected poults[1]

Experiment no.	Group no.[2]	BW (g) at DPI[3]			Mean treatment effect	Age × treatment interaction[4]	BW (% of controls)
		5	10	15			
1	A	477 ± 08.81^{ab}	608 ± 31.22^{bc}	680 ± 15.25^{c}	588 ± 24.99^{b}	4.32 (S)	68.5
	B	450 ± 14.89^{b}	663 ± 52.68^{ab}	811 ± 56.41^{b}	641 ± 46.39^{b}		81.7
	C	403 ± 17.28^{b}	498 ± 36.32^{c}	472 ± 42.32^{d}	458 ± 20.94^{c}		47.5
	D	576 ± 10.99^{a}	765 ± 82.19^{a}	993 ± 52.87^{a}	778 ± 56.21^{a}		—
	Mean age effect	476 ± 15.70^{C}	633 ± 31.40^{B}	738 ± 48.16^{A}	—		—
3	A	516 ± 18.79^{b}	744 ± 41.43^{b}	793 ± 20.68^{bc}	684 ± 35.80^{b}	3.52 (S)	71.8
	B	542 ± 19.87^{b}	768 ± 35.97^{b}	822 ± 14.01^{b}	710 ± 35.16^{b}		74.4
	C	486 ± 11.78^{b}	649 ± 6.31^{c}	715 ± 33.34^{c}	617 ± 28.02^{c}		64.7
	D	663 ± 15.93^{a}	945 ± 37.87^{a}	$1{,}105 \pm 21.52^{a}$	904 ± 50.52^{a}		—
	Mean age effect	551 ± 17.25^{C}	776 ± 28.96^{B}	859 ± 35.50^{A}	—		—
5	A	429 ± 06.97^{b}	536 ± 33.29^{b}	734 ± 23.56^{c}	566 ± 36.05^{c}	7.04 (S)	63.6
	B	464 ± 27.90^{b}	592 ± 32.60^{b}	877 ± 45.73^{b}	644 ± 50.05^{b}		76.0
	C	436 ± 07.87^{b}	569 ± 12.55^{b}	705 ± 45.74^{c}	570 ± 32.91^{c}		61.1
	D	573 ± 12.48^{a}	855 ± 22.60^{a}	$1{,}154 \pm 21.35^{a}$	861 ± 64.22^{a}		—
	Mean age effect	476 ± 15.20^{C}	638 ± 31.56^{B}	868 ± 44.01^{A}	—		—

[a–d]Values with different lowercase superscripts differ significantly ($P \leq 0.05$).

[A–C]Values within a row showing mean age effect with different uppercase superscripts differ significantly ($P \leq 0.05$).

[1]All values are means ± SE of 5 poults.

[2]A = poults inoculated with unfiltered supernatant; B = poults inoculated with filtered supernatant; C = poults inoculated with sediment; D = poults inoculated with PBS (controls).

[3]DPI = days postinoculation.

[4]F-value indicates interaction between treatment and age of turkey poults. S = significant.

RESULTS

Clinical Findings

None of the group D poults (control group) exhibited depression, lethargy, and diarrhea in any of the 5 experiments. In general, PES-inoculated poults in experiments 1, 3, and 5 showed diarrhea (watery feces) from 2 DPI onward. Initially, feces were watery in consistency but subsequently changed to semisolid consistency. Sediment-treated poults (group C) passed watery feces from 2 to 15 DPI and those given unfiltered supernatant (group A) exhibited watery feces from 2 to 12 DPI. Poults given filtered supernatant (group B) exhibited watery feces between 3 and 7 DPI, which subsequently became semisolid. More watery feces for a longer duration were noticed in group C as compared with groups A and B. Frothy feces were seen between 2 and 10 DPI in groups A and C. Mild lameness was observed in 3 poults in group C in experiment 1 and in poults of groups B and C in experiment 5. No lameness was noticed in experiment 3 poults. Dullness and depression were evident in a few poults in all treated groups at different days of observations in these 3 experiments. Lack of uniformity (size of bird) was evident in all treated groups from 5 DPI onward. One poult each died in groups A and C in experiment 1. No mortality was seen in any of the treated groups in experiments 3 and 5.

Clinical findings in experiments 2 and 4 were similar to those recorded in experiments 1, 3, and 5. In both experiments, sediment-treated poults (group C) passed watery feces from 2 to 20 DPI. Group A and B poults exhibited watery feces between 4 and 15 DPI and then semisolid feces till 18 DPI. More watery feces for a longer duration were noticed in group C as compared with groups A and B. Lameness was noticed only in 1 poult of group C starting at 17 DPI in experiment 2 that continued until the end of the experiment. In addition, depression was also noticed in a few birds of group B, but there was no mortality. Neither lameness nor mortality was observed in experiment 4.

BW

In experiment 1, oral inoculation of sediment (group C) or supernatant (groups A and B) retarded the growth of poults from 5 DPI until the end of the experiment. Body weights in poults of groups C and A were significantly lower than those of poults in group D from 5 and 10 DPI onward, respectively (Table 2). Birds inoculated with filtered supernatant (group B) had lower BW than those inoculated with PBS alone (group D) at all intervals and the decrease was statistically significant at 5 and 15 DPI. Among the 3 treatments, sediment-treated poults (group C) had the lowest BW followed by unfiltered supernatant (group A) and filtered supernatant-treated poults (group B). The decrease in BW in group C as compared with group A was statistically significant at 15 DPI, whereas the decrease in group C as compared with group B was significant from 10 DPI onward. Body weights of group A were lower than the poults of group B from 10 DPI onward and the difference was statistically significant at 15 DPI (Table 2).

In experiments 3 and 5, oral inoculations with either sediment or supernatant significantly retarded their growth from 5 DPI onward (Table 2). Among the treated groups in experiment 3, poults in the sediment-

treated group (group C) had lower BW than supernatant-treated groups (groups A and B) at all intervals. The decrease in group C in comparison to group B was significant at 10 and 15 DPI, whereas in comparison to group A, the decrease was significant only at 10 DPI. Among the treated groups in experiment 5, poults in groups A (unfiltered supernatant-treated) and C (sediment treated) had lower BW than group B (filtered supernatant-treated) at all intervals and the decrease was significant at 15 DPI (Table 2).

In experiment 2, inoculation of sediment or filtered supernatant retarded poult growth from 10 DPI onward. Body weights of filtered supernatant-treated (group B) poults were significantly lower than those of the control group (group D) at 20 DPI (Table 3). Although poults in groups A (unfiltered supernatant-treated) and C (sediment-treated) had lower BW than group D from 10 DPI onward and at 20 DPI, respectively, the decrease was not statistically significant at any interval. Among the treated groups, poults in group B had significantly lower BW than groups A and C at 20 DPI. In experiment 4, BW of poults in all treated groups (groups A, B, and C) were significantly lower than BW of poults in group D at 10 and 20 DPI (Table 3). Sediment-treated poults (group C) had the lowest BW followed by unfiltered supernatant-treated (group A) and filtered supernatant-treated (group B). The decrease in group C in comparison to group B was statistically significant at both the intervals.

Mean treatment effect at the end of experiments 1, 3, 4, and 5 revealed a significant decrease in BW of turkey poults in all treated groups (groups A, B, and C) as compared with controls (group D; Tables 2 and 3). Mean treatment effect also revealed a significant decrease in BW in sediment-treated poults (group C) as compared with filtered supernatant-treated poults (group B) in these 4 experiments. Although mean treatment effect revealed a decrease in BW in group C as compared with group A in experiments 1, 3, and 4, the decrease was significant in experiments 1 and 3. The interaction between the treatments and age of poults was also significant in these 4 experiments. Although mean treatment effect revealed lower BW in group C than in group A in experiment 2, the decrease was not statistically significant. However, mean treatment effect revealed a significant decrease in BW in filtered supernatant-treated poults (group B) as compared with the other 3 groups (groups A, C, and D). Age and treatment interaction was statistically nonsignificant in experiment 2.

Body weights of treated groups as a percentage of control group are presented in Tables 2 and 3. Mean BW at the end of experiment 1 in treated poults ranged from 47.5 to 81.7% of controls. Similarly, in experiments 2, 3, 4, and 5, these values ranged from 69.6 to 95.6%, 64.7 to 74.4%, 49.5 to 60.4%, and 61.1 to 76.0% of the controls, respectively. Overall growth depression due to PES (in all treated groups) as compared with control group was 31.8%.

Identification of Agents

Inocula used for experimental reproduction of PES were positive for rotavirus, astrovirus, and *Salmonella*. In addition, enterovirus was also detected by electron microscopy in inocula for experiments 1 and 3. Rotavirus was detected in 4 of 5 inocula by electron microscopy and in all 5 inocula by RT-PCR. Astrovirus was not detected by electron microscopy in any of the inocula, but all of them were positive for astrovirus by RT-PCR. None of the inocula were positive for coronavirus either by electron microscopy or by RT-PCR.

Table 3. Body weights of turkey poults inoculated orally with unfiltered supernatant, filtered supernatant, or sediment prepared from intestinal contents of poult enteritis syndrome-affected poults[1]

Experiment no.	Group no.[2]	BW (g) at DPI[3] 10	BW (g) at DPI[3] 20	Mean treatment effect	Age × treatment interaction[4]	BW (% of controls)
2	A	750 ± 30.33^{a}	1,270 ± 40.57^{a}	1,010 ± 89.90^{a}	3.15 (NS)	88.6
	B	593 ± 37.51^{a}	998 ± 111.10^{b}	795 ± 87.29^{b}		69.6
	C	617 ± 25.40^{a}	1,370 ± 110.55^{a}	993 ± 136.44^{a}		95.6
	D	686 ± 50.18^{a}	1,433 ± 78.04^{a}	1,060 ± 132.07^{a}		—
	Mean age effect	661 ± 22.11^{B}	1,268 ± 56.18^{A}	—		—
4	A	412 ± 24.24^{c}	775 ± 56.97^{c}	593 ± 67.17^{c}	11.76 (S)	51.1
	B	573 ± 28.77^{b}	915 ± 41.27^{b}	744 ± 61.78^{b}		60.4
	C	403 ± 27.43^{c}	750 ± 28.66^{c}	576 ± 60.86^{c}		49.5
	D	761 ± 32.20^{a}	1,516 ± 70.26^{a}	1,139 ± 130.99^{a}		—
	Mean age effect	537 ± 35.92^{B}	989 ± 75.13^{A}	—		—

[a–c]Values with different lowercase superscripts differ significantly ($P \leq 0.05$).

[A,B]Values within a row showing mean age effect with different uppercase superscripts differ significantly ($P \leq 0.05$).

[1]All values are mean ± SE of 5 poults.

[2]A = poults inoculated with unfiltered supernatant; B = poults inoculated with filtered supernatant; C = poults inoculated with sediment; D = poults inoculated with PBS (controls).

[3]DPI = days postinoculation.

[4]F-value indicates interaction between treatment and age of turkey poults. S = significant.

The details of rotavirus detection in pooled intestinal contents and unfiltered supernatant of pooled intestinal contents by electron microscopy, and in unfiltered supernatant of intestinal contents by RT-PCR, are presented in Table 4. Rotavirus was detected in treated poults at all intervals. In these 5 experiments, rotavirus was detected in more samples by RT-PCR than by electron microscopy at different intervals. Astrovirus was not detected by electron microscopy but was detected by RT-PCR. The details of astrovirus detection are presented in Table 5. In experiment 1, enterovirus was also detected in filtered supernatant-treated poults (group B) at 5 DPI both in pooled intestinal contents as well as in unfiltered supernatant of intestinal contents by electron microscopy. Similarly, in group C in this experiment, enterovirus was also detected at 5 and 15 DPI. Although the inoculum for experiment 3 was also positive for enterovirus, this virus could not be detected in pooled intestinal contents from treated poults by electron microscopy.

In experiment 1, *Salmonella* was detected from group C in tissue pools as well as in sediment of pooled intestinal contents at 10 and 15 DPI, respectively. However, *Salmonella* was not detected in poults of groups A, B, and D at all intervals. In experiment 5, *Salmonella* was detected from tissue pools and sediment of pooled intestinal contents from poults of groups A and C at 15 DPI. *Salmonella* could not be detected either from tissue pools or sediment of pooled intestinal contents from poults of all treated groups at all intervals in experiments 2, 3, and 4. None of the pooled intestinal contents in any group (except at 5 DPI in group A in experiment 1) in any of the 5 experiments were found positive for *Eimeria* sp. at any interval.

Table 4. Identification of rotavirus in experimentally inoculated poults

				Group[4]			
Experiment no.	Interval[1] (DPI)	Method[2]	Material tested[3]	A	B	C	D
1	5	EM	IC	+	+	+	ND
			IS	ND	ND	+	ND
		RT-PCR	IS	+	+	+	ND
	10	EM	IC	ND	ND	ND	ND
			IS	ND	ND	ND	ND
		RT-PCR	IS	+	+	+	ND
	15	EM	IC	ND	ND	ND	ND
			IS	ND	ND	ND	ND
		RT-PCR	IS	+	ND	ND	ND
2	10	EM	IC	ND	ND	ND	ND
			IS	ND	ND	ND	ND
		RT-PCR	IS	+	+	ND	ND
	20	EM	IC	+	+	+	ND
			IS	+	+	+	ND
		RT-PCR	IS	+	+	+	ND
3	5	EM	IC	+	+	+	ND
			IS	+	+	ND	ND
		RT-PCR	IS	+	+	+	ND
	10	EM	IC	+	+	+	ND
			IS	+	+	ND	ND
		RT-PCR	IS	+	+	+	ND
	15	EM	IC	+	+	+	ND
			IS	+	ND	ND	ND
		RT-PCR	IS	+	+	+	ND
4	10	EM	IC	ND	ND	ND	ND
			IS	ND	+	ND	ND
		RT-PCR	IS	ND	+	+	ND
	20	EM	IC	+	+	+	ND
			IS	ND	+	ND	ND
		RT-PCR	IS	+	+	+	ND
5	5	EM	IC	+	+	+	ND
			IS	+	+	ND	ND
		RT-PCR	IS	+	+	+	ND
	10	EM	IC	ND	ND	ND	ND
			IS	ND	ND	ND	ND
		RT-PCR	IS	+	ND	+	ND
	15	EM	IC	ND	ND	ND	ND
			IS	ND	ND	ND	ND
		RT-PCR	IS	ND	ND	+	ND

[1]DPI = days postinoculation.

[2]EM = electron microscopy; RT-PCR = reverse transcription-PCR.

[3]IC = pooled intestinal contents; IS = unfiltered supernatant of pooled intestinal contents.

[4]A = poults inoculated with unfiltered supernatant; B = poults inoculated with filtered supernatant; C = poults inoculated with sediment; D = poults inoculated with PBS (controls). + = rotavirus detected; ND = rotavirus not detected.

Table 5. Identification of astrovirus by reverse transcription-PCR in experimentally inoculated poults[1]

Experiment no.	Interval[2] (DPI)	Material tested[3]	Group[4]			
			A	B	C	D
1	5	IS	+	+	+	ND
	10	IS	+	+	+	ND
	15	IS	+	+	+	ND
2	10	IS	+	+	+	ND
	20	IS	ND	ND	ND	ND
3	5	IS	+	+	ND	ND
	10	IS	+	+	ND	ND
	15	IS	+	ND	ND	ND
4	10	IS	+	ND	ND	ND
	20	IS	ND	ND	ND	ND
5	5	IS	+	+	+	ND
	10	IS	ND	ND	ND	ND
	15	IS	ND	ND	ND	ND

[1]Astrovirus was not detected in intestinal contents by electron microscopy in all 5 experiments.

[2]DPI = days postinoculation.

[3]IS = unfiltered supernatant of pooled intestinal contents.

[4]A = poults inoculated with unfiltered supernatant; B = poults inoculated with filtered supernatant; C = poults inoculated with sediment; D = poults inoculated with PBS (controls). + = astrovirus detected; ND = astrovirus not detected.

DISCUSSION

This study was undertaken to reproduce PES experimentally in turkey poults by oral inoculation with intestinal contents from PES-affected birds. We were able to reproduce the disease with intestinal contents from PES-affected poults of less than 2 wk of age and those from older poults (4 to 6 wk of age). No major difference was observed with regards to clinical findings and growth depression when inocula from poults of different age groups were used. Brown et al. (1997) and Davis et al. (1997) reproduced PEMS experimentally by oral inoculation of turkey poults with intestinal contents from PEMS-affected birds. The clinical finding of diarrhea as observed in the present study is similar to that of Yason and Schat (1986, 1987), who also reported diarrhea in turkey poults experimentally infected with rotavirus. The clinical findings of the present study are also similar to the previous reports of Perry et al. (1991a) on poult malabsorption syndrome. Pantin-Jackwood et al. (2008) also reported diarrhea, frothy feces, and depression in turkey poults inoculated orally with turkey astrovirus-2 (TAstV-2). Brown et al. (1997) experimentally reproduced SMT resulting in considerable mortality. However, no mortality was observed in our study except that 2 poults died in experiment 1. Yason and Schat (1986) also did not observe any mortality in turkey poults after experimental infection with rotavirus even though the virus used for experimental inoculation was isolated from flocks with up to 20% mortality. The authors believed that other undetected viruses or secondary infections may have led to high mortality in flocks under field conditions. Brown et al. (1997) and Guy et al. (2000), on the other hand, recorded high mortality in turkey poults inoculated with turkey coronavirus infection. Yu et al. (2000) and Pantin-Jackwood et al. (2008) also reported mortality in astrovirus-challenged turkey poults. The latter authors were of the opinion that the mortality in poults could be because of viral challenge at a very young age leading to depression, lethargy, and failure to take feed and water.

Decreased BW of turkey poults recorded in the present study are similar to those reported in PEMS and SMT (Brown et al., 1997; Davis et al., 1997; Qureshi et al., 2000; Schultz-Cherry et al., 2000). Perry et al. (1991b) also recorded decreased weight gain in turkey poults placed on contaminated litter obtained from poults suffering from poult enteritis. In the present study, inoculation of sediment suspension caused a maximum decrease in BW of turkey poults followed by unfiltered supernatant and filtered supernatant (except experiment 2), indicating that the presence of enteric bacteria in sediment may have increased the severity of growth depression induced by enteric viruses. This observation is supported by Guy et al. (2000), who observed high mortality and increased growth depression in turkey poults inoculated with turkey coronavirus and enteropathogenic *E. coli* than those inoculated with turkey coronavirus or *E. coli* alone. Torres-Medina (1984) reported that calves infected with rotavirus and enterotoxic *E. coli* had a more severe infection than observed with either alone. In the present study, the only exception was experiment 2, in which filtered supernatant-treated poults had the lowest BW followed by sediment-treated and unfiltered supernatant-treated poults. Although it is difficult to explain the reason for such variation from other experiments, it may have been due to variation in the amount of pathogens present in inocula used for different experiments. The difference in the virulence of various strains may also account for some of these differences.

Loss in BW in the present study can be attributed to decreased feed intake, poor feed conversion efficiency, or both. Although feed intake was not monitored, the staff in isolation units empirically observed that poults in treated groups consumed relatively less feed compared with controls. Damage caused to the intestinal tract by viruses or *Salmonella*, or both, may have led to poor feed conversion efficiency. In the present study, we observed an overall growth depression of 31.8% due to PES in all 5 experiments. A growth depression of this magnitude would have serious economic consequences. Barnes et al. (2000) reported that estimated losses to the US turkey industry from 10 to 15% growth depression due to poult enteritis complex (**PEC**, a term that includes infectious intestinal diseases like coronavirus enteritis, maldigestion syndrome, runting and stunting syndrome of turkeys, poult malabsorption syndrome, stunting syndrome, PEMS, SMT, and turkey viral enteritis of poults) would be between US$300 and US$400 million annually. Under field conditions, several stressors operate that may complicate the situation, further affecting the growth of the birds. Davis et al. (1997) observed 49% BW depression in turkey poults inoculated with filtered intestine-pancreas homogenate collected from turkeys suffering from SMT. The homogenate contained rotavirus, enterovirus-like virus, and birnavirus. These authors believed that this growth depression was due to these and other undetected viruses present in the homogenate.

Lameness was noticed in 3 of the 5 experiments in some of the treated poults at about 10 DPI. The duration of experiments 1, 3, and 5 was 15 DPI, but for experiments 2 and 4, it was 20 DPI. The period of observation in experiments 2 and 4 was increased keeping in view the lameness observed in the first experiment at 11 DPI. We extended the observation period to gain a better understanding of progression of the disease, particularly the lameness. Poults undergo rapid skeletal growth during first 4 wk of age and during this period their demand for vitamin D, calcium, and phosphorus for incorporation of mineral into the skeleton is at its maximum (Bar et al., 1982). With early enteric disease, malabsorption of these vital nutrients occurs thus leading to skeletal deformities. Lameness may have contributed to a decrease in BW due to the inability of lame poults to reach up to the feeders or waterers properly.

Statistically significant treatment × age interactions in 4 of 5 experiments indicated that PES material produced significant adverse effects on the growth of turkey poults. Further studies are needed to elucidate the long-term effects of PES on growth potential of turkeys during the brooding or the grow-out phase.

Intestinal contents from PES-affected poults used as inocula in the present study were consistently positive for rotavirus, astrovirus, (and enterovirus in 2 experiments) and *Salmonella*. Rotavirus and astrovirus were detected in the intestinal contents of treated poults at different intervals in all experiments. Similarly, *Salmonella* was isolated from tissue pools of poults in the treated groups but not in all experiments. In addition, in experiment 1, we also detected enterovirus from pooled intestinal contents. Yason and Schat (1986) demonstrated the presence of rotavirus in intestinal contents of rotavirus-inoculated poults even at 24 DPI. Similarly, Pantin-Jackwood et al. (2008) detected astrovirus in cloacal swabs of all inoculated poults at all intervals postinoculation. Increased detection of rotavirus by RT-PCR as compared with electron microscopy is not surprising because electron microscopy is comparatively less sensitive than RT-PCR. Neither inocula nor intestinal contents from treated birds were positive for coronavirus. The risk of misidentification or misclassification of a virus on electron microscopy has been previously recognized (van Regenmortel et al., 2000). Although small round viruses (astrovirus, enterovirus, and enterovirus-like particles) with distinct morphologies are visible by electron microscopy (Caul and Appleton, 1982), their characteristic morphology may change or become less prominent because of the emergence of new subgroups and strains within these viruses; hence, viral diagnosis must not rely solely on morphology but should also include a set of other characteristics (Koci and Schultz-Cherry, 2002). It could be the reason for misidentification of astrovirus by enterovirus in some of the samples on electron microscopy. This finding has the support of Guy et al. (2004), who tentatively identified a small round virus isolated from droppings of turkeys as enterovirus on the basis of size and intracytoplasmic morphogenesis. However, RT-PCR with astrovirus capsid and polymerase-specific primers revealed this isolate as turkey astrovirus. Viruses other than rotavirus and astrovirus that have been found associated with PEC are turkey coronavirus (Guy, 1998; Ismail et al., 2003; Teixeira et al., 2007), reovirus (Heggen-Peay et al., 2002), and adenovirus (Sharma, 1991; Suresh and Sharma, 1996). Among bacteria, *Salmonella* and *E. coli* are the most important pathogens associated with PEC (Edens et al., 1997; Barnes et al., 2000; Pakpinyo et al., 2002).

Rotavirus is one of the major causes of enteritis and diarrhea in mammals and birds. Rotavirus replicates primarily in the cytoplasm of mature epithelial cells lining the upper portions of intestinal villi where they cause destruction of mature villous epithelium leading to its replacement by immature epithelium. The immature epithelium has impaired absorptive ability and lacks disaccharides leading to diarrhea due to malabsorption and maldigestion (Moon, 1994). Rotaviral enteritis in turkeys has been described in many different countries (McNulty et al., 1978, 1979; Saif et al., 1985; Theil and Saif, 1987). Rotavirus has also been detected in cases of runting and stunting syndrome in broiler chicks (Otto et al., 2006). Astroviruses were first identified in 1980 in the United Kingdom from turkey poults suffering with diarrhea (McNulty et al., 1980) and were subsequently reported from the United States (TAstV-1; Saif et al., 1985; Reynolds and Saif, 1986). Later, a second turkey astrovirus (TAstV-2), genetically distinct from TAsV-

1, was characterized (Koci et al., 2000b; Schultz-Cherry et al., 2000, 2001). Astroviruses have also been found to be associated with PEMS (Koci et al., 2000b; Yu et al., 2000). How turkey astroviruses induce diarrhea is still not clear, but Reynolds and Schultz-Cherry (2003) attribute induction of diarrhea to the osmotic effects of undigested, unabsorbed nutrients attracting water to the intestinal lumen. Koci et al. (2003) reported that astrovirus-induced diarrhea does not involve extensive destruction of enterocytes in the villous epithelium or increased inflammatory response, whereas Moser et al. (2007) attributed it to increased intestinal barrier permeability leading to more fluid in the intestinal lumen. Astroviruses cause a significant decrease in D-xylose absorption in turkey poults (Reynolds and Saif, 1986) and a decrease in maltase activity thereby leading to disaccharide maldigestion and malabsorption (Thouvenelle et al., 1995). Enterovirus also replicates in the intestine particularly in the jejunum and ileum, in the villus epithelium located halfway between the tip and base of the villus (McNulty and Guy, 2003). Enterovirus has been detected in the feces of young turkeys by various workers (Saif et al., 1985, 1990; Reynolds et al., 1987). It appears that these viruses act initially in the intestine individually or collectively and later bacterial or protozoa, or both, complicate the situation.

More severe clinical findings and growth depression in sediment-treated poults suggest the possible association of bacteria to cause or to increase, or both, the severity of disease initiated by enteric viruses. Under field conditions, the presence of bacteria, parasites, and stressors in a turkey flock might further increase the severity of virus-induced disease. In conclusion, experimental inoculation of supernatant or sediment from intestinal contents of PES-affected birds leads to diarrhea, dullness, and significant growth depression in turkey poults.

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