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Original article

Simultaneous detection of astrovirus, rotavirus, reovirus and adenovirus type I in broiler chicken flocks

D.A. Roussan¹, I.A. Shaheen¹, G.Y. Khawaldeh¹, W.S. Totanji¹, R.H. Al-Rifai²¹ Technical Department, Provimi, Jordan, P.O. Box 499, Amman 11118, Jordan² Department of International Health Development, Division of Public Health, Tokyo Medical and Dental University, Tokyo, Japan, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

Abstract

Enteric diseases cause substantial economic losses to the poultry industry. Astroviruses, rotaviruses, reoviruses, and adenovirus type 1 have been reported as a significant cause of intestinal symptoms in poultry. In the present study, intestinal samples from 70 commercial broiler chicken flocks were examined for the presence of astroviruses, rotavirus, and reovirus by reverse transcription-polymerase chain reaction, and for the presence of group I adenovirus by polymerase chain reaction. Astroviruses were identified in 38.6% of samples tested. Both avian nephritis virus and chicken astrovirus were identified in the astrovirus positive flocks, where 74.1% of these flocks were positive for only one type of astrovirus, whereas, 25.9% of these flocks were positive for both types of astrovirus. Reoviruses, rotaviruses, and adenoviruses were identified in 21.4, 18.6, and 14.3% of these flocks, respectively. Concomitant infection with two or more viruses in the same flock were also prominent, where 5.7, 5.7, 2.9, 2.9, 1.4, and 1.4% of these flocks were positive with both astrovirus and rotavirus; astrovirus and adenovirus; astrovirus and reovirus; rotavirus and adenovirus; rotavirus and reovirus; and reovirus and adenovirus respectively. Moreover, 4.3 and 2.7% of these flocks were positive for astrovirus, reovirus, and adenovirus; and astrovirus, reovirus, and rotavirus, respectively. Further studies will focus on identifying specific viral factors or subtypes/subgroups associated with disease through pathogenesis studies, economic losses caused by infections and co-infections of these pathogens, and the costs and benefits of countermeasures.

Key words: broiler, enteric viruses, Jordan, molecular test

Introduction

Enteric disease in broiler chicken flocks has an important economic impact because of production losses due to poor feed conversions and poor weight gain. Sporadic outbreaks of enteric disease are seen worldwide in commercial poultry and can vary widely

in severity (Reynolds 2003). The causes of enteric disease have never been definitely established because they are complex and polymicrobial, and similar disease signs can likely be caused by different pathogens (Barnes and Guy 2003, McFerran 2003, Reynolds 2003, Baxendale and Mebatsion 2004). Numerous viruses, including astroviruses, rotaviruses, reoviruses, and aden-

oviruses, have been implicated as causative agents for enteric disease because they have been isolated, from or identified, in the intestines and intestinal contents of affected poultry flocks (Barnes and Guy 2003, Pantin-Jackwood et al. 2008) making disease association difficult to determine.

Detection of avian viruses by molecular techniques has become routine in most diagnostic laboratories. Traditionally, diagnosis of viral enteric infections in poultry has been made by electron microscopy (EM), immunofluorescent assays (IFA), and genome electropherotyping to detect and identify the viruses and by ELISA to detect antibodies. There are many advantages to using reverse transcription PCR (RT-PCR) for detecting enteric viruses over traditional methods, including greater sensitivity and specificity, detection of multiple viruses in one sample, no need for virus propagation, the ability to test a large number of samples quickly, and reduced cost of the assay. Currently, molecular-based diagnostic tests are available to detect astrovirus, rotavirus, reovirus, and adenovirus type I in intestinal samples (Hess et al. 1999, Sellers et al. 2004, Spackman et al. 2005, Tang et al. 2005, Pantin-Jackwood et al. 2008). Runtng stunting syndrome (RSS) is a global problem affecting broiler chicken production, which results in financial losses from increased culling, poor feed conversion and lower uniformity at slaughter with concomitant increased costs from treatment (De Wit 2008). This study was designed to determine the prevalence of the following enteric viruses: astrovirus, rotavirus, reovirus, and adenovirus type I in commercial broiler chicken flocks suffering from enteric conditions (diarrhea, poor weight gain and feed conversions) using molecular tests.

Materials and Methods

Broiler Flocks

During the period from January 2008 to May 2011, we examined 70 commercial broiler farms in Jordan in which the chickens in these flocks were suffering from digestive disease. Each farm selected for investigation had 1 flock. Thirty, 22, and 18 percent of the samples were from broiler flocks less than 1, 2, and 3 wks of age, respectively (Table 2). Intestinal contents were collected from these flocks. Samples consisted of pooled intestines from five birds from each house of a total 70 broiler flocks. Clinical signs observed in these flocks included diarrhea, depression, loss of appetite, dehydration, poor weight gain, and vent past. Mortality and morbidity rates were 4 to 7% and 70 to 85%, respectively. The most common finding at necropsy is the presence of abnormal amounts of fluid and gas in the

intestinal tract and ceca. Duodenum and jejunum generally are pale and flaccid. Emaciation and dehydration were also observed in most examined flocks. All flocks were contacted through the appropriate veterinarians, and after a detailed explanation of the aims, benefits, and protocol of the study all owners agreed voluntarily to participate in this study. All samples were stored at -70°C in the Provimi Jordan Laboratory until examined.

Viral RNA Extraction

Two hundred and fifty microliters of intestinal contents were diluted in 1500 µl phosphate buffer saline (PBS; pH 7.2), homogenized with sterile glass beads in a Fast Prep homogenizer (Savant, Inc, USA), and centrifuged for 10 min at 800 X g. Total RNA was extracted directly from 250 µl of the supernatant using Trizol Reagent (Life Technology, Carlsbd, CA, USA), according to the manufacturer's instructions. Briefly, Trizol® was added to supernatants from homogenized intestinal contents and incubated for 5 min at 25°C. The mixtures were then extracted with chloroform (200 µl per 1000 µl of Trizol® Reagent) and centrifuged at 10000 X g for 15 min at 4°C. The aqueous phases were precipitated with isopropyl alcohol and the pellets were washed with 75% ethanol, dried, and suspended in RNase-free water (Promega Corp., Madison, WI, USA). RNA extracted from the intestinal contents of specific-pathogen-free chickens was used as negative controls for all molecular tests.

Viral DNA Extraction

DNA was purified from samples using Tris-ethylene diamine tetraacetic acid (EDTA) saturated phenol-chloroform (1:1). Briefly, 250 µl of the intestinal homogenate described under the RNA extraction methods was mixed with 250 µl of phenol-chloroform (1:1), vortexed for 10 sec, and centrifuged at 14000 X g for 3 min. The aqueous phase was removed to a fresh tube. An equal volume of chloroform was added, vortexed, and centrifuged at 14000 X g for 3 min. The aqueous phase was removed to a fresh tube, and 3 volumes of 90% ethanol/0.12 M sodium acetate was added, mixed by inversion, incubated on ice for 10 min, and centrifuged at 14000 X g for 15 min at 4°C. The supernatant was decanted; 80% ethanol was added, corresponding to 2 volumes of the original sample, and the samples were then incubated at room temperature for 10 min. Samples were then centrifuged at 14000 X g for 5 min. The supernatant was decanted, and the pellet was dried at room temperature for 15 min.

Table 1. Nucleotide sequences of primers used in this study for detection of enteric viruses.

Enteric virus	Target Gene	Primer name	Primer sequences (5'-3') ^a	Amplicon size (bp)
Avian astrovirus	ORF-1b	TAPG-L1	TGG TGG TGY TTY CTC AAR A	601
		TAPG-R1	GYC KGT CAT CMC CRT ARC A	
Chicken astrovirus	ORF-1b	CAS pol1F	GAY CAR CGA ATG CGR AGR TTG	362
		CAS pol1R	TCA GTG GAA GTG GGK ART CTA	
Avian nephritis virus	ORF-1b	AVN pol1F AVN pol1R	GYT GGG CGC YTC YTT YGAYAC CRT TTG CCC KRT ART CTT TRT	473
Avian reovirus	S4	S4-F13 S4-R1133	GTG CGT GTT GGA GTT TCC CG TAC GCC ATC CTA GCT GGA	1120
Avian rotavirus	NSP4	NSP4-F30 NSP4-R660	GTG CGG AAA GAT GGA GAA C GTT GGG GTA CCA GGA TTA A	630
Avian adenovirus group I	Hexon	H1	TGG ACA TGG GGG CGA CCT A	1219
		H2	AAG GGA TTG ACG TTG TCC A	

^a IUB codes used: Y = pyrimidine, R = purine, K = G or T, M = A or C

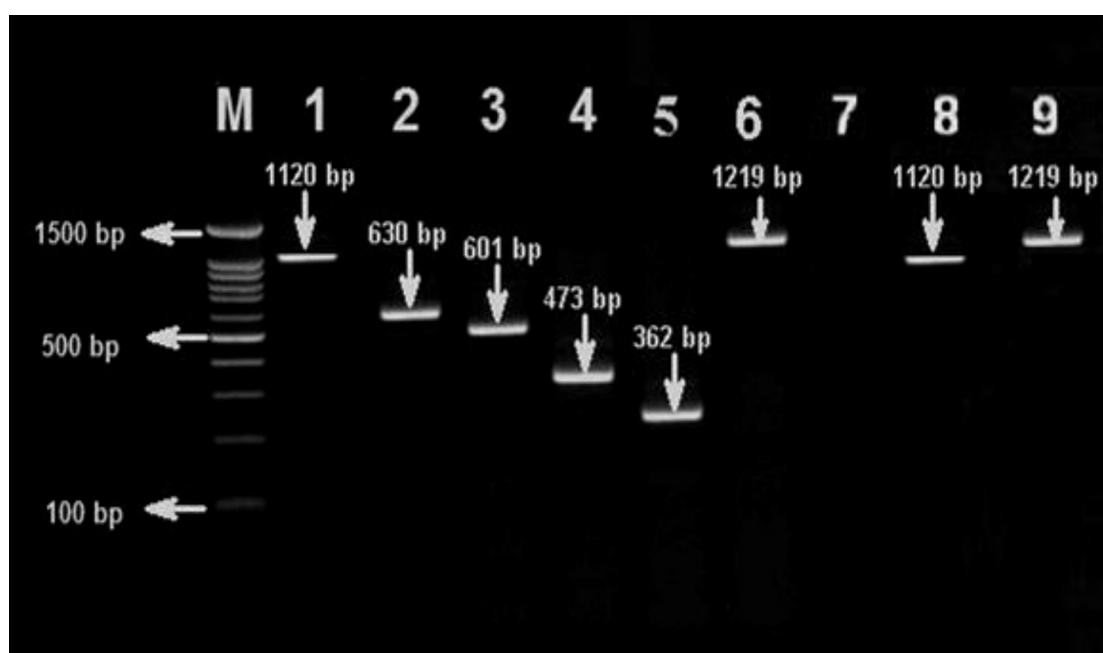


Fig 1. Electrophoresis analysis (2% agarose gel) for enteric viruses. Lane M = 100-bp -1.5-kb DNA ladder marker (Promega Corp., Madison, WI). Lane 1 = Avian reoviruses (positive flock; band at 1120 bp). Lane 2 = Avian rotaviruses (positive flock; band at 630 bp). Lane 3 = Avian astroviruses (positive flock; band at 601 bp). Lane 4 = Avian nephritis viruses (positive flock; band at 473 bp). Lane 5 = Chicken astroviruses (positive flock; band at 362 bp). Lane 6 = Avian adenoviruses group I (positive flock; band at 1219 bp). Lane 7 = Negative control (intestinal content of specific pathogen eggs). Lane 8 = Avian reoviruses (positive control; band at 1120 bp). Lane 9 = Avian adenoviruses group I (positive control; band at 1219 bp).

Purified DNA was hydrated with 50 µl of nuclease free water and stored at -70°C. This method has previously been described and used by Pantin-Jackwood et al. (2008).

RT-PCR for Astroviruses

The test has two phases: first phase, the avian astroviruses were detected by one-step RT-PCR (Promega Corp., Madison, WI, USA), using primer

pairs (Alpha DNA, Montreal, Quebec, Canada) that is directed to polymerase gene (ORF-1b) (Table 1), and generates a 601-bp fragment in a positive sample (Fig. 1) as described by Tang et al. (2005). In the second phase we used primers as described by Day et al. (2007) (Table 1), which differentiates between chicken astrovirus (CAV) and avian nephritis virus (AVN), and generates 362 and 473-bp fragments in a positive sample, respectively (Fig. 1). One-step RT-PCR was performed by using an Access RT-PCR System kit (Promega Corp., Madison, WI, USA) according to the

Table 2. Enteric viruses detection in broiler chicken flocks by agea.

Age (wk-d)	No. of tested flocks	No. of positive flocks	Enteric virus detection						
			Astrovirus				Reovirus (%) ^c	Rotavirus (%) ^c	Adenovirus Type 1 (%) ^c
			Total Positive (%) ^c	ANV ^b (%) ^e	CAsV ^b (%) ^e	ANV+CAsV (%) ^e			
Hatch to 1-0	30	16	9 (30)	5 (55.6)	2 (22.2)	2 (22.2)	3 (10)	10 (33.3)	2 (6.7)
1-1- to 2-0	22	14	13 (59.1)	5 (38.5)	4 (30.8)	4 (30.8)	2 (9.1)	4 (18.2)	5 (22.7)
2-1 to 3-0	18	11	5 (27.8)	2 (40)	2 (40)	1 (20)	8 (44.4)	1 (5.5)	3 (16.7)
Total	70	41	27 (38.6) ^d	12 (44.4) ^f	8 (29.6) ^f	7 (25.9) ^f	13 (18.6) ^d	15 (21.4) ^d	10 (14.3) ^d

^a – All of flocks were suffering from enteric disease.

^b – ANV = Avian nephritis virus; CAsV = Chicken astrovirus.

^c – Values in parentheses represent percentage of positive flocks corresponding to the total number of tested flocks in the same age.

^d – Values in parentheses represent percentage of positive flocks corresponding to the total number of tested flocks.

^e – Values in parentheses represent percentage of flocks corresponding to the total number of astrovirus positive flocks in the same age range.

^f – Values in parentheses represent percentage of flocks corresponding to the total number of astrovirus positive flocks.

manufacturer's procedure. Briefly, a 50 µl reaction volume per sample was prepared by adding 10 µl of avian myeloblastosis virus/reverse transcriptase/*Thermus filiformis* DNA polymerase 5 x reaction buffer, 1 µl of deoxy nucleotide 5'-triphosphate mixture (10 mM of deoxy nucleotide 5'-triphosphate), 1 µl (50 pmol/µl) of each primer (Table 2) (Alpha DNA, Montreal, Quebec, Canada), 2 µl of 25 mM MgSO₄, 1 µl of avian myeloblastosis virus reverse transcriptase (5 U/µl), 1 µl of *T. filiformis* DNA polymerase (5 U/µl), 28 µl of nuclease free water, and 5 µl of RNA template. Reverse transcription PCR was carried out in a DNA DNA Engine[®] thermal cycler (Bio-Rad Laboratories Ltd, Mississauga, ON, Canada) for 1 reverse transcription cycle of 60 min at 50°C, followed by 94°C for 5 min, then 35 PCR cycles at 94°C for 45 sec, 53°C for 45 sec, and 72°C for 90 sec, with a final extension cycle at 72°C for 5 min.

RT-PCR for Rotaviruses

A 630-nt-long region of the NSP4 gene of rotaviruses was amplified with the NSP4 F30 and NSP4 R660 primers (Table 1) that have been reported previously (Day et al. 2007). The RT-PCR program and conditions were performed as described as above except for annealing temperature 57°C, and the generation of 630-bp in a positive sample (Fig. 1).

RT-PCR for Reoviruses

The S4 gene segment was chosen because it detected the most diverse lineages of avian reoviruses (Pantin-Jackwood et al. 2008) (Table 1). The RT-PCR

program and conditions were performed as described as above except for annealing temperature 54°C, and the generation of 1120-bp in positive sample (Fig. 1). The reovirus reference strain S1113 (Chales River Laboratory, North Franklin, USA) was used as a positive control for viral RNA extraction and amplification.

PCR for Adenoviruses

The adenovirus detection primers used in this study were previously evaluated by Hess et al. (1999) and are listed in Table 1. The PCR mix for adenoviruses was prepared in a volume of 50 µl containing 25 µl of master mix (5 units/µl of Taq polymerase, 400 mM deoxynucleotide 5-triphosphate mixture, and 3 mM MgCl₂), 18 µl of nuclease-free water (Promega Corp., Madison, WI, USA), 1 µl (50 pmol/µl) of each primer (Alpha DNA, Montreal, QC, Canada; Table 1), and 5 µl of DNA template. Polymerase chain reaction amplification was carried out in a DNA DNA Engine[®] thermal cycler (Bio-Rad Laboratories Ltd, Mississauga, ON, Canada) for 1 cycle of 5 min at 94°C, followed by 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min for 35 cycles. The PCR generates 1219-bp in a positive sample (Fig. 1). Adenovirus type I (Chales River Laboratory, North Franklin, USA) was used as a positive control for DNA extraction and amplification.

Agarose Gel Electrophoresis

Polymerase chain reaction products were electrophoresed on a 2% agarose gel in Tris-acetate-EDTA buffer (40 mM of Tris and 2 mM of ethylene

Table 3. Enteric virus detection patterns in broiler chicken flocks.

Astrovirus	Rotavirus	Reovirus	Adenovirus	No. of flock (%) ^a
+	-	-	-	12 (17.1)
-	+	-	-	4 (5.7)
-	-	+	-	6 (8.6)
+	+	-	-	4 (5.7)
+	-	+	-	2 (2.9)
-	-	+	+	1 (1.4)
+	-	-	+	4 (5.7)
-	+	+	-	1 (1.4)
-	+	-	+	2 (2.9)
+	-	+	+	3 (4.3)
+	+	+	-	2 (2.7)
-	-	-	-	29 (41.4)

^a Values in parentheses represent percentage of positive flocks corresponding to the total number of tested flocks.

Table 4. Distribution of positive flocks by number of detected viruses.

Age (wk-days)	No. of positive flocks (%) ^a	No. of positive viruses		
		One (%) ^b	Two (%) ^b	Three (%) ^b
Hatch to 1-0	16 (39)	11 (68.75)	2 (12.5)	3 (18.75)
1-1- to 2-0	14 (34.1)	6 (42.85)	6 (42.85)	2 (14.3)
2-1 to 3-0	11 (26.9)	5 (45.4)	6 (54.5)	0
Total	41	22 (53.65) ^c	14 (34.1) ^c	5 (12.2) ^c

^a - Values in parentheses represent percentage of positive flocks in each age range corresponding to the total number of positive flocks.

^b - Values in parentheses represent percentage of tested flocks in corresponding to the total number of positive flocks the same age range.

^c - Values in parentheses represent percentage of flocks corresponding to the total number of positive flocks.

diamine tetraacetic acid [EDTA], pH 8.0) containing ethidium bromide (Promega Corp., Madison, WI, USA) for 45 min at 100 V and visualized under UV light (AlphaImager, AlphaInnotech, San Leandro, CA, USA).

Results

All tested birds were suffering from different enteric symptoms. Thirty (42.9%), 22 (31.4%), and 18 (25.7%) of the examined flocks were within the age groups of hatch to 1-0, 1-1 to 2-0, and 2-1 to 3-0 wk-d of age, respectively (Table 2). PCR testing results showed that all of the above-mentioned viruses were detected in this study (Table 2). Forty one (58.6%) of the tested flocks were positive for at least one of the targeted viruses, where astroviruses, rotavirus, reovirus, and adenovirus type 1 were detected in 27 (38.6%), 13 (18.6), 15 (21.4%), and 10 (14.3%) of the tested flocks, respectively (Table 2). Astroviruses were identified in 38.6% (27/70) of tested flocks, and in 65.9% (27/41) of the total positive flocks, of which ANV was positive in 44.4% (12/27), CAsV in 29.6% (8/27), and 25.9%

(7/27) were co-infected with both ANV and CAsV at the same time (Table 2). Astroviruses were highly predominant in younger ages, where 81.5% (22/27) of astroviruses positive birds were in flocks less than or equal to two wks of age, of these 48.1% (13/27) were in age group 1-1- to 2-0 wk-d (Table 2), while the lowest percentage of astroviruses positive flocks (18.5%) was in age group 2-1 to 3-0 wk-d (Table 2). Furthermore, 29.3% (12/41) of total positive flocks were infected with astroviruses only, and 55.5% (15/27) of astrovirus positive flocks were co-infected with at least one of the above-mentioned viruses (Table 3). Interestingly, 92.9% (13/14) of positive flocks in the age range of 1-1 to 2-0 were positive for astroviruses (Table 2), of which 30.8% (4/13) were co-infected with both ANV and CAsV at the same time (Table 2).

Rotavirus was detected in 18.6% (13/70) of tested flocks, and 31.7% (13/41) of total positive flocks (Table 2). Furthermore, rotavirus detection was higher in older ages than younger ages, where 61.5% (8/13) of rotavirus positive flocks were in age group 2-1 to 3-0 wk-d (Table 2). In addition, 69.2% (9/13) of rotavirus positive flocks were also positive to at least one of the above-mentioned viruses (Table 3).

Reovirus was detected in 21.4% (15/70) of tested flocks, and in 36.9% (15/41) of the total positive flocks (Table 2). Interestingly, reovirus was higher in younger than older ages, where 66.6% (10/15) of reovirus positive flocks were in age group Hatch to 1-0 wk-d (Table 2). Moreover, 60% (9/15) of reovirus positive flocks were also positive to at least one of the other tested viruses (Table 3).

Adenovirus type 1 was detected in 14.3% (10/70) of tested flocks, and 24.4% (10/41) of the total positive flocks (Table 2). 50% (5/10) of adenovirus type 1 positive flocks were in age group 1-1 to 2-0 (Table 2), and 35.7% (5/14) of total positive flocks in this age range were positive to adenovirus type 1 (Table 2). Furthermore, none of the positive flocks were positive only to adenovirus type 1, while co-infection of adenovirus type 1 and reovirus was detected in 40% of the adenovirus type 1 positive flocks (Table 3).

In general, the results outlined here showed that 41.4% (29/70) of tested flocks were negative for all of the above tested viruses (Table 3), whereas 53.6%, 34.1%, and 12.2% of positive flocks were positive for 1, 2, and 3 of the targeted viruses (Table 4). In addition to the above, 39%, 34.1%, and 26.9% of flocks positive for at least one of the tested viruses were within the age groups of Hatch to 1-0, 1-1 to 2-0, and 2-1 to 3-0 wk-d, respectively (Table 4).

Discussion

The results obtained in this study are similar to previously published reports, where the prevalence of astroviruses, reoviruses, and adenoviruses type 1 were the most frequently identified enteric viruses in commercial chicken and turkey flocks (Reynolds et al. (1987a), Reynolds et al. (1987b), Theil and Saif 1987, Yu et al. 2000). However, these earlier studies were conducted using EM and electropherotyping as diagnostic methods, which are not sensitive as molecular techniques used today for viral diagnosis (Pantin-Jackwood et al. 2008). In addition, these earlier studies were performed in both healthy and poorly performing poultry flocks (Reynolds et al. (1987a), Reynolds et al. (1987b), Yu et al. 2000), while this study was conducted in broiler chicken flocks with enteric symptoms and used molecular tests as other recent studies (Pantin-Jackwood et al. 2006, Pantin-Jackwood et al. 2007, Pantin-Jackwood et al. 2008, Smyth et al. 2010) to detect the above-mentioned viruses. Thus, the prevalence of the above enteric viruses as demonstrated in the present study is much higher than previously reported.

The detection of at least one of the above enteric viruses in 73.2% (30/41) of positive flocks in flocks less than or equal to two weeks of age could indicate a high

possibility of vertical transmission of these viruses (Table 4). Previous studies suggested this possibility (Reynolds et al. 1987a) Theil and Saif 1987, Reynolds 2003). Fecal studies showed that hatchery eggs containing rotavirus could also act as potential source of infection for newly hatched chicks (Reynolds et al. 1987a). Further future rigorous studies are required to determine the role of vertical transmission of the above mentioned enteric viruses to newly hatched chicks via the eggs (in the egg or on the surface).

Up to now, two different astrovirus types have been detected in chickens. Avian nephritis virus, originally considered to be a picornavirus, was characterized as an avian astrovirus on the basis of its nucleotide sequence (Imada 2000), while Baxendale and Mebatsion (2004) reported the isolation of a different astrovirus, named CAstV from broiler chickens affected by runt-ing syndrome. The most frequently identified astrovirus in chickens was ANV. No studies on the prevalence of these viruses in broiler flocks in Jordan have been reported previously, however; CAstV and ANV have been detected by serology and RT-PCR in other parts of the world (Pantin-Jackwood et al. 2008, Todd et al. 2009, Smyth et al. 2010). The phylogenetic analysis of these viruses indicated a high diversity (Pantin-Jackwood et al. 2006, Pantin-Jackwood et al. 2007, Pantin-Jackwood et al. 2008). Both ANV and CAstV were identified in this study, where 25.9% (7/27) of astrovirus positive flocks were positive for ANV and CAstV at the same time (Table 2). Because all tested flocks were suffering from enteric symptoms, our data suggest that the astroviruses play an important role in this multifactorial enteric disease in the tested flocks.

Rotavirus infection in avian species is frequently associated with outbreaks of diarrhea and RSS. Rotaviruses from groups A, D, F, and G have been detected in broiler flocks based on electropherotype analysis and serology (Elschner et al. 2005, Otto et al. 2006), and group D avian rotaviruses, also referred to as rotavirus-like virus, have been the most frequently reported rotavirus in poultry (Reynolds 1987a, MuNulty 2003). In previous studies, rotavirus was detected in chicken flocks described in the field as both good and poorly performing flocks with respect to enteric signs; this could be explained by the circulation of more than one avian rotavirus pathotype (Pantin-Jackwood et al. 2008). The higher rate (44.4%) of rotavirus detection in older ages than in younger ages (Table 2), is in agreement with the study published by Yason and Schat (1987) who established that, in general, older chickens and turkeys are more susceptible to rotavirus infection than younger birds. However, group D rotavirus infection has recently been implicated as a contributing factor in the development of RSS in 5-14 d old broilers in Germany (Otto et al. 2006). The re-

sults of this study pave the way for the future typing of rotaviruses in broiler chicken flocks.

Reoviruses have been isolated worldwide from chickens affected by various disease conditions, predominantly including viral arthritis/tenosynovitis, stunting syndrome, enteric disease, malabsorption syndrome and immunosuppression (Jones 2003, Rosenberger 2003). Six (8.6%) of the tested flocks were positive only for reoviruses, whereas in the other flocks reoviruses were combined with one or more other enteric viruses (Table 3). The high detection rate (33.3%) of reoviruses in younger ages indicates a high possibility of viral vertical transmission of these reoviruses (Table 2). Although, as in other countries, breeders in Jordan are usually vaccinated during the rearing period with commercially available live and inactivated reovirus vaccines containing the S1113 strain, we still observe RSS in broiler flocks. Our results documented the involvement of reoviruses as a cause of this multifactorial enteric disease during the first 2 wks of age.

Adenovirus type I has commonly been identified in the feces and tissues of birds with gastrointestinal diseases; however, its role in these diseases remains unproven. Adenovirus type I has been associated in poultry with ventriculitis (Goodwin 1993), proventriculitis (Kouwenhoven 1978), enteritis (Goodwin et al. 1993), and runting/malabsorption syndrome (Kouwenhoven 1978). According to the results of the present study it is clear that this adenovirus plays a role in the multifactorial enteric symptoms which appear in broiler flocks.

Conclusion

We drew three main conclusions from this study. First, this study clearly demonstrates that all of the targeted enteric viruses seem to be widely disseminated and endemic in broiler flocks in Jordan. Second, combined infection with any two of the tested enteric viruses was common in 34.1% (14/41) of positive flocks (Table 4). Finally, the high rate of reovirus (46.6%) and astrovirus (73.3%) detection in positive flocks less than two weeks of age (Table 2) indicates a high possibility of viral vertical transmission, which reaffirms the need for more focused biosecurity and appropriate vaccination programs in broiler breeder flocks.

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