

Prevalence of Rota-and Reoviruses in Turkey Enteritis in Turkey

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Abstract

The objective of this study was to compare the presence of rotavirus (TRotV) and reovirus (TReoV) in clinically healthy turkey flocks and in those with poult enteritis complex (PEC) using reverse transcription-polymerase chain reaction (RT-PCR). TRotV and TReoV were detected in 2.6% (6/230) of the birds each and in 26.08% (6/23) and 13.04% (3/23) of the flocks, respectively. Mixed infection with both agents was found in one sample. None of these two viruses were detected in turkeys originating from clinically healthy flocks.

Keywords: Rotavirus; Reovirus poult enteritis complex; Turkey

Introduction

Gut health of poultry is very important to get maximum returns in terms of weight gain and egg production. Enteric diseases such as Poult Enteritis Complex (PEC) in turkeys do not allow the achievement of their maximum production potential. The PEC is a term that includes all infectious intestinal diseases of young turkeys [1]. The complex is characterized by enteritis, moderate to marked growth depression, retarded development, impaired feed utilization; poor feed conversion efficiency, and sometimes increased mortality, as in Poult Enteritis Mortality Syndrome (PEMS) [2,3].

The etiology of enteric diseases such as PEC, Poult Enteritis Syndrome (PES), PEMS and Light Turkey Syndrome (LTS) in turkeys has been shown to be multifactorial. A number of viruses (adenovirus, astrovirus, coronavirus, reovirus and rotavirus), bacteria (Escherichia coli and species of Salmonella, Clostridium, Campylobacter and Enterococcus) and protozoa (coccidia, cryptosporidium) have been linked to multiple turkey infections [3].

Rotavirus (RotV) is mainly associated with diarrhea in children, other mammals and birds, such as chickens, ducks, pheasants, pigeons and turkeys. A high incidence of rotavirus has been reported in chickens and turkeys in the United States [4-6] but the virus is detected in the feces of both healthy and enteritic poults [7,8]. However, a higher number of PES cases have been determined to be positive for TRotV when compared to astrovirus or reovirus, which indicated that TRotV may play a significant role in the etiology of PEC [9].

Avian reovirus (AReoV) has been linked to a wide range of disease presentations in avian species and can induce enteric disease and immunosuppression (based on the severe bursal atrophy caused by this virus) in turkeys. The AReoV infection may also predispose birds to secondary complications or may act synergistically along with other infections. In turkeys, this virus has been detected in several different enteric disease conditions, namely LTS, PEC, PES, myocarditis, and recently in arthritis/tenosynovitis [10]. Another notable result of TReoV infection was decreased body weight gain. This is of

importance because in commercial turkeys, most of the economic loss from poult enteritis comes from decreases in production where the birds do not grow to the weights expected by their genetic potential [3,11].

In chickens, reovirus causes many syndromes including viral arthritis/ tenosynovitis and runting stunting syndrome (RSS). These are global problems of broiler production, which result in financial losses from increased culling, poor feed conversion and lower uniformity at slaughter with concomitant increase in costs associated with treatment [12].

This study was carried out to investigate the presence of TRotV and TReoV by reverse transcription polymerase chain reaction (RT-PCR) in cloacal swabs collected from both clinically healthy turkey flocks and those associated with PEC.

Materials and Methods

Sample collection

A total of 270 cloacal swab samples were collected from 27 different turkey flocks (10 from each flock) belonging to a commercial turkey company located in the north of Turkey between April and July 2014. The turkeys were white-feathered Californian breed ranging in age from 19 to 119 days. Of the 270 cloacal swab samples, 230 were from turkeys with PEC and the remaining 40 samples were collected from clinically healthy turkeys. The average number of birds was 7,000 in each of the 27 flocks. The birds with PEC showed severe diarrhea, moderate to marked growth depression, retarded development, impaired feed utilization, and poor feed conversion efficiency. Turkeys in the four control flocks were apparently healthy. The cloacal swabs were placed in Stuart Transport Medium and transported on ice to the laboratory within two days. Each swab was processed separately.

RNA extraction and multiplex RT-PCR

RNA extraction was done by using EZ-10 Spin Column (Bio Basic, Canada) according to manufacturer's instructions. The screening of

flocks for TRotV and TReoV was performed by employing a one-step RT-PCR kit combined with specific primer pairs of NSP4 and S4 gene which produce approximately 630-bp and 1120 bp fragments in positive samples, respectively. NSP4 gene is not group specific and may detect all rotavirus groups [5]. S4 gene segment was reported to detect the most diverse lineages of avian reoviruses [8]. Primers used in multiplex RT-PCR listed in Table 1. Amplification was carried out using PrimeScript™ One Step RT-PCR Kit Ver. 2 (TaKaRa Bio Inc, Japan).

Gene	Primer	Oligonucleotide sequence (5'-3')	Fragment Length (bp)	Reference
NSP4	NSP4-F	GGGCGTGCGGAAAGATGGAGAAC	630	[16]
	NSP4-R	GGGGTTGGGTACCAGGGATTAA		
S4	S4-F S4-R	GTGCGTGTGGAGTTTCCCG TACGCCATCCTAGCTGGA	1120	[16]

Table 1: Primer sequences and lengths of PCR amplification products.

One-step RT-PCR was performed in a TC 512 Temperature Cycling System (Techne, Staffordshire, UK) in a reaction volume of 25 µl. RT-PCR was carried out as 1 RT cycle at 50°C for 30 min followed by enzyme inactivation at 94°C for 2 min, then 35 repeated cycles of denaturation at 94°C for 30 s, annealing at 56°C for 1 min and extension at 72°C for 2 min with a final extension cycle at 72°C for 10 min. The amplified products were detected by staining with ethidium bromide (0.5 mg/ml) after electrophoresis at 80 V for 2 h (7 V/cm) in 1.5% agarose gels. Avian reovirus S1143 strain (obtained from Bornova Veterinary Control and Research Institute, Izmir) and TRotV (obtained from Institute of Poultry Diseases Faculty of Veterinary Medicine, Freie Universität Berlin) template RNA were included as positive controls; distilled water was used as negative control in all the assays.

Statistical analysis

A chi-squared test was used to compare the results obtained from PEC-associated flocks and healthy flocks and difference at P<0.05 was considered as statistically significant.

Results

Of the 230 samples collected from PEC flocks, 11 were virus positive; five for TRotV, five for TReoV, and one for both viruses giving an overall proportion of 4.78% and 39.13% at bird and flock levels, respectively. When the results were considered for each virus separately, both TRotV and TReoV were detected in 2.6% (6/230) of the birds and in 26.08% (6/23) and 13.04% (3/23) of the flocks, respectively. No virus was detected in any of the 40 samples obtained from clinically healthy turkeys (Table 2). The difference between the results obtained in PEC-associated flocks and healthy flocks was not significant (P>0.05).

Virus	Flocks	Samples*	Age of virus-positive birds in days	
	No. positive/No. tested (%) positive)	No. positive/No. tested (%) positive)	Nov-28	29-119
Rotavirus	6/23 (26.08)	6/230 (2.6)	3	3
Reovirus	3/23 (13.04)	6/230 (2.6)	2	4
Total	9/23 (39.13)	12/230 (5.22)	5	7

Rotavirus	6/23 (26.08)	6/230 (2.6)	3	3
Reovirus	3/23 (13.04)	6/230 (2.6)	2	4
Total	9/23 (39.13)	12/230 (5.22)	5	7

*Mixed infection with both agents was found in one sample.

Table 2: Detection of Rota and Reovirus in cloacal swabs of turkeys with PEC.

Discussion

Although PEC has been studied comprehensively in countries with intensive turkey breeding such as the USA and Brazil [8-10,13,14], quantitative data toward this complex are rather limited in Turkey. Therefore, cloacal swab samples collected from turkeys suspected of having PEC, which were previously examined for the presence of turkey astrovirus 2 (TAsV-2), turkey coronavirus (TCoV), and hemorrhagic enteritis virus (HEV) [15], were further studied for the presence of TRotV and TReoV in this study.

High rates of rota- and reoviruses have been reported from turkeys suspected to have PEC and PES (18% to 70%) [8,16]. The proportion (4.78%) obtained in the present study for both of the agents was well below the above mentioned studies. Age intervals of samples, seasonal variations and severity of disease (LTS, PES, and PEMS, etc.) might have contributed to the huge differences between our study and the others. The use of pooled samples in most of the studies and time of sampling may also play a role in this difference. In another study, periodical examination of samples belonging to commercial turkey flocks for enteric viruses at 2, 4, 6, 8, 10 and 12 weeks of age revealed the presence of TAsV (89.5%) and TRotV (67.7%) at high proportion in all the flocks between 2 and 6 week time periods [4]. This may be due to the lack of intestinal epithelium development in the early weeks of life which can increase susceptibility to enteric viruses [17].

On the other hand, in a study conducted by Moura-Alvarez et al. [14], neither reovirus nor HEV could be detected in 10 to 104 day old turkey flocks, while other enteric viruses and bacteria were found. The age intervals of samples examined in this study were similar (11-119 days) to the above mentioned study and while reovirus was determined at low percentage, HEV was not detected likewise. In addition, no positivity was reported for either rota or reovirus in a study conducted in Brazil [18]. Less than 10% positivity rates were determined for both agents in several studies based on chicken originated samples [12,19].

The comparison of positivity rates by age revealed that the enteric viruses were more prevalent in turkeys at finishing phase (5-14 weeks) than those at growing phase (1-4 weeks) [17]. Similar results were obtained in this study even though the numbers of positive samples were rather small to draw a firm conclusion. In previous studies, the most frequently detected agent in turkeys with PEC has been reported as TAsV, which usually consisted of having co-infection with other agents rather than individually [2,8]. In addition to mixed infection with both TRotV and TReoV in one sample, TAsV-2 was found to get involved in two samples, one of which was positive for TRotV and the other for TReoV (data not shown). In previous studies, two or more enteric viruses (mostly Rotavirus and TAsV) have frequently been detected in turkey flocks with enteritis, particularly PEC. The effects of mixed infections are expected to be more severe [4].

In previous studies conducted in turkeys with enteric diseases, reovirus was the least frequently detected agent compared to other enteric viruses [6,20]. Therefore, the role of TReoV as primary pathogen in poult enteritis was thought to be less likely, but it may contribute to poult enteritis, and perhaps other diseases, as a predisposing agent. It is probable that poults infected at a young age would experience transient, and possibly permanent, immunosuppression. Induction of immunosuppression at an early age could increase susceptibility to infection and disease caused by other infectious agents and in production where the birds do not grow to the weights expected by their genetic potential.

It is known that viral enteritis due to the agents associated with PEC can cause important disorders such as poor feed conversion rate and marked growth depression, which result in great economic losses in meat-type turkeys. The absence of effective vaccines for enteric viruses is one of the major constraints against the control of PEC-associated viral enteritis. Large-scale studies are, therefore, required to obtain comprehensive data which will help us to better understand the etiology of PEC-cases, to develop effective vaccines, and improve biosecurity procedures.

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