Survival of turkey arthritis reovirus in poultry litter and drinking water

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ABSTRACT Turkey reoviruses (TRVs) can cause arthritis, tenosynovitis, and enteric diseases in turkeys, leading to huge economic losses. The TRVs are tentatively divided into turkey arthritis reoviruses (TARVs) and turkey enteric reoviruses (TERVs) depending on the type of disease they produce. This study was conducted to determine the survival of these viruses in autoclaved and nonautoclaved poultry litter and drinking water at room temperature (approx. 25°C). Three isolates of TARV (TARV-O’Neil, TARV-MN2, and TARV-MN4) and one each of TERV (TERV-MN1) and chicken arthritis reovirus (CARV) were used in this study. The viruses were propagated and titrated on QT-35 cells. In autoclaved dechlorinated tap water, all 5 viruses were able to survive for 9 to 13 wk. In nonautoclaved water, all 5 viruses survived for at least 2 wk. In autoclaved litter, the viruses survived for 6 to 8 wk, and in nonautoclaved litter, they survived for 6 to 8 d only. The implications of these results are discussed below.

Key words: survivability, turkey reovirus, poultry litter, drinking water

INTRODUCTION

Avian reoviruses (ARVs) are ubiquitous in domestic poultry, and 85 to 90% of them are nonpathogenic (Jones, 2000). Of the diseases caused by ARVs, viral arthritis and running-stunting syndrome (RSS) in chickens are the most common, but respiratory and enteric diseases and immunosuppression can also occur (Jones, 2008). Reovirus is a nonenveloped, double-stranded RNA virus with icosahedral symmetry and a particle size of 70 to 80 nm (Varela and Benavente, 1994). In addition to affecting chickens, ARVs can also infect turkeys, geese, ducks, and wild birds. Turkey enteric reovirus (TERV) forms a distinct group within the Reoviridae family based on the genetic analysis of S class genome segments (Day et al., 2007; Pantin-Jackwood et al., 2008; Jindal et al., 2010a). The TERVs have been detected in the gastrointestinal tracts of both apparently healthy and enteric turkeys (Pantin-Jackwood et al., 2008; Jindal et al., 2010a,b; Mor et al., 2013a).

In late 2009, the problem of lameness and arthritis was observed in market age toms turkeys (Mor et al., 2013b; Sharafeldin et al., 2014) in the upper Midwest area of the United States. Reoviruses tentatively named turkey arthritis reoviruses (TARVs) were isolated from gastrocnemius and digital flexor tendons of lame turkeys (Mor et al., 2013b; Mor et al., 2014). In addition, the disease was experimentally reproduced by oral inoculation of turkey poults (Sharafeldin et al., 2014). The problem of lameness and arthritis caused by TARVs appears to be re-emerging in U.S. turkeys; the disease was not seen for over 20 y after it was initially reported in late 1980s and early 1990s (Al Afaleq and Jones, 1989, 1991).

We have genetically characterized TARVs based on S class gene segments and found that TARVs group differently from chicken arthritis reoviruses (CARVs; Mor et al., 2014). Since the fecal-oral route appears to be the most common route of infection for both TARVs and TERVs, it is important to determine the environmental stability of these viruses. Hence, we conducted this study to determine the comparative survival of TARVs, TERV, and CARV in poultry litter and drinking water.

MATERIALS AND METHODS

Source of Virus Strains

Three TARVs isolates (TARV-O’Neil, TARV-MN2, and TARV-MN4), one TERV (TERV-MN1), and one CARV were used in this study. All 5 isolates were grown in QT-35 cells as described previously (Mor et al., 2013b). Initial virus titers were 10^5.8, 10^4.5, 10^5.2, 10^5.8, and 10^5.5 (tissue culture infective dose [TCID]_{50}/mL).
for TARV-MN2, TARV-MN4, TARV-O’Neil, TERV-MN1, and CARV, respectively.

Survival in Drinking Water

Two experiments were conducted, using autoclaved water in experiment 1 and nonautoclaved water in experiment 2. In experiment 1, tap water was dechlorinated and then sterilized by autoclaving for 15 min. After cooling, five 50 mL aliquots of water were prepared in sterile vials. Then 2.5 mL of appropriate virus was added to each. After mixing well, an aliquot was removed from each vial to determine baseline virus titers. The vials were stored at room temperature (approx. 25°C) for 14 wk. Aliquots (2 mL amounts) were removed from all vials weekly. Serial 10-fold dilutions of the aliquots were inoculated in QT-35 cells followed by incubation at 37°C. Viral titers were calculated by the method of Reed and Muench (1938). In experiment 2, to simulate field conditions, nonautoclaved water collected from drinkers at a turkey farm was used. Aliquots of water (5 mL amounts) were spiked with 200 μL of appropriate virus. Samples were collected at zero time and daily thereafter for 10 d followed by virus titration in QT-35 cells. The experiment was done in triplicate. A longer-term survival study with nonautoclaved water was also conducted in which virus survival was tested for 7 wk. The experimental design was the same as for the autoclaved water. Briefly, five 50 mL aliquots of nonautoclaved water were prepared in sterile vials. Then 2.5 mL of appropriate virus was added to each. After mixing well, an aliquot was removed from each vial to determine baseline virus titers. The vials were stored at room temperature (approx. 25°C), and aliquots (2 mL amounts) were removed from all vials weekly for 7 wk.

Survival in Poultry Litter

Again, 2 experiments were conducted using autoclaved and nonautoclaved litter. Litter samples were collected from 6 commercial turkey farms. Equal portions of the 6 litter samples were mixed together and used as a pool. In experiment 1, the pooled litter was autoclaved followed by distribution in 135 vials at 2 g per vial. All aliquots were spiked with 100 μL of the appropriate virus. After sufficient mixing, the vials were stored at room temperature for 8 wk. Three vials per virus were removed at zero time (as a baseline) and then weekly thereafter. Viruses present in these samples were eluted by adding 5 mL eluent solution (3% beef extract, 0.05 M glycine solution, pH 7.2, Sigma-Aldrich, St. Louis, MO) followed by vortexing and centrifugation at 2,500 × g for 10 min 4°C. Serial 10-fold dilutions of the supernatants were used for virus titration in QT-35 cells. In experiment 2, nonautoclaved litter was tested and found to be negative for reoviruses. Aliquots of the litter were distributed in 165 vials at 2 g per vial. Each vial was then spiked with 100 μL of the appropriate viruses. Three vials from each virus group were removed at zero time and daily thereafter for 10 d. Titers of surviving virus in these vials were determined as was done for the autoclaved litter samples.

RESULTS

Survival of Viruses in Drinking Water

In autoclaved dechlorinated tap water, all 5 reoviruses (CARV, TARVs, and TERV) were able to survive for 9 to 13 wk with minor differences (Figure 1); TERV-MN1 and TARV-MN4 survived for 9 and 10 wk, respectively, while TARV-O’Neil and CARV survived for 12 and 13 wk, respectively. For up to 4 wk, there was no reduction in virus titers; a gradual decrease was seen thereafter (Figure 1). In nonautoclaved water, all 5 viruses were able to survive for up to 10 d, although there was a small decrease in virus titers after 4 d of incubation at room temperature (Figure 2). In all experiments, the viral titers decreased gradually over time.

Survival of Viruses in Litter

In autoclaved litter, TARV-O’Neil, TARV-MN2, and CARV were able to survive for 7 wk but not for 8 wk. TARV-MN4 and TERV-MN1 were the least resistant; they were inactivated within 6 and 7 wk, respectively (Figure 3). In nonautoclaved litter, TARV-O’Neil, TARV-MN2, and CARV-1 survived for 8 d, while TERV-MN1 and TARV-MN4 survived for 6 and 7 d, respectively (Figure 4). In all experiments, the viral titers decreased gradually over time.

DISCUSSION

Savage and Jones (2003) reported that CARV strains were capable of surviving for at least 10 wk in drinking water. However, no similar information is available on the survival of TARV and TERV in either water or litter. All 5 viruses in our study were able to survive for...
9 to 12 wk in autoclaved water, and none survived for 13 or 14 wk. These results are in contrast to those of Savage and Jones (2003), who reported little decrease in titer of CARV-R2 after 10 wk. In our study, there were minor differences in the survival of different viruses. TERV-MN1 and TARV-MN4 survived for relatively less time than TARV-O’Neil, TARV-MN2, and CARV-1. These results are interesting because the latter three viruses are more pathogenic than TERV-MN1 and TARV-MN4 are (Sharafeldin et al., 2014).

In nonautoclaved water, the virus titers continued to decrease, and live viruses were still detected after 10 d of incubation. In a subsequent experiment, the viruses survived for at least 2 wk. These data indicate that all 5 viruses survive for less time in nonautoclaved water than in sterile water. This is not surprising because the flora in nonautoclaved water may present competition for these viruses. Savage and Jones (2003) have also reported a decrease in the survival of CARV from 10 to 5 wk in fecal material suspended in PBS.

In autoclaved poultry litter, the viruses were able to survive for 5 to 7 wk. Again, TERV-MN1 and TARV-MN4 survived for less time than the other 3 viruses. This could also be due to lower starting titers of these two viruses. No study is available on the survival of avian reoviruses in poultry litter, and hence a direct comparison with the results of this study is not possible.

The overall conclusion of this study is that TARVs and TERV survive longer in autoclaved water and litter as compared to nonautoclaved water and litter. It should be realized that poor poultry house management may lead to contamination of the drinking system, in which the virus may survive for a period of time and present a health hazard. Also, using contaminated water as a diluent for routine vaccines may also help spread the virus. In the case of litter, we observed a decrease in virus titers over time; however, the presence of fecal matter on solid surfaces such as paper, cotton, and wood may help prolong survival time by protecting the virus from the effects of dehydration and disinfectants (Savage and Jones, 2003).

It should also be noted that results obtained in the laboratory may not be fully applicable in field situations; in our laboratory experiments, the virus was added to the water only once, while under field conditions, the infected birds may shed virus continuously, and hence the virus may survive for the whole life of a particular flock. Al Afaleq and Jones (1994) reported prolonged persistence of disease in repeated exposure to reovirus as compared to a single infection. It should also be noted that even a small amount of surviving virus under field conditions is important because the bird ingesting a small amount of virus may lead to replication in the gut to the point that it may cause disease.

To the best of our knowledge, this is the first study on survival of TARV and TERV in drinking water and poultry litter. The results should be helpful in proper planning of preventive and control measures against these viruses.
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REFERENCES


