IMMUNOLOGY, HEALTH, AND DISEASE

Relationship between different enteric viral infections and the occurrence of diarrhea in broiler flocks in Jordan

A. M. Lobani,[1] S. M. Gharaibeh, and A. M. Al-Majali

Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan

ABSTRACT   The aim of this study is to determine if enteric viruses are the cause of diarrhea in broiler flocks in Jordan. Intestinal content samples were collected from 101 broiler flocks from several regions of Jordan to detect the presence of astrovirus, coronavirus, reovirus, and rotavirus, by using reverse transcriptase polymerase chain reaction (RT-PCR). Forty-six of these flocks were clinically healthy with no enteric disease, and the other 55 flocks were clinically suffering from diarrhea. The samples were collected between 5 and 16 d of age. The results show that 79% of total 101 flocks tested were infected with one or more of the above enteric viruses. Coronavirus was the most common virus, detected in 56.4% of these flocks, with astrovirus in 29.7% of the flocks, and rotavirus (9.9%) and reovirus (5.6%) being the least common. None of these flocks were found to be infected with all four viruses, but one of the flocks was found to be infected with astrovirus, coronavirus, and rotavirus simultaneously. Individual infection was noted with astrovirus, coronavirus and rotavirus but not with reovirus, whereas all flocks infected with reovirus were also infected with coronavirus. There was no statistical evidence to link these viruses as the main cause of diarrhea in the flocks tested. This is the first study in Jordan to detect all of these viruses and to correlate their presence with diarrhea in chicken flocks.

Key words: chicken astrovirus, avian coronavirus, avian reovirus, avian rotavirus, enteric viruses

INTRODUCTION

Enteric viruses infect the gastrointestinal tract (GIT) of avian species, causing a primary insult that provides the milieu to secondary agents like bacteria to invade and penetrate GIT tissue, leading to further damage (Saif, 2008). These viruses were first detected in turkey flocks worldwide, and were shown to cause severe enteric disorders in these flocks, leading to disease conditions such as poult enteritis and mortality syndrome (**PEMS**) or poult enteritis complex (**PEC**) (Jindal et al., 2010a,b).

Numerous viruses were implicated as a causative agent for enteric disease, all of which were detected in the intestine or intestinal content of chicken and turkey flocks (Day et al., 2007; Pantain-Jackwood et al., 2008). The most common enteric avian viruses are astroviruses (**AstV**), coronaviruses (**CoV**), reoviruses (**RV**), and rotaviruses (**RoV**) (Pantain-Jackwood et al., 2008; Jindal et al., 2010b). In most recent studies, these viruses have been detected in commercial broiler flocks in the United States, causing significant economic loss

due to mortality and low feed conversion rates (**FCR**) as a result of enteritis or diarrhea. Also, this may lead to the disease condition called runting-stunting syndrome or malabsorption syndrome (**RSS** or **MAS**) in broiler flocks (Pantain-Jackwood et al., 2008; Saif, 2008).

Historically, electron microscopy has been used as a diagnostic technique to detect these viruses, which is considered the oldest and simplest method (McNulty et al., 1979; Lozano et al., 1990; Lin et al., 2002; Saif, 2008). Enteric virus isolation and detection by conventional methods has many limitations due to the fact that some of these viruses are very difficult to propagate and isolate using SPF eggs, as in the case of rotaviruses (Jones, 2008). Also, antigen-capture ELISA has shown difficulty in detecting some of these viruses, indicating that this technique is not reliable (Minamoto et al., 1988; Saif, 2008).

Nowadays, molecular techniques represented by polymerase chain reaction (**PCR**) test are considered the most sensitive and accurate tests to detect enteric viruses at the level of the flock. This has paved the way to the molecular characterization and genotyping of these viruses through phylogenetic analysis (Pantain-Jackwood et al., 2008).

Field observations in broiler flocks suffering from enteric disorders like diarrhea and/or enteritis that

negatively respond to chemotherapy in Jordan have highlighted the idea of enteric viral infections. The objective of this study is to detect the following enteric viruses: chicken astrovirus, avian coronavirus, avian reovirus, and avian rotavirus, and to correlate each virus with the diarrhea incidence in our broiler flocks.

MATERIAL AND METHODS

Sample Collection

Samples were collected from various regions of Jordan, and several integrated companies agreed to participate in this study. Field data about the health status of the flocks tested were supplied in order to consolidate experimental parameters for all flocks screened. The small intestine contents were collected from a total of 101 broiler flocks at the age range 5 to 16 d old; 46 flocks were clinically normal with no signs of diarrhea, and the remaining 55 flocks were suffering from diarrhea at the time of collection. Samples from healthy and diseased flocks were collected from each geographical area; the fresh intestinal contents of 5 birds per flock were collected after necropsy and directly shipped with ice bags at 4°C to the laboratory to be stored at −70°C until testing.

Sample Preparation and RNA Extraction

Samples were thawed at room temperature, homogenised using cotton swabs, and 0.25 g was taken and diluted in 1.2 mL of sterile phosphate buffered saline. Samples were then directly vortexed and centrifuged at $3,000 \times g$ for 10 min to eliminate possible inhibitors (protein, mucus, organic matter, etc.), then 150 μL of the supernatant was subjected to RNA extraction using QiaAmp RNA extraction kit (Qiagen, Valencia, CA). Purified RNA was directly subjected to one-step reverse transcriptase PCR (RT-PCR) and the remaining of purified RNA stored at −70°C.

One Step RT-PCR for Chicken Astrovirus

The test was carried out to detect chicken astrovirus by using a primer that was previously described by Day et al. (2007). The primer set targeted a conserved region of the polymerase gene (Table 1), and was able to detect all types of chicken astrovirus (**CAstV**) with a product of 362 nucleotides (**nt**) (Pantain-Jackwood et al., 2006). The reaction mixture volume was 25 μL using a specific kit (Qiagen, Valencia, CA) and contained 5 μL of 5X reaction buffer with MgCl$_2$ included, 4 μL of nuclease free water, 1 μL of dNTPs, 5 μL of 5X Q solution, 3 μL (10 pmol/mL) of each forward and reverse primer, 1 μL of Qiagen One-Step RT-PCR enzyme mix, and 3 μL of extracted RNA. The same reaction mixture was used as standard for the other viruses tested in this study. Thermal cycling conditions using Mycycler thermal cycler (Biorad, Hercules, CA) were as follows: 1 cycle of 50°C for 30 min, 1 cycle of 94°C for 15 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec 72°C for 1 min, and a final extension step at 72°C for 10 min (Day et al., 2007). Some of positive bands of astrovirus (KU253625) were extracted from agarose gel and directly subjected to sequencing in order to confirm their identity (revealed 91% identity to CAstV). These positive samples were used as positive control for subsequent reactions.

One-Step RT-PCR for ACoV

RT-PCR was conducted as previously described in astroviruses by using specific primer targeting a conserved region of the 5′ UTR end of the S1 gene (143 nt) (Table 1). The deduced amino acid sequence detected by this primer set in previous study was identical for M41 (Mass strain of IBV), but this method is unable to detect mammalian coronaviruses (Pantain-Jackwood et al., 2008). Mass strain vaccine strain (Ceva Santé Animal, Hungary) was used as a positive control for coronavirus by using the same thermal cycler (Biorad, Hercules, CA); thermal cycling conditions were as

Table 1. RT-PCR primers used to detect enteric viruses.

Target virus	Target gen	Primer name	Primer sequence	Amplicon length	Reference
CAstV	**ORF-1b**	*CASPOL-F1* *CASPOL-F2*	*5′-GAYCARCGAATGCGRAGR TTG-3′* *5′-TCAGTGGAAGTGGGKARTCTA C-3*	*362*	*Day et al., 2007*
ACoV	**5-UTR**	*IBV5′GU391* *IBV5′GL533*	*5′-GCT TTT GAG CCT AGC GTT-3′* *5′-GCC ATG TTG TCA CTG TCT ATT-3*	*143*	*Callison et al., 2006*
ARV	**S1**	*MK87* *MK88*	*5′-GGTGCGACT GCT GTATTT GGTAAC-3′* *5′-AAT GGA ACG ATA GCG TGT GGG-3′*	*532*	*Catrina et al., 2004*
ARoV	**NSP4**	*NSP4-30* *NSP4-660*	*5′-GTGCGGAAAGATGGAGAA C-3′* *5′-GTTGGGGTACCAGGGATTAA3′*	*630*	*Day et al., 2007*

CAstV = chicken astrovirus; ACoV = avian coronavirus; ARV = avian reovirus; ARoV = avian rotavirus.
ORF-1b = open reading frame 1b; 5UTR = 5 untranslated region; S1 = structural protein 1; NSP4 = non structural protein 4.

follows: 1 cycle of 50°C for 30 min, 1 cycle of 94°C for 15 min followed by 40 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 1 min (Callison et al., 2006).

One-Step RT-PCR for ARV

A set of sensitive primers targeting a 532 nt long region of the S1 gene in a conserved region of ARV was used to detect a wide range of reference and field strains known to cause enteritis (Table 1); the S1 gene of avian species is dissimilar to the mammalian counterpart (Xie et al., 1997). Thermal cycle conditions were as follows: 1 cycle of 50°C for 30 min, 95°C for 15 min followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, with an additional final extension of 72°C for 10 min (Catrina et al., 2004). The positive control used was the S1133 vaccine strain (Merial, France).

One Step RT-PCR for ARoV

A set of primers targeted a 630 nt region of the NSP4 gene (Table 1), which greatly differs from the mammalian counterpart but shows similarity between avian species. It was recently shown that this gene has enterotoxin activity leading to diarrhea in suckling mice (Mori et al., 2002). Thermal cycle condition were as follows: 1 cycle of 50°C for 30 min, 94°C for 15 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min, and the final extension at 72°C for 10 min (Pantain-Jackwood et al., 2008). First positive bands were extracted and subjected to sequencing and revealed >96% identity to other ARoVs (KU253626). These positive samples were used as positive control for subsequent reactions.

Gel Electrophoresis of PCR Products

PCR products were separated using 1.7% agarose gel electrophoresis and stained with 10 μL ethidium bromide. Electrophoresis was run at 120 V for 60 min (Invitrogen Carls Lab, Burlington, Canada) and gels were visualized in an ultraviolet light cabinet (Alphamager-Alphainnotech, San Leandro, CA, USA) to check band size, and a few positive samples were excised for sequencing to confirm what was detected.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS 17) (SPSS Inc., Chicago, Illinois) was used to perform statistical analysis. Chi-square was used to compare the rate of detection for each virus between the normal and clinically affected flocks.

RESULTS

One-Step RT-PCR Result

Enteric viruses screened were detected in 79% of total 101 flocks tested, regardless of the health situation of the flocks (Table 2). Avian coronavirus (ACoV) was the most prevalent virus among those tested in the diarrhea and non-diarrhea groups with prevelance rat (56.4%). Furthermore, there was a significant statistical difference between the two groups; the detection rate was 69% in flocks suffering from diarrhea, higher than in healthy group (approximately 41%). The band size, 143 bp, represents the most conserved region in the 5′ end of the virus genome among avian strains; it is completely different from other, mammalian strains (Pantain-Jackwood et al., 2008).

Chicken astrovirus was the second most common virus (29.7%); in flocks that were found infected with this virus, the test targeted the conserved region of polymerase gene to detect all chicken strains, that diverge from their counterpart in mammalian types, with product size about 362 bp (Day et al., 2007). Some of sequenced positive samples showed ∼91% identity to other chicken astroviruses (KU253625); also, additional molecular characterization is needed to differentiate among avian strains. However, there was no correlation with diarrhea incidence. ARoV was detected in 9.9% of tested flocks, with product size of 630 bp targeting conserved region of NSP4 gene (not a group-specific gene) used to detect all avian strains of RV (Day et al., 2007). Blast analysis revealed 96% identity to ARoV (KU253626). There was difference between diarrhea and non-diarrhea groups. Regarding ARV, this virus was detected with low rate, with around 6% of surveyed flocks found infected with this virus; the abstracted band size 532 bp located in conserved region of the S1 gene can detect a wide range of ARV strains, especially enteric isolates. Also, there were no significant statistical differences between the two groups.

Table 2. RT-PCR results for enteric viruses in broiler flocks with or without diarrhea.

	Enteric virus detected			
	CAstV	ACoV	ARV	ARoV
Flocks with diarrhea	13/55 (23.6%)	38/55 (69%)	3/55 (5.4%)	7/55 (12.7%)
Flocks without diarrhea	17/46 (36.9%)	19/46 (41.3%)	3/46 (6%)	3/46 (6%)
P-value	0.19	0.008	0.572	0.243
All flocks	30/101 (29.7%)	57/101 (56.4%)	6/101 (5.9%)	10/101 (9.9%)

CAstV = chicken astrovirus; ACoV = avian coronavirus; ARV = avian reovirus; ARoV = avian rotavirus.

Table 3. Pattern of concomitant infection with astrovirus, coronavirus, reovirus, and rotavirus in broiler flocks.

CAstV	ACoV	ARV	ARoV	No. of flocks (%)
–	–	–	–	21 /101 (20.8%)
+	–	–	–	16/101 (15.8)
–	+	–	–	35/101 (34.6)
+	+	–	–	10/101 (0.099%)
+	–	–	+	3/101 (0.029%)
+	+	–	+	1/101 (0.0099%)
–	–	–	+	1/101 (0.0099%)
–	+	+	–	6/101 (0.059%)
+	–	–	+	5/101 (0.049%)

CAstV = chicken astrovirus; ACoV = avian coronavirus; ARV = avian reovirus; ARoV = avian rotavirus.

Pattern of Infection

Individual infection by one of the enteric viruses tested was noted in flocks infected with ACoV and CAstV; only one flock was found to be infected with ARoV alone, whereas ARV was never detected alone in any of the flocks tested. On the other hand, flocks positive for ARV were often negative for ARoV and vice versa. In case of mixed infection, the majority of flocks were found infected by two of the enteric viruses, and one flock of the diarrheic group was confirmed infection with three viruses: CAstV, ACoV, and ARoV. None of the flocks investigated in our study were infected by all of the viruses screened simultaneously (Table 3).

DISCUSSION

Intestinal lumen of the chickens have an extensive surface that is mostly exposed to numerous agents or factor lead to diarrhea incidence such as bacterial, parasitic, and viral agents as well as nutrition factors (Yegani et al., 2008). Enteric viruses are among the most problematic microorganisms implicated with diarrhea incidence in broiler flocks (Koo et al., 2013). RT-PCR is currently the most accurate test to detect those viruses worldwide; furthermore. studying the nature of infection, pathogenicity, virulance, and viral diversity that help to predict outbreaks and discovering new strains that could be emerges of these viruses (Pantain-Jackwood et al., 2008).

This study was carried out to investigate prevalence of the enteric viruses (CAstV, ACoV, ARV, and ARoV) in broiler flocks, That potentially a causative agent of diarrhea, broiler flocks in several geographical areas of Jordan investigated for presence the above viruses, to establish a basic data about these viruses infection and circulation in Jordan.

Generally, 79% of the total 101 flocks were found to be infected by at least one of the enteric viruses tested in our study, which may reflect the importance of these viruses regarding performance of broiler flocks. A similar prevalence rate was found by a previous studies in the United States and Korea to detect enteric viruses (Pantain-Jackwood et al., 2008; Koo et al., 2013), but there was a variation of detection for each virus

alone among these studies. In our study, ACoV was the most prevalent among these viruses (ACoV, AstV, ARoV, ARV), which may be due to the intensive use of the IBV vaccine at early age in our broiler farms, potentially increasing the possibility of field virus load and rolling among flocks; hence mutation and diversity may occur due to vaccine virus. Based on S1 gene molecular characteristics study, some of field isolates rolling among broiler flocks were found highly similar to vaccine strains (H120, Mass 41), which are the same used to immunize these flocks against IBV (Gelb et al., 2005). Also, an evolutionary trend from respiratory to enteric and vice versa has already reported (Cavanagh et al., 1992; Cook et al., 1999), with the virus detected at higher proportions in the diarrhea group than in the healthy group. Villarreal et al. (2007) found ACoV to be the main causative agent for enteritis in broiler flocks; other caustive agents were not detected in the surveyed flocks.

CAstVs are known to cause diarrhea in poultry flocks (Baxendal and Mebatsion, 2004). However, the virus prevalance rate was more in healthy flocks than flocks suffering from diarrhea with no significant difference between both groups. In previous studies CAstV was detected in healthy and diseased chicken flocks (Pantain-Jackwood et al., 2006; Pantain-Jackwood et al., 2008), the variation of virus virulence and co-infection with other agents also age of infection may be affected clinical manifestation (Baxendal and Mebatsion, 2004), so the necessity for further molecular characterisation and phylogenetic analysis as well as genotyping are substatantial,to determine and understand pathogenicity and nature of infection for this virus, which remains unclear to date.

Avian reovirus was detected by targeting the S1 gene, which is the most important gene and plays a role in viral pathogenicity (Xie et al., 1997). The virus was detected in our study with no signeficant differences between the two groups, which may be due to the transient nature and age-associated infection of the virus (Rosenberger, 1983; Jones and Guneratnee, 1984), or may be because samples were collected too late for the virus to be present. On the other hand, some ARV strains do not cause diarrhea, but may cause disease conditions in broiler flocks called RSS or MAS; this suggests that the presence of the virus in flocks did not result in diarrhea.

Avian rotavirus groups A, D, F, and G have been confirmed to infect broiler flocks in previous studies using electropherotyping and serology studies (McNulty et al., 1984; Elschner et al., 2005; Otto et al., 2006). Group D rotaviruses are the most frequently identified group in poultry flocks (Saif, 2008). In our study, we detected ARoV by targeting NSP4, which is a non-group-specific gene; the virus was detected in about 12% of the diarrhoeic group and significantly higher than non-diarrhoeic group (6%). The pathogenicity of the virus is sometimes not evident, and subclinical infection frequently occurs (Minamoto et al., 1988). Furthermore,

the age of the flock may play a role in the severity of infection, with older age flocks being more susceptible than younger ones (Yason and Schat, 1987). On the other hand, ARoV is more pathogenic in turkeys than in chickens (Saif et al., 2008; Jindal et al., 2010a), and is known to cause the poult enteritis and mortality syndrome in turkeys. Since Jordan does not have any turkey farms, this may have led to decreased viral load in the field, or may have even resulted in the pathogenicity becoming mild in the environment. A previous study to detect these enteric viruses was carried out by Roussan et al. (2012), but we could not take their results into consideration due to weakness of the work and important missing data.

As a conclusion, enteric viruses are widespread in Jordan, but clinical observations of diarrhea as a result of infection with these viruses are still unclear and depend on other factors such as immunity status of infected flocks, nutrition, presence of other infectious agents, management practecies, and enviromental factors. Such factors make enteric virus infection sometimes difficult to interpret (Guy, 1998).

ACKNOWLEDGMENTS

REFERENCES

Baxendal, W., and T. Mebatsion. 2004. Isolation and characterisation of astroviruses from chicken. Avian Pathol. 33:364–370.

Callison, S., D. Hilt, T. Boynton, B. Sample, R. Robison, D. Swayne, and M. W. Jackwood. 2006. Development and evaluation of a real-time Taqman RT-PCR assay for the detection of infectious bronchitis virus from infected chickens. J. Virol. Methods. 138:60–65.

Catrina, K. M., S., Frasca Jr, T. Girshick, and M. Khan. 2004. Development of a multiplex PCR for detection of avian adenovirus, avian reovirus, infectious bursal disease virus, and chicken anemia virus. Mol. Cell. Probes. 18:298–230.

Cavanagh, D., P. J. Davis, K. A. Janek, A. Cook, D. Li, A. Kant, and G. Koch. 1992. Location of amino acid differences in the S1 spike glycoprotein subunit of closely related serotype of infectious bronchitis virus. Avian Pathol. 21:33–43.

Cook, J. K. A., S. J. Orbell, M. A. Woods, and M. B. Huggins. 1999. Breadth of protection of respiratory tract provided by different live attenuated infectious bronchitis vaccine against challenge with infectious bronchitis viruses of heterologous types. Avian Pathol. 28:471–479.

Day, J. M., E. Spackman, and M. Pantain-Jackwood. 2007. A multiplex RT-PCR test for the differential identification of turkey astrovirus type-1, turkey astrovirus type-2, chicken astrovirus, avian nephritis virus and avian rotavirus. Avian Dis. 51:681–684.

Elschner, M., H. Hotzel, J. R. Diller, and P. Otto. 2005. Isolation, identification and characterization of group a rotavirus from a chicken: the inner capsid protein sequence shows only a distant phylogenetic relationship to most other avian group A rotaviruses. J. Vet. Med. Ser. B. 52:211–213.

Gelb Jr, J., Y. Wiesman, B. S. Ladman, and R. Meir. 2005. S1 gene characteristics and efficacy of vaccination against infectious bronchitis virus field isolates from United States and Israel (1996–2000). Avian Pathol. 34:194–203.

Guy, J. S. 1998. Virus infections of gastrointestinal tract of poultry. Poult. Sci. 77:1166–1175.

Jindal, N., D. P. Patnayak, Y. Chander, A. F. Ziegler, and S. M. Goyal. 2010a. Detection and molecular characterization of enteric viruses from poult enteritis syndrome in turkeys Poult. Sci. 89:217–226.

Jindal, N., D. P. Patnayak, Y. Chander, A. F. Ziegler, and S. M. Goyal. 2010b. Detection and molecular characterization of enteric viruses in breeder turkeys. Avian Pathol. 39:53–61.

Jones, R. C, 2008. Pages 309–328 In: Diseases of Poultry. 12th ed. Y. M. Saif, Blackwell Publishing, Ames, IA.

Jones, R. C., and J. R. Guneratne, 1984. The pathogenicity of some avian reoviruses with particular reference to tenosynovitis. Avian Pathol. 13:173–189.

Koo, B. S., H. R. Lee, E. O. Jeon, M. S. Han, K. C. Min, S. B. Lee, and I. P. Mo. 2013. Molecular survey of enteric viruses in commercial chicken farms in Korea with a history of enteritis. Poult. Sci. 92:2876–2885.

Lin, T. I., C. Loa, S. C. Tsai, C. Wu, T. A. Brayan, H. L. Thacker, T. Hooper, and D. Schrader. 2002. Characterisation of turkey coronavirus from turkey poults with acute enteritis. Vet. Microbiol. 84:179–186.

Lozano, L., S. Hammami, A. E. Castro, and B. Osburn. 1990. Comparison of electron microscopy and polyacrylamide gel electrophoresis in the diagnosis of avian reovus and rotavirus infection. Avian Dis. 36:183–188.

McNulty, M. S., D. Todd, G. M. Allan, J. B. McFerran, and J. A. Greene. 1984. Epidemiology of rotavirus infection in broiler chickens: recognition of four serogroups. Arch. Virol. 81:113–121.

McNulty, M. S., W. L. Curran, D. Todd, and J. B. McFerran. 1979. Detection of viruses in avian faeces by direct electron microscopy. Avian Pathol. 8:239–247.

Minamoto, N., K. Oki, M. Tomita, T. Kinjo, and Y. Suzuki. 1988. Isolation and characterisation of rotavirus from feral pigeon in mammalian cell cultures. Epidemiol. Infect. 100:481–492.

Mori, Y., M. A. Borgan, N. Ito, M. Sugiyama, and N. Minamoto. 2002. Diarrhea-inducing activity of avian rotavirus NSP4 glycoproteins, which differ greatly from mammalian rotavirus NSP4 glycoproteins in deduced amino acid sequence, in suckling mice. J. Virol. 76:5829–5834.

Otto, P., E. M. Liebler-Tenorio, M. Elschner, J. Reetz, U. Löhren, and R. Diller. 2006. Detection of rotaviruses and intestinal lesions in broiler chicks 243 from flocks with runting and stunting syndrome (RSS). Avian Dis. 50:411–418.

Pantain-Jackwood, M. J., E. Spackman, and P. R. Woolcock. 2006. Molecular characterization and typing of chicken and turkey astroviruses circulating in the United States: Implication for diagnostics. Avian Dis. 50:397–404.

Pantain-Jackwood, M. J., M. Day, M. W. Jackwood, and E. Spackman. 2008. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005–2006. Avian Dis. 52:235–244.

Rosenberger, K. 1983. Characterisation of reoviruses associated with runting syndrome in chickens. In: Proc. No 66. International Union of the Immunological Society: Sydney, Australia pp. 141–152.

Roussan, D., I. Shaheen, G. Khawaldeh, W. Totanji, and R. Al-Rifai. 2012. Simultaneous detection of astrovirus, rotavirus, reovirus and adenovirus type I in broiler chicken flocks. Polish J. Vet. Sci. 15:337–244.

Saif, Y. M., 2008, Pages 329–338 In: Diseases of Poultry. 12th ed. Blackwell Publishing, Ames, AI.

Villarreal, L. Y. B., P. E. Brandão, J. L. Chacón, A. B. S. Saidenberg, M. S. Assayag, R. C. Jones, and A. J. P. Ferreira. 2007. Molecular characterisation of infectious bronchitis virus strains isolated from the enteric content of Brazilian laying hens and broiler. Avian Dis. 51:974–978.

Xie, Z., A. A. Fadl, T. Girshick, and M. I. Khan. 1997. Amplification of avian reovirus RNA using the reverse transcription-polymerase chain reaction. Avian Dis. 43:98–105.

Yason, C., and K. Schat. 1987. Pathogenesis of rotavirus infection in various age groups of chicken and turkeys: clinical signs and virology. Am. J. Vet. Res. 48:977–983.

Yegani, M., and D. Korver. 2008. Factors affecting intestinal health in poultry. Poult. Sci. 87:2052–2063.