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Mortality Patterns Associated with Poult Enteritis
Mortality Syndrome (PEMS) and Coronaviral Enteritis in Turkey Flocks Raised in PEMS-Affected Regions

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SUMMARY. Poult enteritis mortality syndrome (PEMS) is an economically devastating disease. To date, many questions about the syndrome remain unanswered, including its cause, transmission of causative agent(s), and control methods. Turkey coronavirus (TCV) infection has been associated with some outbreaks of PEMS, with areas having a higher prevalence of TCV infection also experiencing an increased incidence of PEMS. This study was designed to establish mortality patterns for flocks experiencing excess mortality and TCV infection in PEMS-affected regions and to delineate the possible role of TCV in PEMS-affected flocks. Fifty-four commercial turkey flocks on farms in areas with and without a history of TCV infection were monitored for weekly mortality and for antibodies to TCV. Flocks were chosen on the basis of placement dates and were monitored from day of placement until processing. All flocks were tested for TCV by an indirect fluorescent antibody assay. PEMS status was determined with the use of the clinical definition of mortality greater than 2% during any 3-wk period from wk 2 of age through the end of brooding due to unknown cause. Of the 54 flocks, 24 remained healthy, 23 experienced PEMS, and 7 tested positive for TCV but did not experience PEMS. Ten flocks experienced PEMS and tested positive for TCV, whereas 13 flocks experienced PEMS and did not test positive for TCV. Four health status groups were evident: healthy, PEMS positive, TCV positive, and PEMS + TCV positive. Distinct mortality patterns were seen for each of the four health status groups. Whereas TCV was associated with PEMS in 43% of PEMS cases, 13 cases (57%) of PEMS did not involve TCV. Additionally, 7 out of 17 cases of TCV (41%) did not experience excess mortality (PEMS) at any time during brooding of the flock. The results of this study indicate that TCV can be associated with PEMS but is neither necessary nor sufficient to cause PEMS.

RESUMEN. Patrones de mortalidad asociados con el síndrome de mortalidad y enteritis de los pavitos y de la enteritis por coronavirus, en lotes de pavos criados en regiones afectadas por dicho síndrome.

El síndrome de mortalidad y enteritis de los pavitos es una enfermedad importante económicamente. Hasta la fecha, muchas preguntas acerca del síndrome de mortalidad y enteritis permanecen sin responderse, incluyendo su agente o agentes causales, la forma de transmisión y los métodos de control. La infección por coronavirus del pavo ha sido asociada con algunos brotes del síndrome de mortalidad y enteritis en ciertas áreas con una alta incidencia de infecciones por coronavirus del pavo presentando al mismo tiempo un aumento en la incidencia del síndrome de mortalidad y enteritis. Este estudio fue diseñado con el fin de establecer los patrones de mortalidad en aquellos lotes con una mortalidad alta y con infecciones por coronavirus del pavo en zonas afectadas por el síndrome de mortalidad y enteritis, así como para delinear la posible incidencia del coronavirus del pavo en lotes afectados por el síndrome de mortalidad y enteritis. Se determinó semanalmente la mortalidad y la presencia de anticuerpos contra el coronavirus del pavo en 54 lotes comerciales de pavos provenientes de granjas ubicadas en zonas con y sin historial de infecciones por coronavirus del pavo. Se seleccionaron lotes con base en la fecha de encasetamiento, siendo supervisados desde el día de edad hasta la fecha de sacrificio. Se determinó la presencia de anticuerpos contra el...
coronavirus del pavo mediante la técnica indirecta de anticuerpos fluorescentes. Se determinó la presencia del síndrome de mortalidad y enteritis mediante el uso de la definición clínica de una mortalidad mayor del 2% por causa desconocida durante cualquier período de 3 semanas, desde las 2 semanas de edad hasta el final del periodo de cría. De los 54 lotes, 24 se conservaron sanos. 23 presentaron el síndrome de mortalidad y enteritis, y 7 fueron positivos al coronavirus del pavo, sin observarse la presencia del síndrome de mortalidad y enteritis. Diez lotes presentaron el síndrome de mortalidad y enteritis y fueron positivos al coronavirus del pavo, mientras que 13 lotes presentaron el síndrome de mortalidad y enteritis y fueron negativos al coronavirus del pavo. De acuerdo con el estado de salud observado, cuatro grupos fueron evidentes: Sanos, positivos al síndrome de mortalidad y enteritis, positivos al coronavirus del pavo, y positivos al síndrome de mortalidad y enteritis y al coronavirus del pavo. En cada uno de estos grupos se observaron distintos patrones de mortalidad. Se observó una asociación entre el coronavirus del pavo y el síndrome de mortalidad y enteritis en el 43% de los casos, mientras que en 13 casos (57%), la presencia del síndrome de mortalidad y enteritis no fue asociada con el coronavirus del pavo. Adicionalmente, en 7 de 17 casos de coronavirus del pavo (41%), no se observó un aumento en la mortalidad por el síndrome de mortalidad y enteritis durante la crianza de los lotes. Los resultados de este estudio indican que el coronavirus del pavo puede encontrarse asociado con el síndrome de mortalidad y enteritis, no siendo necesaria o suficiente la presencia del coronavirus del pavo para causar el síndrome de mortalidad y enteritis.

Key words: mortality, turkey, poult enteritis mortality syndrome, coronavirus, avian, disease, enteric

Abbreviations: EMT = excess mortality of turkeys; IFAT = indirect fluorescent antibody technique; PBS = phosphate-buffered saline; PEMS = poult enteritis mortality syndrome; PEMS(+) = flocks positive for poult enteritis mortality syndrome; SMT = spiking mortality of turkeys; TCV = turkey coronavirus; TCV(+) = flocks positive for turkey coronavirus; TCV(+)PEMS(+) = flocks positive for both turkey coronavirus and poult enteritis mortality syndrome

Poult enteritis mortality syndrome (PEMS) is an economically devastating disease (1,2). To date, many questions about the syndrome remain unanswered, including its cause (3,4,5,7,8,11,12,13,14), transmission of causative agent(s), and control methods. Because there is no specific diagnostic test, PEMS is identified by a characteristic mortality pattern (2). The clinical syndrome associated with the two forms of PEMS has been characterized as excess mortality of turkeys (EMT) and spiking mortality of turkeys (SMT) (2). EMT is defined as mortality greater than 2% for any 3-wk period, excluding the first week, during the brooding phase of production, and SMT is defined as mortality greater than 9% for any 3-wk period, excluding the first week, during the brooding phase of production. PEMS has been associated with turkey coronavirus (TCV) infections. Areas having a higher incidence of TCV infection also typically have an increased incidence of PEMS. Understanding the relationship of TCV to PEMS is important in order to better understand the natural history of PEMS and to establish and maintain control efforts for PEMS. The objectives of this study were to determine mortality patterns for flocks experiencing PEMS and/or TCV infection in PEMS-affected regions and to delineate the role of TCV in PEMS-affected flocks.

MATERIALS AND METHODS

Study design. A prospective longitudinal study was conducted with commercial turkey flocks reared in two areas of North Carolina. Both areas had historic evidence of PEMS and TCV. PEMS occurred in these areas starting in mid-May and continuing through October. Flocks were selected for the study on the basis of placement dates. Flocks placed between May 15 and July 15 were eligible for inclusion in the study so they could be closely monitored for at least 6 wk with mortality data collection continuing until processing. Previous disease status with regard to PEMS and TCV for individual farms was not revealed to investigators prior to flock selection. Flocks from four commercial companies were monitored.

Mortality. Mortality was monitored daily and was reported as number of birds dead each day including cull birds. Weekly mortality was used for the analysis.
Table 1. Mortality for the different health status groups.

<table>
<thead>
<tr>
<th>Health status</th>
<th>Weeks 2–6</th>
<th>Weeks 7–13</th>
<th>Total</th>
<th>Difference^ weeks 2–6</th>
<th>Difference^ weeks 7–13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>4.6%</td>
<td>1.9%</td>
<td>6.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCV(+)</td>
<td>7.3%</td>
<td>2.9%</td>
<td>10.2%</td>
<td>2.7%</td>
<td>1.0%</td>
</tr>
<tr>
<td>PEMS(+)</td>
<td>12.0%</td>
<td>2.6%</td>
<td>14.6%</td>
<td>7.4%</td>
<td>0.7%</td>
</tr>
<tr>
<td>TCV(+)PEMS(+)</td>
<td>10.3%</td>
<td>4.5%</td>
<td>14.8%</td>
<td>5.7%</td>
<td>2.6%</td>
</tr>
</tbody>
</table>

^Difference in mortality percentage between infected group and healthy group.

and was calculated as the number of birds that died over a 7-day period. This number was then divided by the number of birds placed and multiplied by 100 to obtain weekly mortality percentage.

**TCV status.** TCV status was monitored for each flock by obtaining six blood samples per turkey house on each farm. Age at first testing varied from 6 to 14 wk of age. Serum collected from each flock was tested by an indirect fluorescent antibody test. If any one serum sample tested positive for TCV antibodies, the flock was called positive. Flocks that tested negative were retested approximately 4 wk after the initial test and again before processing. TCV antibody titers were determined for each serum by an indirect fluorescent antibody technique (IFAT). This test has proved reliable over the years in determining TCV status for turkeys and has been used as a gold standard in development of another serologic test for TCV (9,10). Antigen for the IFAT consisted of epithelial cells exfoliated from the bursae of Fabricius of turkeys experimentally infected with TCV (6). TCV-infected epithelial cells were spotted onto glass microscope slides, air dried, and fixed in cold (4°C) absolute acetone for 10 min. Twofold serum dilutions were prepared in phosphate-buffered saline (PBS) starting at a 1:20 dilution. Diluted sera were overlaid onto cells and incubated at 37°C for 15 min. Slides were washed briefly in two changes of PBS, and cells were overlaid with a 1:40 dilution of fluorescein isothiocyanate–labeled rabbit anti-chicken immunoglobulin G (ICN Biomedicals, Inc., Costa Mesa, CA). Slides were incubated at 37°C for 15 min, washed twice with PBS, and examined by epifluorescence.

**PEMS status.** PEMS status was determined with the use of clinical definitions previously provided by Barnes and Guy, combining EMT and SMT into one category referred to as PEMS. Mortality experienced the first week after placement was not included in the analysis because of possible effects of hatchery, holding, shipment, and placement on mortality. Mortality rates from weeks 2–6 (8–42 days of age) were analyzed and those flocks experiencing unexplained 3-wk cumulative mortality greater than 2% in weeks 2–4, 3 and 4, or 4–6 were considered positive for PEMS.

A chi-square test was used to determine if the occurrence of PEMS affected the timing of the first test for TCV.

**Health status.** Flocks were categorized as healthy, TCV positive (TCV(+)), PEMS positive (PEMS(+)), and TCV and PEMS positive (TCV(+)PEMS(+)). Healthy flocks were defined as those that experienced neither TCV nor PEMS throughout the life of the flock. TCV(+) flocks were those that tested positive for TCV but did not experience PEMS. PEMS(+) flocks were those that experienced PEMS but never tested positive for TCV. Flocks that experienced PEMS and tested positive for TCV were classified as TCV(+)PEMS().

**RESULTS**

**Healthy flocks.** Twenty-four of the 54 turkey flocks remained healthy and did not seroconvert to TCV or experience PEMS. Healthy flocks had cumulative mortality rates (Table 1) of 4.6% at 6 wk of age and 6.5% at 13 wk of age. These rates equate to a cumulative mortality increase of 1.9% between 6 and 13 wk of age in healthy flocks.

**TCV(+).** Seventeen flocks tested positive for TCV antibodies. TCV(+) flocks had detectable antibodies on the first test at an average of 7.7 wk of age. Of the 17 flocks with TCV antibodies, 10 experienced PEMS and are referred to as TCV(+)PEMS(+) flocks. No correlation was found between age of first test and presence or absence of PEMS (P = 0.82). The seven remaining flocks with no evidence of PEMS are referred to as TCV(+). TCV(+) flocks experienced greater mortality than did healthy flocks. Mortality patterns differed for TCV(+) flocks relative to healthy flocks (Fig. 1). Mortality in TCV(+) flocks was essentially the same as in healthy flocks from week 2 through week 5. At week 6, mortality in TCV(+) flocks started to increase above that for healthy flocks and remained higher throughout the remainder of the
growout period. Cumulative mortality (Table 1) was greater at 6 wk of age in TCV(+) flocks (mean = 7.3%) relative to healthy flocks (mean = 4.6%). Cumulative mortality continued to diverge from the pattern seen in healthy flocks and averaged 10.2% in TCV(+) flocks compared with 6.5% in healthy flocks at 13 wk of age. The cumulative mortality increase in TCV(+) flocks between weeks 6 and 13 was 2.9%. This increase was 1% greater than that expected for a healthy flock.

PEMS(+). A total of 23 flocks experienced PEMS. Of these, 10 tested positive for antibodies to TCV and are referred to as TCV(+)PEMS(+) flocks. The remaining 13 flocks that experienced PEMS with no seroconversion to TCV are referred to as PEMS(+) flocks. PEMS(+) flocks experienced greater mortality than did healthy flocks. Mortality patterns differed in PEMS(+) flocks relative to healthy flocks (Fig. 2). Mortality in PEMS(+) flocks was greater than in healthy flocks from

Fig. 1. Weekly mortality patterns of healthy and TCV-positive turkey flocks.

Fig. 2. Weekly mortality patterns of healthy and PEMS-positive turkey flocks.
placement and remained much higher until week 9. At weeks 6 and 7, the mortality began to approach that of healthy flocks. By week 10, mortality patterns of PEMS(+) and healthy flocks were essentially the same. Cumulative mortality (Table 1) was greater at 6 wk of age in PEMS(+) flocks (mean = 12%) relative to healthy flocks (mean = 4.6%). Cumulative mortality was also greater at 13 wk of age in PEMS(+) flocks (mean = 14.6%) when compared with healthy flocks (mean = 6.5%). The cumulative mortality increase in PEMS(+) flocks between weeks 6 and 13 was 2.6%. This increase was 0.7% greater than expected for healthy flocks.

**Flocks positive for PEMS and TCV.** Ten flocks were positive for both PEMS and TCV and are referred to as TCV(+)PEMS(+) flocks. These flocks also experienced greater mortality than healthy flocks. Mortality patterns differed in TCV(+)PEMS(+) flocks relative to healthy flocks (Fig. 3). Like the PEMS(+) flocks, mortality in TCV(+)PEMS(+) flocks was greater than that in healthy flocks from placement, then approached mortality levels for healthy flocks by week 8. Like the TCV(+) flocks, the mortality pattern for the TCV(+)PEMS(+) flocks started to diverge from the pattern of the healthy flocks at 9 wk of age and remained elevated throughout the remainder of the growout period in these flocks. Cumulative mortality (Table 1) was greater at 6 wk of age in TCV(+)PEMS(+) flocks (mean = 10.3%) relative to healthy flocks (mean = 4.6%). Cumulative mortality was also greater at 13 wk of age in TCV(+)PEMS(+) flocks (mean = 14.8%) compared with healthy flocks (mean = 6.5%). The cumulative mortality increase in TCV(+)PEMS(+) flocks between weeks 6 and 13 was 4.5%. This increase was 2.6% greater than that in healthy flocks.

**DISCUSSION**

Three basic mortality patterns were evident in clinically affected flocks. One was associated with the brooding phase of production (≤6 wk of age), one with the growout phase of production (>6 wk of age), and one occurred in both brooding and growout periods. Early in the brooding phase, a mortality pattern started very high and remained relatively elevated for several weeks, then gradually declined (Fig. 2). Once birds were moved to the growout phase of production, the daily mortality curve was similar to that of healthy flocks. This mortality pattern had only one peak and was typical for flocks that experienced PEMS. The second mortality pattern was one that occurred later in the production phase and was persistent. This
pattern began to diverge from the healthy flock curve after 6 wk of age (Fig. 1). This mortality pattern did not have a distinct peak and continued to slowly diverge from that of the healthy flocks throughout the remainder of the growout period. Unlike the previous pattern, this pattern did not approximate that of healthy flocks late in the production phase. Birds in these flocks continued to experience increased mortality until market. TCV(+) flocks exhibited this mortality pattern. A third mortality pattern that was apparent in TCV(+)PEMS(+) flocks involved an increase in mortality very early followed by a merging with the healthy flock curve (Fig. 3). This brief merging was followed by another increase during the growout phase. This bimodal distribution was the apparent result of both disease patterns occurring within the same flocks.

Cumulative mortality (Table 1) for clinically affected flocks quantifies losses due to mortality. Though the overall cumulative mortality clearly showed an increase in mortality for all health status groups, again there were differences in mortality specific to health status and phase of production.

The role of TCV in PEMS and the existence of PEMS as a separate clinical disease are controversial. This study showed that TCV does play a role in some but not all cases of PEMS. Of those flocks experiencing PEMS, almost half did not have TCV. Similarly, about one-fourth of TCV(+) flocks did not experience PEMS. Additionally, this study provides evidence of mortality patterns that differ by health status. PEMS(+) flocks experienced mortality in the early phase of production and eventually exhibited mortality equal to that of healthy flocks. TCV(+) flocks experienced mortality during growout, and the mortality continued until processing. These mortality patterns are distinct and suggest that TCV can be a disease entity separate from PEMS. The mortality of TCV(+) PEMS(+) flocks suggest that these were flocks that experienced PEMS in conjunction with TCV. Mortality during brooding was similar to the that of PEMS(+) flocks, and mortality during growout was similar to, though more extreme than, that of TCV(+) flocks.

Data from this study suggest that there are instances when TCV infection is superimposed on PEMS. These data also show that there are situations when TCV plays no role in PEMS. TCV appears to be neither necessary nor sufficient to cause PEMS.

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