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Specific-pathogen-free Turkey model for reoviral arthritis

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ABSTRACT

Turkey arthritis reovirus (TARV) infections have been recognized since 2011 to cause disease and significant economic losses to the U.S. turkey industry. Reoviral arthritis has been reproduced in commercial-origin turkeys. However, determination of pathogenesis or vaccine efficacy in these turkeys can be complicated by enteric reovirus strains and other pathogens that ubiquitously exist at subclinical levels among commercial turkey flocks. In this study, turkeys from a specific-pathogen-free (SPF) flock were evaluated for use as a turkey reoviral arthritis model. One-day-old or 1-week-old poults were orally inoculated with TARV (O'Neil strain) and monitored for disease onset and progression. A gut isolate of turkey reovirus (MN1 strain) was also tested for comparison. Disease was observed only in TARV-infected birds. Features of reoviral arthritis in SPF turkeys included swelling of hock joints, tenosynovitis, distal tibiotarsal cartilage erosion, and gait defects (lameness). Moreover, TARV infection resulted in a significant depression of body weights during the early times post-infection. Age-dependent susceptibility to TARV infection was unclear. TARV was transmitted to all sentinel birds, which manifested high levels of tenosynovitis and tibiotarsal cartilage erosion. Simulation of stressful conditions by dexamethasone treatment did not affect the viral load or exacerbate the disease. Collectively, the clinical and pathological features of reoviral arthritis in the SPF turkey model generally resembled those induced in commercial turkeys under field and/or experimental conditions. The SPF turkey reoviral arthritis model will be instrumental in evaluation of TARV pathogenesis and reoviral vaccine efficacy.

1. Introduction

Avian reoviruses are ubiquitously present in clinically normal commercial poultry flocks (Jones, 2000, 2013; Pantin-Jackwood et al., 2008; Porter, 2018). However, some reovirus strains are associated with disease outbreaks in poultry farms. In chickens, reoviruses have been isolated from a variety of tissues and organs affected by an assortment of disease conditions including viral arthritis/tenosynovitis, enteric disease and stunting syndrome (Jones, 2013). Reoviruses have been implicated in several enteric diseases in turkeys (Heggen-Peay et al., 2002; Jindal et al., 2010, 2009; Mor et al., 2013a; Woolcock and Shivaprasad, 2008) and their ability to induce immune suppression (Day et al., 2008; Pantin-Jackwood et al., 2007a; Spackman et al., 2005) can increase susceptibility to other viral, bacterial and parasitic diseases, and interfere with vaccine efficacy (Hoerr, 2010). Turkey arthritis reoviruses (TARVs) have been recognized in the U.S. since 2011 to cause disease in market age turkeys (Lu et al., 2015; Mor et al., 2014; Tang et al., 2015). The disease has continued to induce economic losses in turkey producing states as a result of excessive culling, diminished carcass quality and reduced market weights (Lu et al., 2015; Porter, 2018).

Field cases of reoviral arthritis in turkeys are characterized by lameness associated with hock (tibiotarsal) joint swelling in one or both legs, excessive fluid in hock joints and tendon sheaths, periarticular fibrosis, tenosynovitis, occasional articular cartilage erosion on distal tibiotarsus and gastrocnemius or digital flexor tendon rupture (Mor et al., 2013b; Porter, 2018). Experimental reproduction of these features in commercial-origin turkeys, in the absence of other known arthritis-causing agents, has confirmed TARV as a causative agent of arthritis (Sharafeldin et al., 2014, 2015a; Sharafeldin et al., 2015b). After oral inoculation in experimental turkeys, TARV replicated early and to high levels in the intestines and cloacal bursa (bursa of Fabricius) before translocating to other organs (such as liver, spleen, and kidney) and tendons (Sharafeldin et al., 2015b). Virus from inoculated turkeys

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is shed in feces (Sharafeldin et al., 2015b) and transmitted to contact birds at high rates (Sharafeldin et al., 2014). As of yet there is no published epidemiologic data or scientific study to demonstrate vertical transmission of TARV from breeder flock to poults through contaminated eggs. However, it is likely for TARV to be transmitted vertically, similarly to chicken reoviruses (Al-Muffarej et al., 1996; Menendez et al., 1975). For this reason, the turkey industry is vaccinating breeder flocks with autogenous vaccines containing TARV strains that are identified as antigenically unique through virus cross-neutralization analysis performed at private entities. The information gained is generally proprietary and the turkey industry has not shared or published information on the efficacy of these vaccines. Despite these vaccination efforts, TARV cases continue to be reported (Porter, 2018).

TARV pathogenesis appears to be restricted to turkeys. For example, although the O'Neil strain is highly arthrotropic in both turkeys and chickens (Sharafeldin et al., 2014, 2015a; Sharafeldin et al., 2015b, c), it only produces clinical signs in turkeys (Sharafeldin et al., 2014, 2015a; Sharafeldin et al., 2015b) but not in chickens (Sharafeldin et al., 2015c). The immunologic mechanisms of TARV pathogenesis are largely unknown. In the intestines of commercial-origin poults, IFN-α/β cytokine gene upregulation corresponded with a remarkable reduction of TARV replication, highlighting a possible role of innate immune responses in controlling viremia and tendon infection (Sharafeldin et al., 2015b). In the leg tendons, upregulation of IL-6 and IFN-γ cytokine gene expression corresponded with lymphocytic infiltration in gastrocnemius tendon sheath, indicating these cytokines may be involved in the development of tenosynovitis (Sharafeldin et al., 2015b). Our understanding of TARV pathogenesis can be enhanced through multi-omic research approaches using appropriate experimental models.

All TARVs are grouped into two lineages based on phylogenetic analysis of S2, S3 and S4 genome segments and one lineage based on S1 genome segment (Mor et al., 2014). Since TARVs also cluster with some isolates from enteric samples (Mor et al., 2014), and even though emerging evidence suggests TARV may be differentiated based on the M2 (μB) outer capsid protein (Kumar et al., 2018; Markis et al., 2018), it is probable that TARVs are unusual mutants of the seemingly non-pathogenic enteric reoviruses that are ubiquitously present in flocks of commercial poultry (Jones, 2000, 2008; Pantin-Jackwood et al., 2008; Porter, 2018). However, TARVs have continued to undergo rapid genetic and antigenic diversification as recent isolates have shown low cross-neutralization with the viruses isolated in 2011 (Markis et al., 2015). Consequently, the effectiveness of autogenous vaccines is reduced (Porter, 2018). The pathogenesis and immune escape mechanisms of these viruses need to be investigated through controlled experiments in an appropriate turkey arthritis model that is free of the several confounding factors associated with field conditions. Note that commercial-origin chickens may be infected with nonpathogenic reovirus strains vertically via infected eggs (Al-Muffarej et al., 1996; Menendez et al., 1975) and/or horizontally through feces or aerosolized virus (Jones and Georgiou, 1984; Macdonald et al., 1978; Roessler and Rosenberger, 1989); thus, it is likely that the same phenomenon can occur in turkeys. These birds may also be co-infected at subclinical levels with several viral and bacterial pathogens (Barnes et al., 1982; Johnson et al., 2018; Ngunjiri et al., 2019; Pantin-Jackwood et al., 2008, 2007b).

In this study, turkeys from a reovirus-negative specific-pathogen-free (SPF) research flock were investigated for use as a turkey reoviral arthritis model to support TARV pathogenesis and vaccine efficacy studies. Overall, the clinical and pathological features of reoviral arthritis produced in the SPF turkey model were similar to those observed in commercial turkeys under field and experimental conditions. The SPF turkey reoviral arthritis model will be useful for unequivocal determination of TARV pathogenesis and evaluation of reoviral vaccine efficacy.

2. Materials and methods

2.1. Turkeys and ethics statement

Specific pathogen free (SPF) turkeys were obtained from the Food Animal Health Research Program (OARDC, OSU) flock, which is maintained free from known turkey pathogens including reovirus, avian encephalomyelitis virus, infectious bursal disease virus, infectious bronchitis virus, Newcastle disease virus, avian influenza virus, hemorrhagic enteritis virus, *Mycoplasma gallisepticum*, *M. synoviae*, *M. meleagridis, Bordetella avium* and *Salmonella pullorum*. The flock consists of random-bred turkeys (about 10 generations) that were originally derived from a line selected for egg production (about 50 generations). The animals were maintained, inoculated and euthanized in accordance with protocol #2011A00000109-R2 approved by The Ohio State University Institutional Animal Care and Use Committee. The turkeys were housed in a BSL2 facility with forced air ventilation and adequate air exchanges to prevent ammonia build up. Air entering or leaving the facility is HEPA filtered. The birds were raised on floor with wood shavings litter. Room temperatures were maintained at 25 ± 3 °C. Birds had *ad libitum* access to feed and water. The wellbeing and health status of the animals was monitored twice daily throughout the experiments. Animals were humanely euthanized by carbon dioxide (CO2) exposure when they displayed symptoms such as ruffled feathers and reluctance to move, not moving when prodded, respiratory distress, or injuries that were not related to experimental treatment. Humane euthanasia was also applied to all animals before tendon and hock joint collection.

2.2. Viruses

TARV (O'Neil strain) isolated from leg tendons of lame turkeys in Minnesota was kindly provided by Dr. Jack Rosenberger (AviServe LLC, Newark, Delaware). The MN1 strain of reovirus was isolated from feces of turkeys with enteritis at the Minnesota Veterinary Diagnostic Laboratory and kindly provided by Dr. Sagar Goyal (University of Minnesota, St. Paul, Minnesota). Although these strains are phylogenetically more closely related to other turkey reoviruses than to chicken, duck, and goose reoviruses, they display genomic differences based on the sequences of the S class gene segments (Mor et al., 2014). The viruses were propagated and titrated on Japanese quail fibrosarcoma cell line (QT-35). Determination of $TCID_{50}$ (median Tissue Culture Infectious Dose) titer of each stock was performed in 96-well plates following the Reed-Muench method (Reed and Muench, 1938).

2.3. Detection of virus in samples

Reovirus was detected from tendon and intestinal tissue homogenates through a combination of a previously published RT-PCR of the S4 gene segment (Pantin-Jackwood et al., 2008) and isolation in QT-35 cells, or a quantitative RT-PCR (qRT-PCR) of a 145 bp fragment of the S4 segment. RNA was extracted using RNeasy Mini Kit (Qiagen Sciences Inc., Germantown, MD). The qRT-PCR was performed with the following primers: Forward 5′-CATGATGGCGGCTCAACT-3′ and Reverse 5′- CATCAGCTCACGATCAATAGG-3′. Reactions were performed in Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) using the QuantiTect SYBR Green PCR Kit (Qiagen Sciences Inc., Germantown, MD). The cycling parameters were: 50 °C for 30 min; 95 °C for 15 min; 40 cycles of 94 °C for 15 s, 61 °C for 60 s, and 72 °C for 45 s; 95 °C for 15 s, 60 °C for 60 s, 95 °C for 15 s, and 60 °C for 60 s. A standard curve was created by plotting cycle threshold (Ct) values generated with RNA extracted from serial 10-fold dilutions of the same virus stock used to inoculate the poults as a function of virus dilution. The curve was used to convert Ct values of tissue homogenate viral RNA to EID_{50} equivalent titers.

2.4. Serology

Levels of reovirus antibodies were measured before and after infection by ELISA (IDEXX, Westbrook, Maine, USA) at the Animal Disease Diagnostic Lab, Ohio Department of Agriculture. Although the ELISA assay was developed for detection of chicken IgG, and has had variable results in turkeys depending on strains (our observations), it has been consistently sensitive in detecting serum antibodies induced by the strains used in this study (our unpublished data and data presented herein).

2.5. Histologic inflammation scoring

Formalin-fixed and decalcified hock joints with attached tendons were sent to the Comparative Pathology and Mouse Phenotyping facility at The Ohio State University for H&E slide preparation. The slides were read by a pathologist who was blinded to the grouping and the infection status of individual birds. Inflammation of the intertarsal (gastrocnemius) tendon complex and sheaths was measured by a previously described scoring system (Sharafeldin et al., 2015a).

2.6. Gait scoring and lameness quantification

The six-point gait scoring system for turkeys was followed as previously described (Sharafeldin et al., 2015a). This system is based on the following clinical signs: inability to flex legs, shaking legs, reluctance in movement, staggered movement, inability to stand and walk, dropped keel bone, valgus or varus, and hock joint (tendon) swelling. Each bird was observed separately from other birds by 3 personnel who gave independent scores for each clinical sign. These scores were averaged to obtain the final gait score for each bird.

2.7. Experimental design to assess reoviral arthritis in SPF turkeys inoculated at 1 week of age

1-week-old poults were placed in three groups in separate isolation rooms as follows: Mock (n = 26), TARV (n = 24), and MN1 (n = 24). At 1 week of age (WOA), birds in the Mock group were orally inoculated with growth medium while those in TARV and MN1 groups were inoculated with TARV O'Neil and the enteric MN1 virus, respectively. The maximum available inoculation doses were $10^{6.7}$ and $10^{5.7}$ TCID50 in a 200 µL volume per bird for TARV and MN1 viruses, respectively. Eight birds per group were euthanized at 7, 28 days post-infection (dpi) to collect samples for serology, histopathology, and virus detection. All the remaining birds were euthanized at 63 dpi. Formalin-fixed hock joints were decalcified prior to histopathological slide preparation.

2.8. Experimental design to evaluate factors associated with reoviral arthritis in SPF turkeys

1-day-old poults were placed in six groups: Mock (n = 21) and TARV-1day (n = 17 inoculated); and TARV-1wk, TARV-1wk + DEX, MN1:1wk, and MN1:1wk + DEX (n = 18 inoculated + 6 contacts per group). The virus inoculum was standardized to $10^{7.7}$ TCID50 per bird for both viruses. Poults in TARV-1day were inoculated with TARV at 1 day of age. The other groups were inoculated at 1 WOA: Mock group was given growth medium; TARV-1wk and TARV-1wk + DEX groups were given TARV; MN1:1wk and MN1:1wk + DEX groups were inoculated with MN1 virus. The virus infection was allowed to establish in the inoculated birds for 24 h before placing contact (sentinel) birds in TARV-1wk, TARV-1wk + DEX, MN1:1wk, and MN1:1wk + DEX groups. Groups indicated with DEX suffix were intramuscularly administered with 3 doses of dexamethasone (DEX) (2 mg/kg body weight) on alternating days to simulate stress prior to infection with TARV or MN1 viruses (Ali et al., 2013). Live body weights of all groups were taken at 0, 2, 3, 4, and 7 WOA, which corresponded with 0, 1–2, 2–3, 3–4, and 6–7 weeks post-infection (WPI), respectively. Cloacal swabs were taken from six randomly-selected birds at 4, 7, and 14 dpi to assess virus shedding and transmission to the sentinel birds. Four to six birds per group were euthanized at 5 WOA while the remaining birds are euthanized at 17 WOA.

2.9. Statistical analysis

Statistical comparisons involving only 2 groups were performed using Unpaired *t*-test, whereas comparisons involving 3 or more groups were performed using One-way analysis of variance (ANOVA) followed by post-hoc Tukey test (GraphPad Software, San Diego California USA). Statistical significance was determined at p value < 0.05.

3. Results

3.1. Assessment of reoviral arthritis in SPF turkeys inoculated at 1 week of age

This trial was conducted to assess virus tropism, immune responses, gross and histologic lesions, and clinical disease in SPF turkeys inoculated at 1 week of age as previously demonstrated in commercial-origin turkeys of the same age (Sharafeldin et al., 2014, 2015c). Procedures utilized in the commercial turkey studies were broadly followed except that the SPF birds were housed in isolation rooms with wood shavings litter. Following oral inoculation, 8 birds per group were euthanized at 7, 28 and 63 days post-infection for histopathology and virus detection in tendons and intestines. None of the birds died of reovirus infection or other unrelated causes.

3.1.1. TARV is more arthrotropic and induces higher humoral immune responses

Following inoculation through the oral route, TARV replicates in the gastrointestinal tract and migrates to tendons and other body sites (Sharafeldin et al., 2015b). Tendon and intestinal samples were initially tested for the presence of reovirus through RT-PCR of an 1120 bp fragment of the S4 gene segment (Pantin-Jackwood et al., 2008). We suspected that reovirus titer was too low for detection in some samples and may not be detected by RT-PCR that amplifies a long size of S4 gene fragment. Accordingly, all tendon samples from the infected groups were subjected to 2–5 blind passages in QT-35 cells to isolate the virus. The RT-PCR did not result in higher detection of positive samples than the virus isolation method (Table 1), likely because the RT-PCR was designed to amplify a large fragment of the S4 gene segment (Pantin-Jackwood et al., 2008). Clear differences in reovirus detection in gastrocnemius tendons were observed between the inoculated groups at 7 and 28 days post-infection (dpi). At 7 dpi, the TARV:1wk group showed a higher rate of detection (5 out of 8) compared to the MN1:1wk group

Table 1
Detection and re-isolation of reovirus from tendons of birds.

Group[a]	Virus detection					
	7 dpi		28 dpi		63 dpi	
	RT-PCR[b]	Isolation	RT-PCR	Isolation	RT-PCR	Isolation
Mock	0/4	ND	0/4	ND	0/4	ND
TARV:1wk	1/8	5/8	7/8	8/8	2/8	5/8
MN1:1wk	1/8	2/8	2/8	2/8	2/8	6/8

ND, not determined.
[a] All groups were inoculated orally with growth medium (Mock) or reovirus (TARV:1wk and MN1:1w) groups] at 1 week of age.
[b] RT-PCR of S4 gene: an 1120 bp product was amplified using a previously published primer set as described in the Materials and Methods section.

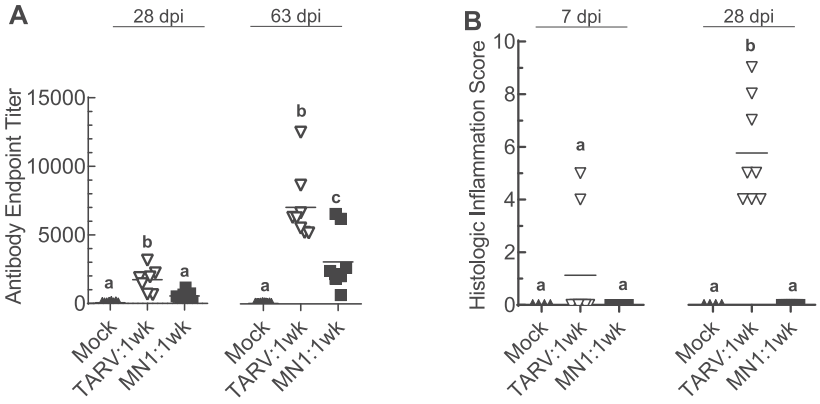

Fig. 1. Serum IgG antibody responses and histologic inflammation of tendon sheaths. Birds were inoculated with growth medium (Mock) or reovirus (TARV:1wk and MN1:1wk groups) at 1 week of age. A. ELISA endpoint titers. B. Histologic inflammation scores. dpi, days post infection. 7, 28 and 63 dpi corresponded with 2, 5 and 10 weeks of age, respectively. Statistical comparisons were performed separately for each age. Different letters indicate statistically significant differences between groups (ANOVA, $p < 0.05$).

(2 out of 8). At 28 dpi, reovirus was detected in tendons of all 8 poults from the TARV:1wk group, but only from 2 poults in the MN1:1wk group. However, both viruses were detected at similar rates at 63 dpi (Table 1). The re-isolated viruses were identical with the inoculated strains based on the consensus sequences of the S4 gene. Our attempt to detect reovirus from intestinal samples of the inoculated groups by RT-PCR was unsuccessful and the cell culture method was not applied to these samples. As expected, the virus was not detected in tendons or intestines of mock-infected birds.

Turkey reovirus infection via the gastrointestinal route and spread to tendons and other body sites/organs is associated with induction of humoral immune responses (Nersessian et al., 1985). Hence, reovirus infection was monitored by measuring serum IgG antibody titers at 28 and 63 dpi. The Mock group did not have detectable levels of avian reovirus-specific IgG antibodies at both sampling time points (Fig. 1A). All the inoculated birds, on the other hand, displayed high reovirus IgG titers at both sampling time points, with the TARV:1wk group demonstrating significantly higher mean titers compared to the MN1:1wk group (Fig. 1A).

3.1.2. Histopathologic lesions and body weight depression were observed only in TARV-infected birds while gross lesions and lameness were absent

Field and experimental reoviral arthritis in commercial turkeys is characterized by lameness in addition to gross and histological lesions at the hock joints (Mor et al., 2013b; Porter, 2018; Sharafeldin et al., 2015a). In this trial, no obvious rupture/hemorrhage of digital flexor and gastrocnemius tendons was observed. Histological evidence of lymphocytic tenosynovitis was present in the TARV:1wk group, especially at 5 weeks of age (WOA) when all birds in this group demonstrated high tendon sheath inflammation scores, which were significantly higher compared to Mock and MN1:1wk groups (Fig. 1B). The primary histological changes were lymphoplasmacytic infiltration of the subsynovium of the intertarsal (gastrocnemius) tendon complex and hypertrophy of the synovium. Tenosynovitis was not assessed at 10 WOA. Clinical manifestations of arthritis including lameness or swollen hock joints were not observed in any bird for the entire duration of the experiment.

Since TARV was previously shown to cause significant weight gain depression in commercial-origin turkeys (Sharafeldin et al., 2015a), body weights were taken immediately after euthanasia before removing tissues from the birds. Mean body weights were statistically indistinguishable among the groups at 7 and 28 dpi (Table 2). Conversely, birds in the TARV:1wk group were significantly lighter compared to the Mock and MN1:1wk groups at 63 dpi (Table 2).

3.2. Evaluation of factors associated with reoviral arthritis in SPF turkeys

Based on the data generated in the experiment presented above, we were able to codify the two reovirus strains based on differences in tendon sheath inflammation, arthrotropism and replication in tendons,

Table 2
Average body weights of birds.

Group[a]	Average body weight in grams (*n* per group)		
	7 dpi	28 dpi	63 dpi
Mock	183 (8)	706 (8)	2500 (10)
TARV:1wk	191 (8)	698 (4)	2125 (8)[b]
MN1:1wk	171 (8)	696 (8)	2400 (8)

[a] All groups were inoculated orally with growth medium (Mock) or reovirus (TARV:1wk and MN1:1wk) groups] at 1 week of age.
[b] Average body weight was significantly lower compared to Mock ($p < 0.05$).

serum IgG titers and suppression of weight gain (Tables 1 and 2, Fig. 1). In this trial, our focus was on how the reovirus pathotypes are impacted by factors such as bird age at inoculation, stress, and extension of experimental timeline beyond 10 WOA. The viral dose was standardized to $10^{7.7}$ $TCID_{50}$ per bird for both viruses to eliminate a possible influence of unequal inoculum doses on the pathotype differences displayed by the two strains (Tables 1 and 2, Fig. 1) (Sharafeldin et al., 2014). Moreover, the experimental timeline was extended to 17 WOA to demonstrate lameness, dexamethasone was used to simulate field stress and immunosuppression as previously shown with influenza virus and other pathogens (Ali et al., 2013; Huff et al., 1998, 2014; Isobe and Lillehoj, 1993; Thachil et al., 2014), the effect of age at inoculation on the pathogenesis of TARV was demonstrated in 1 day- and 1 week-old poults, contact birds were introduced to evaluate virus transmission, and a qRT-PCR was used for virus detection. Early mortalities (within 3 WOA) were observed as follows: TARV:1day (5/17 inoculated), TARV:1wk (2/18 inoculated), TARV:1wk + DEX (1/6 contacts), MN1:1wk (1/18 inoculated, 1/6 contacts), and Mock (1/21). In addition, some birds from TARV:1day (2 birds), TARV:1wk groups (2 birds), and MN1:1wk + DEX (1 bird) groups were euthanized between 8 and 16 WOA due to development of severe gait defects.

3.2.1. TARV and MN1 were shed from inoculated birds and efficiently transmitted to contacts

Reovirus from inoculated commercial-origin turkeys is shed in feces and transmitted to contact birds at high rates (Sharafeldin et al., 2014, 2015b). Hence, we took cloacal swabs to evaluate the dynamics of virus shedding and transmission in the SPF turkey model. Cloacal swab eluates from all inoculated poults were virus positive at 4 and 7 dpi indicating that reovirus was being shed to the environment through feces (Fig. 2A). Cloacal virus shedding declined over time and reovirus was not shed by most of the poults sampled at 14 dpi. Notably, the mean viral titers observed at 4 dpi were significantly higher in the MN1 groups compared to the TARV groups, independently of bird age during inoculation or dexamethasone treatment. The impact of dexamethasone treatment on virus shedding was minimal and not significant for both

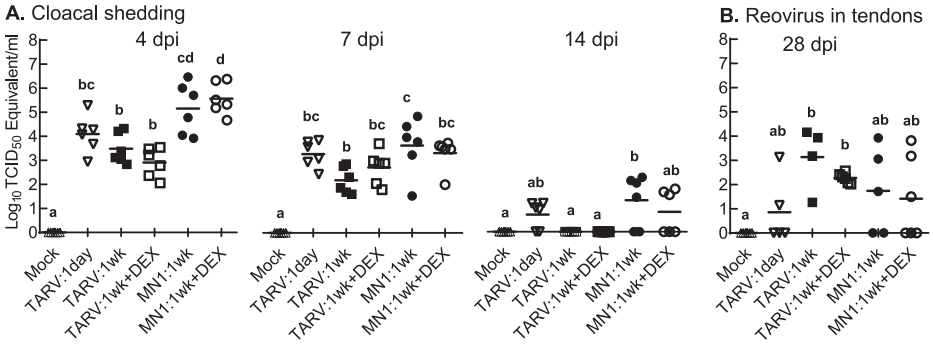

Fig. 2. Cloacal shedding and tendon viremia in inoculated groups. Comparison between viruses with regard to dexamethasone treatment status and age of inoculation. Viral titers were extrapolated from cycle threshold (Ct) values obtained through qRT-PCR of the S4 gene segment as described in the Materials and Methods. Statistical comparisons were performed separately for each age. Different letters indicate statistically significant differences between groups (ANOVA, $p < 0.05$). dpi, days post-infection. DEX, dexamethasone.

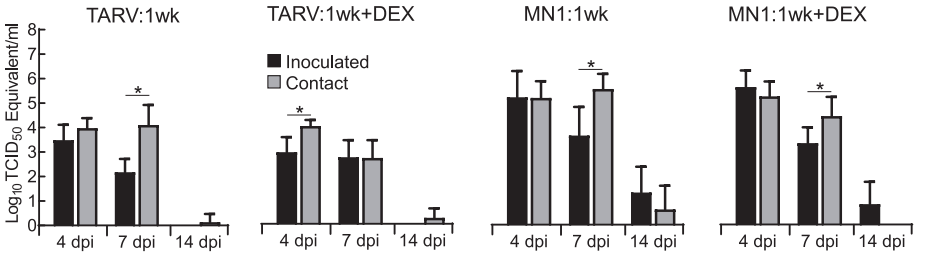

Fig. 3. Virus transmission to contact birds. Comparison of cloacal shedding between inoculated and contacts birds. Viral titers were extrapolated from cycle threshold (Ct) values obtained through qRT-PCR of the S4 gene segment as described in the Materials and Methods. Asterisks indicate statistically significant differences between groups (unpaired t test, $p < 0.05$). dpi, days post infection. DEX, dexamethasone.

viruses. Likewise, inoculation at 1 day or 1 week of age did not significantly affect shedding of TARV. Yet, TARV-1day titer was consistently ˜0.5 log higher than TARV-1wk at all three sampling timepoints.

Fig. 3 compares cloacal shedding between birds inoculated at 1 WOA and contact birds placed in the same room. The virus shed from the inoculated birds was horizontally transmitted to contact birds, which also shed at high rates. For both viruses, the contact birds in both groups (TARV:1wk and MN1:1wk groups) shed significantly higher titers (˜1.5-2 logs higher) at 7 dpi compared to the inoculated roommates. Dexamethasone treatment resulted in similar trends, where the contacts shed significantly higher virus titers (˜1 log higher) than the inoculated roommates at 4 and 7 dpi for TARV:1wk + DEX and MN1:1wk + DEX groups, respectively.

3.2.2. Bird age at inoculation affects TARV detection in tendons at 28 dpi

Some birds were euthanized at 28 dpi to determine whether TARV arthrotropism is affected by inoculation age as has been reported for chicken arthritis reovirus (Jones and Georgiou, 1984; Rosenberger et al., 1989). The two strains were expected to have the biggest difference at that time. In accordance with data presented in Table 1, and despite standardization of the inoculation dose, the TARV strain was detected in tendons of all poults infected at 1 WOA (TARV:1wk and TARV:1wk + DEX groups) independently of dexamethasone treatment (Fig. 2B). Birds inoculated with the TARV strain at 1 day of age (TARV:1day group) did not follow this pattern as only 2 of 5 poults had detectable levels of the virus in their tendons. The rates of virus detection in tendons of MN1-infected groups (MN1:1wk and MN1:1wk + DEX groups) were lower compared to the TARV groups inoculated at the same age (TARV:1wk and TARV:1wk + DEX groups). It is worth noting that only the groups inoculated with the TARV strain at 1 WOA (TARV:1wk and TARV:1wk + DEX groups) had significantly higher mean tendon virus titers compared to Mock. Nonetheless, the average virus titers in tendon homogenates did not differ significantly among the inoculated groups (Fig. 2B).

3.2.3. TARV strain elicited higher serum IgG antibody responses in inoculated and contact birds

Humoral antibody responses were evaluated as a confirmation for successful establishment of the viral infection and transmission in addition to displaying strain-specific differences in antibody titers. As observed in the previous trial (Fig. 1A), both viruses induced detectable levels of serum IgG antibodies as early as 5 WOA (28–35 dpi), but strain-specific differences in mean antibody titers could not be discerned likely due to the small number of birds euthanized at that time point (Fig. 4). Strain-specific trends in serum IgG responses were observed at 17 WOA (112–119 dpi), with TARV-inoculated groups (TARV:1day, TARV:1wk and TARV:1wk + DEX) displaying

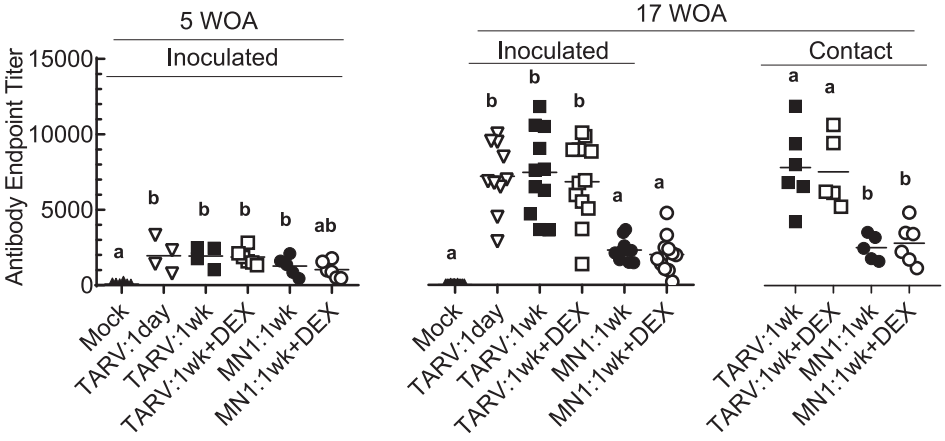

Fig. 4. Serum IgG antibody responses following inoculation and natural transmission. 5 and 17 WOA corresponded with 5 and 17 weeks post infection, respectively, for TARV:1day group and 4 and 16 weeks post infection for all other groups. Statistical comparisons were performed separately for inoculated and contact groups. Different letters indicate statistically significant differences between groups (ANOVA, $p < 0.05$). WOA, weeks of age.

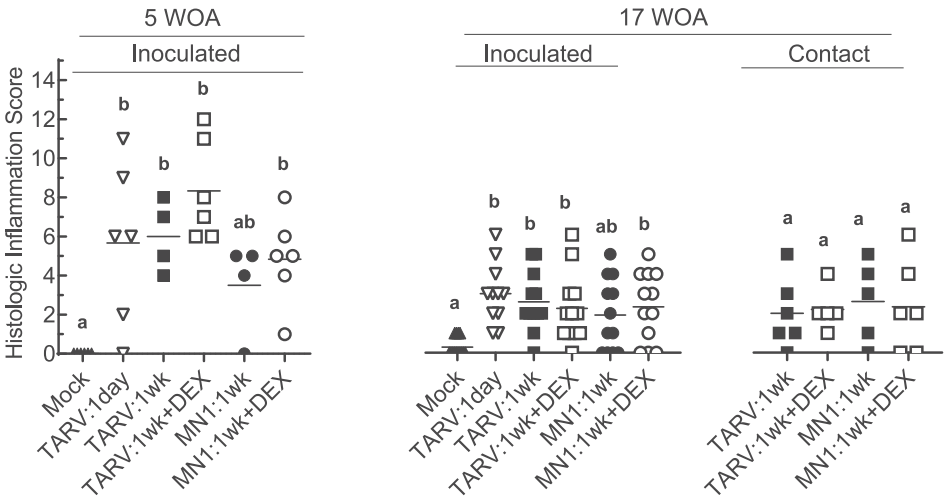

Fig. 5. Histologic inflammation of gastrocnemius tendon sheaths. 5 and 17 WOA corresponded with 5 and 17 weeks post infection, respectively, for TARV:1day group and 4 and 16 weeks post infection for all other groups. Statistical comparisons were performed separately for inoculated and contact groups. Different letters indicate statistically significant differences between groups (ANOVA, $p < 0.05$). WOA, weeks of age. DEX, dexamethasone.

significantly higher mean titers compared to MN1 groups (MN1:1wk and MN1:1wk + DEX). The strain-specific trends in mean IgG titers were reproduced in contact birds. Further, dexamethasone treatment did not significantly affect IgG response to viral infection (Fig. 4).

3.2.4. Gross and histopathologic lesions, weight depression and clinical lameness were generally more pronounced in TARV-infected birds

The intrinsic strain differences in induction of pathologic lesions were expected to be clearer in this trial based on the increased and standardized virus dose together with the extended experimental timeline. While tendon rupture and hemorrhage were not observed, macroscopic pitted erosions were found in the distal articular cartilage of the tibiotarsus at the hock joints of more than 65% of birds in the TARV-infected groups (Fig. 6). This pathologic feature of turkey reoviral arthritis clearly distinguished the TARV strain from the MN1 strain or mock-infected birds (Fig. 6). The histopathological evidence of tenosynovitis associated with TARV infection was observed independently of bird age at infection or dexamethasone treatment (Fig. 5). However, higher scores were generally observed at 5 WOA compared to 17 WOA. Histologic inflammation scores were above zero for most of the inoculated birds in MN1 groups (MN1:1wk and MN1:1wk + DEX) (Fig. 5), which was in stark contrast to the observation made in the previous trial (Fig. 1). These scores were statistically indistinguishable from those of the TARV groups or the Mock. Still, strain-specific signatures were evident when the inoculated groups were compared with the Mock, the TARV groups displayed significantly higher mean scores at both sampling time points while the MN1 groups did not. Dexamethasone treatment did not significantly affect tenosynovitis induction in the inoculated birds. Tenosynovitis was assessed in the contact birds at 17 WOA and no significant differences were observed among all four groups regardless of the infecting strain or dexamethasone treatment status of the inoculated roommates (Fig. 5).

Turkey reovirus infections can induce depression of body weights depending on intrinsic characteristics of the infecting strain and time post-infection (Spackman et al., 2005). We suspected that the apparent lack of weight depression of reovirus-infected birds group at 7 and 28 dpi in the previous trial (Table 2) was partly due to the small number of birds (8 birds per group) compared at each sampling time point. In this trial, live body weights were taken from a larger number of birds (at least 12 birds per group) at 0, 2, 3, 4, and 7 WOA, which corresponded with 0, 1–2, 2–3, 3–4, and 6–7 WPI, respectively, depending on the experimental group. The average body weight of all 1-day-old poults was 42.6 g and there were no significant differences among birds assigned to different groups. Following virus infection, the average body weights of the MN1 groups (MN1:1wk and MN1:1wk + DEX) were statistically indistinguishable from the Mock (Fig. 7). In contrast, significant body weight depression was observed in the TARV groups (TARV:1day, TARV:1wk and TARV:1wk + DEX) relative to the Mock group at 2 and 3 WOA. At the same time, the ability of TARV to induce body weight depression was not significantly affected by bird age at inoculation or dexamethasone treatment. Depression of body weights was reversed over time for most birds. As such, and with exception of

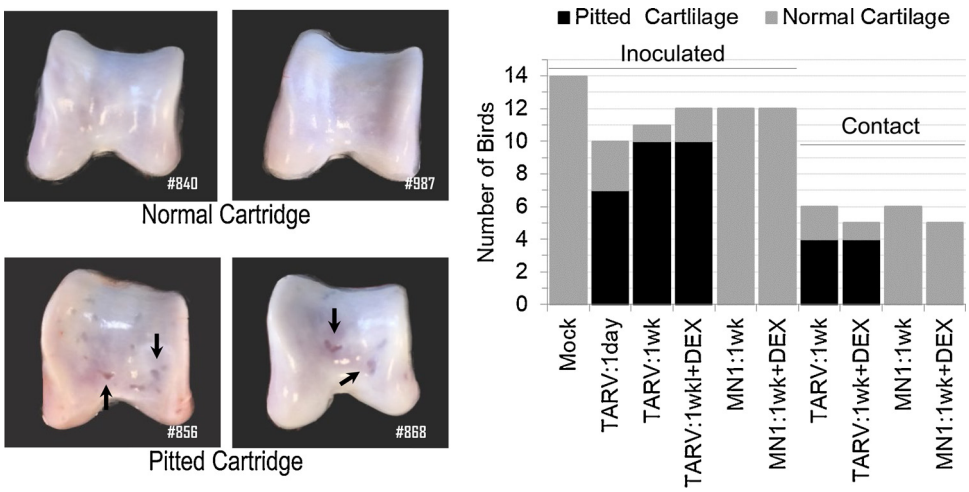

Fig. 6. Pitted erosions in the distal articular cartilage of the tibiotarsus at the hock joint. Cartilage erosions were observed during euthanasia at 17 weeks of age.

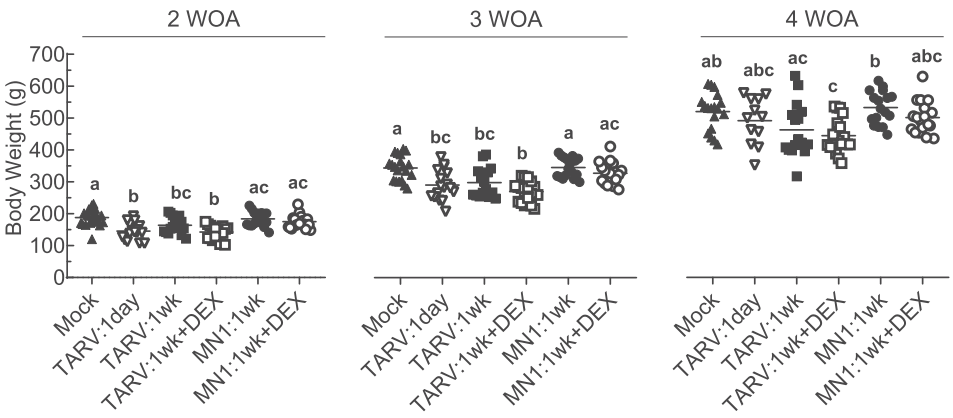

Fig. 7. Change in body weight following reovirus infection. WOA, weeks of age. 2, 3, and 4 WOA corresponded with 2, 3 and 4 weeks post infection, respectively, for TARV:1day group and 1, 2, and 3 weeks post infection for all other groups. Statistical comparisons were performed separately for each age. Different letters indicate statistically significant differences between groups (ANOVA, $p < 0.05$). DEX, dexamethasone.

the TARV:1wk + DEX group, the mean body weights of inoculated birds were similar to the Mock at 4 WOA (Fig. 7). At 7 WOA, the mean body weights for the groups ranged from 921 g to 1051 g but there were no significant differences among groups.

Lameness is a key feature of TARV-associated arthritis that has been reported in commercial turkeys as early as 10–12 weeks of age in the field (Mor et al., 2013b; Porter, 2018) and 8 WPI under experimental conditions (Sharafeldin et al., 2015a). Starting from 8 weeks after infection onward, the TARV-infected groups of SPF turkeys had individuals with various degrees of clinical signs such as severe valgus and swollen hock joints with edema (Fig. 8), which corresponded with gait abnormalities. A previously described six-point (0–5) turkey gait scoring system was used to calculate gait scores for individual birds (Sharafeldin et al., 2015a). Fig. 9 shows that only groups infected with the TARV strain had significantly higher ($p < 0.05$) gait scores (compared to the Mock and MN1-infected groups) and clinical lameness appeared to progress as a function of age as previously reported in commercial turkeys (Sharafeldin et al., 2015a). Early infection (at 1 day of age) or dexamethasone treatment did not affect the number of lame birds or the degree of lameness. Four TARV-infected birds, 2 from the TARV:1day and TARV:1wk groups, respectively, were removed early from the experiment (between 8 to 16 WOA) due to severe lameness. Besides, the contact birds in the TARV:1wk group were starting to show some gait defects by the time the experiment was terminated (Fig. 9). Virtually all birds inoculated with the MN1 strain scored zero on the six-point gait scale at all three assessment time points. However, one contact bird in the MN1:1wk + DEX group was euthanized at 13 WOA due to development of severe valgus and recumbence.

4. Discussion

Reoviruses are ubiquitously present among commercial poultry flocks (Jones, 2000; Pantin-Jackwood et al., 2008) and poults may get infected vertically via infected eggs and/or horizontally through feces or aerosolized virus as previously observed in chickens (Al-Muffarej et al., 1996; Jones and Georgiou, 1984; Macdonald et al., 1978; Menendez et al., 1975; Roessler and Rosenberger, 1989). Therefore, evaluation of reoviral pathogenesis or reoviral arthritis vaccine efficacy in these turkeys can be complicated by the presence of an active reovirus infection or reovirus-specific antibodies. In this study, the SPF turkey model of reoviral arthritis was evaluated using two reovirus strains with known pathotypes (Sharafeldin et al., 2014, 2015a). Reoviral arthritis in SPF turkeys generally resembles the disease reported in commercial turkeys under field and/or experimental conditions (Mor et al., 2013b; Sharafeldin et al., 2014, 2015a; Sharafeldin et al., 2015b).

In accordance with previous observations in commercial-origin turkeys (Sharafeldin et al., 2014, 2015a), the TARV-induced tenosynovitis emerged as early as 1 WPI, peaked at 4 WPI and decreased thereafter (Figs. 1B and 5). The tenosynovitis corresponded well with the *sine qua non* features of avian reoviral arthritis including hock joint swelling, erosion of distal tibiotarsus cartilage at the hock joint, and high gait scores (Figs. 6, 8 and 9) (Afaleq and Jones, 1989; Jones and Guneratne, 1984; Mor et al., 2013b; Sharafeldin et al., 2015a). Lameness (hock joint swelling and gait defects) was first observed in a few birds at 8 WOA and progressed with age as previously reported in commercial-origin turkeys (Sharafeldin et al., 2015a). Surprisingly, the MN1 virus induced high tendon inflammation scores in one of the trials (Fig. 5), likely due to inoculation with a virus dose that was more than 4 logs higher than that used in a previous commercial-origin turkey study (Sharafeldin et al., 2014). It is intriguing that the high level of tenosynovitis did not generally correspond with clinical lameness or gross pathological lesions in MN1 virus-infected birds (Figs. 6 and 9). However, we cannot rule out the possibility of arthritis development if the experimental timeline were to be extended beyond the endpoint reached in the current study. For instance, one bird in the MN1:1wk + DEX group developed severe valgus and became

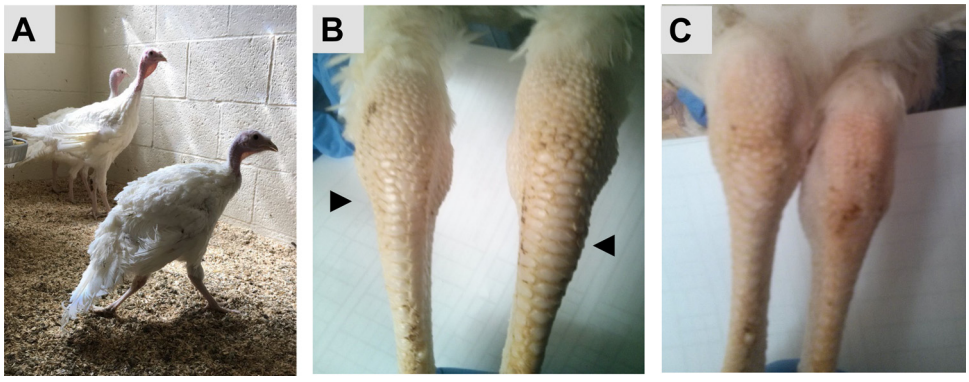

Fig. 8. Clinical signs of arthritis. A. Bird showing severe valgus. B. Swollen hock joint and tendons C. Normal hock joint.

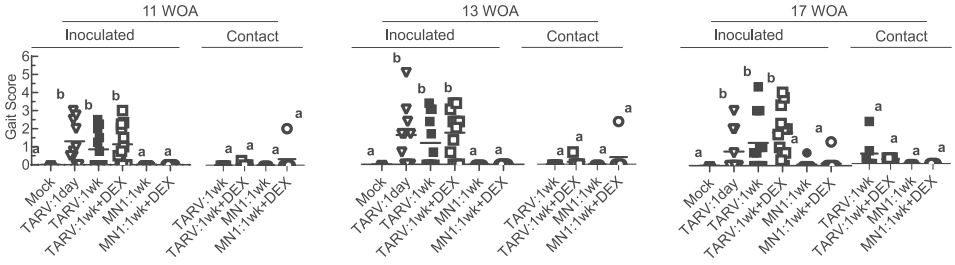

Fig. 9. Average gait scores at different time points. WOA, weeks of age. 11, 13, and 17 WOA corresponded with 11, 13 and 17 weeks post infection, respectively, for TARV:1day group and 10, 12, and 16 weeks post infection for all other groups. Statistical comparisons were performed separately for inoculated and contact groups. Different letters indicate statistically significant differences between groups (ANOVA, $p < 0.05$). DEX, dexamethasone.

recumbent, suggesting the MN1 virus may cause arthritis in turkeys under stress conditions. Arthrotropic reoviruses vary in pathogenesis depending on intrinsic strain characteristics and the magnitude of infectious dose (Gouvea and Schnitzer, 1982). Still, the pathotypic differences between TARV and MN1 observed in commercial-origin turkeys could stem from inoculation with unequal virus doses (Sharafeldin et al., 2014). By standardizing the inoculum size in this study we strongly proved that the observed pathotypes are inherent to the virus strains.

Following oral inoculation of 1 week-old commercial-origin turkeys, TARV O'Neil replicated in the intestines and migrated to the tendons within 7 days (Sharafeldin et al., 2014, 2015a; Sharafeldin et al., 2015b). Unlike the commercial-origin turkeys (Sharafeldin et al., 2015a), the SPF turkeys displayed higher rates of TARV detection in tendons at 1, 4, and 9 WPI (rates > 60%), with a virus detection rate peak of 100% at 4 WPI (Table 1, Fig. 2B). The higher rate of virus detection in SPF turkey tendons may be attributed to the high inoculation doses used in this study, which were 1–2 logs higher than the dose used in the commercial turkey studies (Sharafeldin et al., 2014, 2015a; Sharafeldin et al., 2015b). Compared to the TARV, detection of the MN1 virus in tendons was rarer in both SPF (this study) and commercial-origin turkeys (Sharafeldin et al., 2014) and never reached 100% of inoculated birds in this study (Table 1, Fig. 2B). However, tendon viral titers in MN1 virus-positive birds were similar to those of TARV groups inoculated at the same age (1 WOA), suggesting that detection of reovirus in tendons is not sufficient to predict arthropathogenicity of TARVs. For example, although TARV O'Neil strain is highly arthrotropic in both turkeys and chickens (Sharafeldin et al., 2014, 2015a; Sharafeldin et al., 2015b, c), it is pathogenic in turkeys (Sharafeldin et al., 2014, 2015a; Sharafeldin et al., 2015b) but not in chickens (Sharafeldin et al., 2015c). It remains to be determined whether reoviral arthrotropism is also dependent on the turkey breed. Nevertheless, the rate of virus detection in tendons corresponded well with the level of serum anti-reovirus IgG titers (Figs. 1 and 4), indicating an enhanced systemic spread of TARV compared to the MN1 virus.

All inoculated birds and the sentinel roommates shed reovirus from cloaca, with the peak titers being observed between 4 and 7 dpi and continually declining to low levels at 14 dpi (Fig. 2A and 3). The shedding kinetics were reversed in the commercial-origin turkeys (Sharafeldin et al., 2015b), likely due to some unexplained experimental variables between the two studies. Virus transmission to contact birds is likely to have occurred through ingestion of fecal material (Jones and Georgiou, 1984; Macdonald et al., 1978), contaminated litter and drinking water (Mor et al., 2015) or aerosolized dust (Roessler and Rosenberger, 1989). Although the contacts were not evaluated for gastrocnemius tendon viral titers due to the small number of individuals allocated to each experimental group in this study, TARV was previously shown to translocate to hock joints of contact birds in the commercial-origin turkey arthritis model (Sharafeldin et al., 2014). However, the significance of viral titers in tendons of contact birds is unclear since virus-positive contacts did not show tenosynovitis, and that particular experiment was marred by problems such as presence of reovirus and reovirus-specific antibodies before inoculation and several isolator management issues (Sharafeldin et al., 2014).

In the SPF turkey arthritis model, the majority of contact birds showed tenosynovitis at 17 WOA (Fig. 5) and the hock joint cartilage erosion associated with TARV infection was evident in both inoculated and contact birds (Fig. 6). In stark contrast to the more rapid pathogenesis induced by transmitted TARV in commercial turkey flocks (as early as 10–12 WOA) (Porter, 2018), the onset of gait defects in the TARV-infected contacts was significantly delayed and only a few birds displayed gait scores above zero at 17 WOA (Fig. 9). This discrepancy can be partly explained by the genetic limitation of weight gain between the two types of turkeys, where the high body weight of commercial turkeys (~3 times that of the egg-line SPF turkeys) is deemed to exacerbate TARV-induced decrease in the tensile strength and elasticity of gastrocnemius tendons (Sharafeldin et al., 2016).

Another notable result from this study was the display of depressed body weights in TARV-infected groups during the early times after infection (up to 4 WOA) (Fig. 7) and at 10 WOA (Table 2). We believe the discrepancy in temporal trends of weight depression between the trials is due to difference in the number of birds weighed (4–8 birds in one trial versus 12 birds or more in the other). Body weight depression was not observed in MN1 virus-infected birds. It is not uncommon for turkey reoviruses to cause various degrees of weight depression in the early times after infection, depending on the infecting strain (Spackman et al., 2005). While histological evidence of intestinal pathogenesis was not gathered in this study, mild to moderate enteritis was observed in commercial-origin turkeys infected with MN1, TARV O'Neil, and other reoviruses (Sharafeldin et al., 2014). Whether or not the body weight depression is related to enteritis is a subject that needs further investigation. Nevertheless, in commercial-origin turkeys, the depression of body weights in TARV-infected birds was observed at 12 and 16 WOA (11 and 15 WPI, respectively) and was thought to result from lameness-induced stress (Sharafeldin et al., 2015a). This will be addressed in future studies as we did not find such a connection in the SPF turkey arthritis model.

Age-linked susceptibility to reovirus infection has been reported in chickens where birds are most susceptible at young age (Jones and Georgiou, 1984; Rosenberger et al., 1989). However, it is unclear whether resistance to arthritis reoviruses occurs in turkeys since field samples used for virus isolation are usually obtained from clinically lame birds approaching the market age (10 weeks or older) (Mor et al., 2013b; Porter, 2018; Sharafeldin et al., 2014). A side-by-side comparison of birds inoculated with TARV at 1 day and 1 week of age did not reveal statistical differences in body weights, cloacal shedding, tendon viral titers, tenosynovitis and cartilage pitting and erosion, gait scores, and anti-reovirus IgG responses (Figs. 2,4,5,6,7,9). Even so, the dynamics of TARV replication in the gut and translocation to the tendons were noticeably altered in birds inoculated at 1 day of age, they were consistently comparable to the dynamics in MN1 virus-infected birds (Fig. 2). These data generally contrast the chicken studies in which birds inoculated at the age of 1 week or older were clearly more resistant compared to those infected at 1 day of age as demonstrated by a decrease in virus re-isolations and a concurrent reduction in the severity of lesions in tissues (Jones and Georgiou, 1984; Rosenberger et al., 1989). The apparent species difference in age-linked resistance to reovirus is also likely a result of the huge genetic differences between TARVs and chicken arthritis reoviruses (Mor et al., 2014; Porter, 2018).

Nevertheless, age-linked resistance to reovirus warrants further clarification by inoculating turkeys that are older than 1 week.

Reovirus isolates from severe cases of turkey arthritis outbreaks differ in the degree of pathogenesis under experimental conditions (Afaleq and Jones, 1989; Sharafeldin et al., 2014). It is possible that some arthrotropic strains (e.g., the MN1 virus) that normally cause asymptomatic infection could induce arthritis under conditions, such as stress and immunosuppression, which favor virus replication and spread. Treatment of turkeys with dexamethasone, a glucocorticoid that induces T cell-mediated immunosuppression and lowers resistance to several pathogens including the influenza virus (Ali et al., 2013; Huff et al., 1998, 2014; Isobe and Lillehoj, 1993; Thachil et al., 2014), did not increase the virulence of the MN1 virus nor exacerbate the pathological effects of the TARV strain. This finding suggests that the MN1 virus is intrinsically unable to cause arthritis but does not prove that "asymptomatic" turkey reoviruses cannot cause occasional outbreaks of arthritis under stressful farm conditions. For example, factors such as co-infection with other enteric viruses and non-viral pathogens may trigger virulence of otherwise "asymptomatic" viruses (Barnes et al., 1982; Johnson et al., 2018; Mor et al., 2013a; Ngunjiri et al., 2019; Ongor et al., 2015; Pantin-Jackwood et al., 2007b; Spackman et al., 2010).

5. Conclusion

SPF turkeys can clearly distinguish TARV from other reoviral pathotypes. The *sine qua non* features of turkey reoviral arthritis were clear and prominent in TARV-infected birds. However, age-dependent resistance to disease and other host and environmental factors that may affect the virulence of avian reovirus warrants further investigation. Reovirus-negative SPF turkeys will play a critical role in evaluation of TARV pathogenesis and the development of vaccines and other preventive measures against the disease.

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References

Afaleq, A.A., Jones, R.C., 1989. Pathogenicity of three turkey and three chicken reoviruses for poults and chicks with particular reference to arthritis/tenosynovitis. Avian Pathol. 18, 433–440.
Al-Muffarej, S.I., Savage, C.E., Jones, R.C., 1996. Egg transmission of avian reoviruses in chickens: comparison of a trypsin-sensitive and a trypsin-resistant strain. Avian Pathol. 25, 469–480.
Ali, A., Ibrahim, M., Eladl, A.E., Saif, Y.M., Lee, C.W., 2013. Enhanced replication of swine influenza viruses in dexamethasone-treated juvenile and layer turkeys. Vet. Microbiol. 162, 353–359.
Barnes, H.J., Wheeler, J., Reed, D., 1982. Serologic evidence of infectious bursal disease virus infection in Iowa turkeys. Avian Dis. 26, 560–565.
Day, J.M., Spackman, E., Pantin-Jackwood, M.J., 2008. Turkey origin reovirus-induced immune dysfunction in specific pathogen free and commercial turkey poults. Avian Dis. 52, 387–391.
Gouvea, V., Schnitzer, T.J., 1982. Pathogenicity of avian reoviruses: examination of six isolates and a vaccine strain. Infect. Immun. 38, 731–738.
Heggen-Peay, C.L., Qureshi, M.A., Edens, F.W., Sherry, B., Wakenell, P.S., O'Connell, P.H., Schat, K.A., 2002. Isolation of a reovirus from poult enteritis and mortality syndrome and its pathogenicity in turkey poults. Avian Dis. 46, 32–47.
Hoerr, F.J., 2010. Clinical aspects of immunosuppression in poultry. Avian Dis. 54, 2–15.
Huff, G.R., Huff, W.E., Balog, J.M., Rath, N.C., 1998. The effects of dexamethasone immunosuppression on turkey osteomyelitis complex in an experimental Escherichia coli respiratory infection. Poult. Sci. 77, 654–661.
Huff, G.R., Huff, W.E., Ratha, N.C., 2014. Effects of vitamin D and yeast extract supplementation on turkey mortality and clostridial dermatitis incidence in a dexamethasone immunosuppression model. Avian Dis. 58, 572–578.
Isobe, T., Lillehoj, H.S., 1993. Dexamethasone suppresses T cell-mediated immunity and enhances disease susceptibility to Eimeria mivati infection. Vet. Immunol. Immunopathol. 39, 431–446.
Jindal, N., Patnayak, D.P., Chander, Y., Ziegler, A.F., Goyal, S.M., 2010. Detection and molecular characterization of enteric viruses from poult enteritis syndrome in turkeys. Poult. Sci. 89, 217–226.
Jindal, N., Patnayak, D.P., Ziegler, A.F., Lago, A., Goyal, S.M., 2009. A retrospective study on poult enteritis syndrome in Minnesota. Avian Dis. 53, 268–275.
Johnson, T.J., Youmans, B.P., Noll, S., Cardona, C., Evans, N.P., Karnezos, T.P., Ngunjiri, J.M., Abundo, M.C., Lee, C.W., 2018. A consistent and predictable commercial broiler chicken bacterial microbiota in antibiotic-free production displays strong correlations with performance. Appl. Environ. Microbiol. 84.
Jones, R.C., 2000. Reovirus infections. Rev. Sci. Tech. Off. Int. Epiz. 19, 614–625.
Jones, R.C., 2008. Reovirus infections. In: Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R., Swayne, D.E. (Eds.), Diseases of Poultry. Iowa State University Press, Ames, Iowa, USA.
Jones, R.C., 2013. Reovirus infections. In: Swayne, D., Glisson, J., McDougald, L., Nolan, L., Suarez, D., Nair, V. (Eds.), Diseases of Poultry. John Wiley & Sons, Inc., Ames, Iowa, USA, pp. 351–373.
Jones, R.C., Georgiou, K., 1984. Reovirus-induced tenosynovitis in chickens the influence of age at infection. Avian Pathol. 13, 441–457.
Jones, R.C., Guneratne, J.R., 1984. The pathogenicity of some avian reoviruses with particular reference to tenosynovitis. Avian Pathol. 13, 173–189.
Kumar, P., Porter, R., Goyal, S., Mor, S., 2018. Whole genome sequencing - a key to solve the problem of re-emerging turkey arthritis reovirus. The American Association of Avian Pathologists (AAAP) Annual Meeting, Denver.
Lu, H., Tang, Y., Dunn, P.A., Wallner-Pendleton, E.A., Lin, L., Knoll, E.A., 2015. Isolation and molecular characterization of newly emerging avian reovirus variants and novel strains in Pennsylvania, USA, 2011-2014. Sci. Rep. 5, 14727.
Macdonald, J.W., Randall, C.J., Dagless, M.D., McMartin, D.A., 1978. Observations on viral tenosynovitis (viral arthritis) in Scotland. Avian Pathol. 7, 471–482.
Markis, M., Rosenberger, J., Rosenberger, S., 2015. Antigenic and genetic characterization of a variant viral arthritis Turkey reovirus isolated in 2014. The 2015 American Association of Avian Pathologist Annual Convention, Boston, Massachusetts, USA.
Markis, M., Rosenberger, J., Rosenberger, S., 2018. Genomic characterization of turkey arthritis reoviruses. The 69th North Central Avian Disease Conference And Symposium, Minneapolis, IN, USA, March 12-13.
Menendez, N.A., Calnek, B.W., Cowen, B.S., 1975. Experimental egg-transmission of avian reovirus. Avian Dis. 19, 104–111.
Mor, S.K., Sharafeldin, T.A., Abin, M., Kromm, M., Porter, R.E., Goyal, S.M., Patnayak, D.P., 2013a. The occurrence of enteric viruses in Light Turkey Syndrome. Avian Pathol. 42, 497–501.
Mor, S.K., Sharafeldin, T.A., Porter, R.E., Ziegler, A., Patnayak, D.P., Goyal, S.M., 2013b. Isolation and characterization of a turkey arthritis reovirus. Avian Dis. 57, 97–103.
Mor, S.K., Verma, H., Sharafeldin, T.A., Porter, R.E., Jindal, N., Ziegler, A., Goyal, S.M., 2014. Characterization of S class gene segments of a newly isolated turkey arthritis reovirus. Virology 464-465, 33–44.
Mor, S.K., Verma, H., Sharafeldin, T.A., Porter, R.E., Ziegler, A.F., Noll, S.L., Goyal, S.M., 2015. Survival of turkey arthritis reovirus in poultry litter and drinking water. Poult. Sci. 94, 639–642.
Nersessian, B.N., Goodwin, M.A., Page, R.K., Kleven, S.H., 1985. Studies on orthoreoviruses isolated from young turkeys. II. Virus distribution in organs and serological response of poults inoculated orally. Avian Dis. 963–969.
Ngunjiri, J.M., Taylor, K.J.M., Abundo, M.C., Jang, H., Elaish, M., Kc, M., Ghorbani, A., Wijeratne, S., Weber, B.P., Johnson, T.J., Lee, C.W., 2019. Farm stage, bird age and body site dominantly affect the quantity, taxonomic composition, and dynamics of respiratory and gut microbiota of commercial layer chickens. Appl. Environ. Microbiol.
Ongor, H., Bulut, H., Cetinkaya, B., Akan, M., Tonbak, S., Mor, S., Goyal, S., 2015. Detection of astrovirus, coronavirus and haemorrhagic enteritis virus in turkeys with poult enteritis mortality syndrome in Turkey. J. Poult. Sci. 52, 232–237.
Pantin-Jackwood, M.J., Day, J.M., Jackwood, M.W., Spackman, E., 2008. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Dis. 52, 235–244.
Pantin-Jackwood, M.J., Spackman, E., Day, J.M., 2007a. Pathology and virus tissue distribution of Turkey origin reoviruses in experimentally infected Turkey poults. Vet. Pathol. 44, 185–195.
Pantin-Jackwood, M.J., Spackman, E., Day, J.M., Rives, D., 2007b. Periodic monitoring of commercial turkeys for enteric viruses indicates continuous presence of astrovirus and rotavirus on the farms. Avian Dis. 51, 674–680.
Porter, R., 2018. Turkey reoviral arthritis update. 2018 MPF Convention Proceedings: Turkey Health Workshop (Midwest Poultry Federation).
Reed, L., Muench, H., 1938. A simple method of estimating fifty percent endpoints. Am. J. Epidemiol. 27, 493–497.
Roessler, D.E., Rosenberger, J.K., 1989. In vitro and in vivo characterization of avian reoviruses. III. Host factors affecting virulence and persistence. Avian Dis. 33, 555–565.
Rosenberger, J.K., Sterner, F.J., Botts, S., Lee, K.P., Margolin, A., 1989. In vitro and in vivo characterization of avian reoviruses. I. Pathogenicity and antigenic relatedness of several avian reovirus isolates. Avian Dis. 33, 535–544.
Sharafeldin, T.A., Chen, Q., Mor, S.K., Goyal, S.M., Porter, R.E., 2016. Altered biomechanical properties of gastrocnemius tendons of turkeys infected with Turkey arthritis reovirus. Vet. Med. Int. 2016, 7829138.
Sharafeldin, T.A., Mor, S.K., Bekele, A.Z., Verma, H., Goyal, S.M., Porter, R.E., 2014. The role of avian reoviruses in turkey tenosynovitis/arthritis. Avian Pathol. 43, 371–378.
Sharafeldin, T.A., Mor, S.K., Bekele, A.Z., Verma, H., Noll, S.L., Goyal, S.M., Porter, R.E., 2015a. Experimentally induced lameness in turkeys inoculated with a newly emergent turkey reovirus. Vet. Res. 46, 11.
Sharafeldin, T.A., Mor, S.K., Sobhy, N.M., Xing, Z., Reed, K.M., Goyal, S.M., Porter, R.E.,

2015b. A newly emergent Turkey arthritis reovirus shows dominant enteric tropism and induces significantly elevated innate antiviral and t Helper-1 cytokine responses. PLoS One 10, e0144085.
Sharafeldin, T.A., Mor, S.K., Verma, H., Bekele, A.Z., Ismagilova, L., Goyal, S.M., Porter, R.E., 2015c. Pathogenicity of newly emergent turkey arthritis reoviruses in chickens. Poult. Sci. 94, 2369–2374.
Spackman, E., Day, J.M., Pantin-Jackwood, M.J., 2010. Astrovirus, reovirus, and rotavirus concomitant infection causes decreased weight gain in broad-breasted white poults. Avian Dis. 54, 16–21.
Spackman, E., Pantin-Jackwood, M., Day, J.M., Sellers, H., 2005. The pathogenesis of turkey origin reoviruses in turkeys and chickens. Avian Pathol. 34, 291–296.
Tang, Y., Lu, H., Sebastian, A., Yeh, Y.T., Praul, C.A., Albert, I.U., Zheng, S.Y., 2015. Genomic characterization of a turkey reovirus field strain by Next-Generation Sequencing. Infect. Genet. Evol. 32, 313–321.
Thachil, A.J., Shaw, D.P., Nagaraja, K.V., 2014. Effects of dexamethasone immunosuppression on turkey clostridial dermatitis. Avian Dis. 58, 433–436.
Woolcock, P.R., Shivaprasad, H.L., 2008. Electron microscopic identification of viruses associated with poult enteritis in turkeys grown in California 1993-2003. Avian Dis. 52, 209–213.