Research Note—

Mechanical Transmission of Turkey Coronavirus by Domestic Houseflies (Musca domestica Linnaeaeus)


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SUMMARY. Domestic houseflies (Musca domestica Linnaeaeus) were examined for their ability to harbor and transmit turkey coronavirus (TCV). Laboratory-reared flies were experimentally exposed to TCV by allowing flies to imbibe an inoculum comprised of turkey embryo–propagated virus (NC95 strain). TCV was detected in dissected crops from exposed flies for up to 9 hr postexposure; no virus was detected in crops of sham-exposed flies. TCV was not detected in dissected intestinal tissues collected from exposed or sham-exposed flies at any time postexposure. The potential of the housefly to directly transmit TCV to live turkey poults was examined by placing 7-day-old turkey poults in contact with TCV-exposed houseflies 3 hr after flies consumed TCV inoculum. TCV infection was detected in turkeys placed in contact with TCV-exposed flies at densities as low as one fly/bird (TCV antigens detected at 3 days post fly contact in tissues of 3/12 turkeys); however, increased rates of infection were observed with higher fly densities (TCV antigens detected in 9/12 turkeys after contact with 10 flies/bird). This study demonstrates the potential of the housefly to serve as a mechanical vector of TCV.

RESUMEN. Nota de Investigación—Transmisión mecánica del coronavirus de los pavos mediada por la mosca doméstica (Musca domestica Linnaeaeus).

Se examinó la capacidad de la mosca doméstica (Musca domestica Linnaeaeus) para alojar y transmitir el coronavirus de los pavos. Se expusieron experimentalmente moscas criadas en el laboratorio al coronavirus de los pavos permitiendo que las mismas se alimentaran con material obtenido de embriones de pavos inoculados con la cepa NC95 del virus. El coronavirus fue detectado hasta 9 horas después de la administración del virus, en muestras de tracto digestivo superior de las moscas expuestas. No se detectó virus en el tracto digestivo superior de las moscas no inoculadas en ninguna de las muestras obtenidas de este grupo. Tampoco se detectó el virus en ninguna de las muestras de intestinos obtenidas a partir de los grupos expuestos ni de los grupos control. La capacidad de las moscas para transmitir el virus en forma directa a pavos jóvenes se determinó mediante la exposición de pavos de 7 días de edad a las moscas infectadas con el virus, 3 horas después de la exposición inicial de las moscas al coronavirus. Se detectaron antígenos virales en 3 de 12 de los pavos expuestos a las moscas infectadas 3 días después de la exposición, en el grupo experimental donde la densidad de la exposición fue de una mosca por ave. Se observaron niveles de infección más elevados en los grupos de pavos expuestos a densidades de moscas más altas (los antígenos virales se detectaron en 9 de 12 de los pavos del grupo expuesto a una densidad de 10 moscas por ave). Este estudio demuestra el potencial de la mosca doméstica como vector mecánico en la transmisión del coronavirus de los pavos.

Key words: turkey coronavirus, housefly

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Turkey coronavirus (TCV) is the cause of an acute, highly contagious enteric disease of turkeys (11). In recent years, TCV has been increasingly recognized in North America as an important cause of enteric disease in young turkeys, resulting in economic loss due to impaired growth and poor feed conversion. The virus also has been associated as a cause of poult enteritis and mortality syndrome, a disease characterized by high mortality, severe growth depression, and immune dysfunction (2).

Prevention and control of TCV in TCV-endemic areas has proven to be difficult. Outbreaks of TCV in Minnesota spanned two decades before strictly adhered-to depopulation and decontamination requirements eventually eliminated the disease (11). The movement of personnel, vehicles, and equipment was recognized as a contributing factor in the spread of the virus (11). During recent TCV outbreaks in North Carolina, implemented biosecurity measures failed to prevent spread of TCV from infected farms, suggesting a potential vector. Additionally, the incidence of TCV infection in North Carolina turkey flocks increased dramatically during summer months, coinciding with increased fly populations during these months (pers. obs.).

The housefly, Musca domestica (L.), is a common poultry farm pest (1). Houseflies reproduce by depositing eggs in a wide variety of organic substances including manure, mixtures of manure and bedding, and spoiled feeds. The growth and development of the fly are temperature dependent, but, in general, the flies complete their life cycle in as little as 7–10 days during the summer. Adult houseflies subsist on semisolid and liquid diets obtained from the environment. Manure and excreted body fluids provide the housefly with much of the nutrition required to sustain the insect’s life. Upon encountering such materials on the poultry farm, the adult fly, using sponging mouthparts, consumes fluids that are stored temporally in the insect crop (diverticulum). To help liquefy solid foods before ingestion, the fly may regurgitate crop contents onto foods and surfaces (16). The food mixture then passes from the crop into the insect midgut (stomach) and is digested over the next 5 hr (10). Similarly, flies frequently defecate while feeding and resting on surfaces. As a result of such feeding habits, the housefly has been implicated in the transmission of over 30 bacterial, protozoan, and viral diseases including transmissible gastroenteritis virus, a coronavirus of pigs (6,7,8). The purpose of the present study was to determine whether flies could serve as vectors of TCV.

**MATERIALS AND METHODS**

**Virus.** TCV (NC95) was propagated in embryonated turkey eggs as described (9). An inoculum was prepared by amniotic inoculation of 21-day-old embryonated turkey eggs with TCV (NC95) at the 15th embryo passage. At 3 days postinoculation (PI), embryo intestines were harvested and prepared as a 10% suspension in Dulbecco minimal essential medium (DMEM) (Sigma Chemical Co., St. Louis, MO). The suspension was homogenized and clarified by centrifugation for 10 min at 1200 × g. Virus was titrated by inoculation of 10-fold dilutions into each of three 22-day-old embryonated turkey eggs with examination of individual embryo intestines on day 3 PI by indirect immunofluorescence (FA); virus titer was calculated by the method of Reed and Muench (12). An inoculum was prepared to contain approximately 5 × 10⁴ 50% embryo infectious dose/0.1 ml and stored at −70 C.

**Flies.** Houseflies were obtained from a colony maintained at the Department of Entomology, North Carolina State University. All flies used in this study were 5 days old.

**Turkeys.** Commercial medium white turkeys were obtained at 1 day of age from a primary breeder company (British United Turkeys of America, Lewisburg, WV). Turkeys were fed nonmedicated game bird starter (Granville Milling, Creedmoor, NC). Feed and water were provided ad libitum. Turkeys were reared to 7 days of age in electrically heated brooders.

**Experimental design.** Three experiments were conducted to examine survival of TCV in alimentary tract tissues, crops, and intestines (mid- and hindgut) of flies after exposure to TCV and ability of TCV-exposed flies to transmit the virus to susceptible turkeys. In each experiment, 5-day-old flies of mixed sex were used. Flies were held for 18 hr at 22 C in screened containers without food or water, then anes-
thetized by placement in a −20°C freezer for 5 min. Small plastic reservoirs containing approximately 1500 μl of TCV inoculum (TCV exposed) or DMEM (sham exposed) were placed in cages with flies. Flies consumed the TCV inoculum or DMEM within 10 min after recovery from cold-induced anesthesia.

**Experiment 1.** Survival of TCV in fly crops after TCV exposure was evaluated. Flies were separated into two groups of 240 flies each. At 0.5, 1, 3, 6, 9, and 12 hr postexposure, 40 flies were removed from each group. These flies were placed in sterile petri dishes and held on ice. Whole crops were aseptically removed from flies; placed in chilled (4°C) DMEM containing 1% fetal bovine serum, 0.15 mg/ml gentamicin, and 5 μg/ml amphotericin B; and stored at −70°C. Dissection instruments were sterilized after each dissection. The presence of TCV in pooled fly crops was determined by inoculation of embryonated turkey eggs as described (4). Briefly, pooled fly crops were removed from frozen storage and clarified by centrifugation at 1500 × g for 20 min at 4°C, then two 21- to 24-day-old embryonated turkey eggs were each inoculated with 0.2 ml of sample. Three days PI, embryo intestines were collected and examined for presence of TCV antigens by an indirect immunofluorescent antibody technique (IFAT) as described (9). The experiment was replicated three times.

**Experiment 2.** Survival of TCV in fly intestines after TCV exposure was evaluated. Flies were separated into two groups of 240 flies each; flies were fed TCV inoculum or DMEM as described above. Fly intestinal tissues, including mid- and hindguts, were removed at 6, 9, 12, and 24 hr postfeeding from TCV- and sham-exposed flies. Intestinal tissues were aseptically removed from the fly by excising the terminal abdominal plate and gently pulling the intestinal tract through the opening. Fly intestinal contents and tissues were placed in chilled (4°C) DMEM containing antibiotics, homogenized by vortex mixer, and frozen at −70°C. Fly intestinal tissues were evaluated for presence of TCV as described above. The experiment was replicated three times.

**Experiment 3.** The potential of the housefly to directly transmit TCV to live turkey pouls was evaluated. Seven-day-old turkeys were randomly allocated to eight groups of 15 birds each and placed in Horsfall isolation units with negative-pressure ventilation. Birds were exposed to fly densities of zero (control), 1, 10, and 100 flies/bird; each of the four treatments was done in duplicate. Flies selected for the study were separated into two groups of 15 flies, two groups of 150, and two groups of 1500. Flies were exposed to TCV inoculum as described above; 3 hr after consumption of TCV inoculum, flies were released into isolation units with turkeys. One day after the flies were released into isolation units, 0.30% pyrethrin insecticide synergized with 2.40% piperonyl butoxide (CB-38 Extra®; Waterbury Companies Inc., Waterbury, CT) was sprayed through the fresh air intake to kill the flies. Dead flies were removed from the chambers.

Three days after fly challenge, six birds were selected randomly from each treatment group. These birds were humanely euthanatized and necropsied; tissues (ileum and bursa of Fabricius) were collected and examined for presence of TCV antibodies by indirect FA as described (4). At 21 days after fly challenge, the remaining birds were tested for TCV-specific antibodies.

**Serology.** TCV-specific antibody was detected in turkey sera by indirect FA. Antigen for the indirect FA procedure consisted of epithelial cells exfoliated from the bursae of Fabricius of experimentally infected turkeys (5). TCV-infected epithelial cells were spotted onto glass microscope slides, air dried, and fixed in cold (4°C) absolute acetone for 10 min. Sera were diluted 1:20 in phosphate-buffered saline (PBS), 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.

**RESULTS**

**Experiment 1.** Flies readily consumed TCV inoculum after overnight water and feed deprivation. In a preliminary experiment, flies consumed approximately 0.002 ml of inoculum after overnight water and feed deprivation on the basis of weight of dissected crops immediately after flies imbibed inoculum. TCV was detected by virus isolation in dissected fly crops from TCV-exposed flies for up to 9 hr postexposure (Table 1). No virus was detected in dissected fly crops from sham-inoculated flies.

**Experiment 2.** TCV was not detected by virus isolation in dissected intestines, including mid- and hindgut tissues, of TCV-exposed flies at any time postexposure (data not shown). No virus was detected in dissected intestines collected from sham-exposed flies.

**Experiment 3.** Seven-day-old turkey pouls placed in contact with TCV-exposed flies responded attentively to their presence, and pouls caught and ate flies in isolation units containing high densities of flies (10 and 100 flies/bird). TCV infection was detected in all groups of turkeys placed in contact with fly.
densities of 100 flies/bird, and 10 flies/bird but in only one of two groups placed in contact with fly densities of one fly/bird (Table 2). At 3 days post fly contact, TCV infection was demonstrated in three of six (50%) turkeys (replicate A) placed in contact with fly densities of one fly/bird on the basis of detection of TCV antigens in bursa of Fabricius and ileum. In this same group, serologic evidence of TCV infection was demonstrated in seven of eight (87%) turkeys at 14 days post fly contact. Increased rates of TCV infection were observed with higher fly densities; TCV antigens were detected at 3 days post fly contact 67%–83% and 100% of turkeys after contact with fly densities of 10 flies/bird and 100 flies/bird, respectively.

**DISCUSSION**

The present study demonstrates the potential of the housefly to harbor and support TCV transmission. There was no evidence in our study of TCV propagation in exposed flies, indicating that the housefly functions strictly as a mechanical vector. TCV remained viable in the crop of the housefly for up to 9 hr after a single feeding but could not be detected in dissected intestines. These findings indicate that the fly crop provides a relatively hospitable environment for survival of TCV and potentiates mechanical transmission of the virus.

Although the fly crop provided time-limited protection for TCV, our study suggests that the virus was readily inactivated in the gut of the fly. Adult houseflies produce a variety of digestive enzymes including amylase, alpha glucosidases, alpha galactosidase, beta glucosidase, beta fructosidases, pepsin, trypsin, chymosin, peptidases, dipeptidases, and lysozymes (13,14). The presence of these digestive enzymes likely contributed to the inactivation of TCV in the gut of the fly.

Transmission of TCV to turkeys by TCV-exposed flies was demonstrated at various fly densities. Turkey poult exposed to TCV-exposed flies at fly densities of 1, 10, and 100 flies/bird became infected with TCV. A dose-dependent effect was seen when birds were examined at 3 days post fly contact for presence of TCV antigens in bursa of Fabricius and ileum. These results suggested that as fly density rises, there is a greater likelihood for disease transmission. The dose-dependent effect was

### Table 1. Expt. 1. Detection of TCV in crops of flies after experimental exposure in virus-containing media.

<table>
<thead>
<tr>
<th>Hours postexposure</th>
<th>A</th>
<th>0.5</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 1                |+|+|+|+|+|+|-
| 2                | |+|+|+|+|+|-
| 3                | |+|+|+|+|+|-

*Flies were exposed to TCV by allowing them to imbibe a TCV inoculum containing approximately $1.5 \times 10^6$ 50% egg infectious dose/ml. At selected times postexposure, crops were removed from euthanatized flies and examined for presence of TCV by virus isolation. TCV was not detected in crops of sham-exposed flies collected at the same times postexposure.

$^a$+ = TCV isolated from crop; − = no virus isolated.

### Table 2. TCV detection in turkeys placed in contact with TCV-exposed houseflies.

<table>
<thead>
<tr>
<th>Fly treatment (no. flies/bird)</th>
<th>Replica</th>
<th>TCV detection: no. positive/no. tested (% positive)</th>
<th>TCV antigens $^a$</th>
<th>Seroconversion $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-exposed flies (100 flies/bird)</td>
<td>A</td>
<td>0/6 (0%)</td>
<td>0/9 (0%)</td>
<td></td>
</tr>
<tr>
<td>TCV-exposed flies (1 fly/bird)</td>
<td>B</td>
<td>3/6 (50%)</td>
<td>7/8 (87%)</td>
<td></td>
</tr>
<tr>
<td>TCV-exposed flies (10 flies/bird)</td>
<td>A</td>
<td>4/6 (67%)</td>
<td>6/8 (75%)</td>
<td></td>
</tr>
<tr>
<td>TCV-exposed flies (100 flies/bird)</td>
<td>B</td>
<td>5/6 (83%)</td>
<td>9/9 (100%)</td>
<td></td>
</tr>
<tr>
<td>TCV-exposed flies (100 flies/bird)</td>
<td>A</td>
<td>1/1 (100%)</td>
<td>7/8 (87%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6/6 (100%)</td>
<td>6/6 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$TCV antigens detected by IFAT with TCV-specific monoclonal antibody 3 days after commingling with TCV-exposed flies.

$^a$TCV-specific antibodies detected by IFAT 14 days after commingling with TCV-exposed flies.
lost, however, by 14 days post fly contact when infection was detected on the basis of serology. This finding was expected because of the potential of TCV to spread from bird to bird, independent of the housefly.

Whereas mechanical transmission of TCV by flies was demonstrated in the present study, the exact mechanism whereby TCV was transmitted to turkeys was not determined. Consumption of TCV-exposed flies was evident in isolation units containing high densities of flies (10 and 100 flies/bird) and this is a likely method for transmission of TCV from flies to turkeys. However, TCV transmission also may have occurred by contamination of feed, feeders, water, and drinkers by flies. Further study is needed to determine the relative importance of these different modes of transmission.

The relative risk of insects contributing to a TCV epizootic depends on the habits and mobility of the insect (15). The housefly is found on farms resting on the walls, drinkers, and feeders. Flies are attracted to manure and dead birds; thus they have ample opportunity for exposure to TCV and other avian pathogens. The flies annoy birds with their activities and often are eaten. More importantly, the potential of the fly to disperse from the farm is great. Bishop and Laake (3) reported that flies are capable of dispersing up to 20 km. Greenberg (8) reported houseflies were capable of flights ranging from 2.3 to 11.8 km within 24 hr. Dispersal of houseflies from an infected farm is an important biosecurity concern for TCV and other avian diseases.

The findings of the present study indicate that houseflies may be important mechanical vectors of TCV. As a consequence, effective control of TCV in TCV-endemic areas is dependent upon managing fly populations via litter management, proper dead bird disposal, and use of insecticides.

REFERENCES


