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REGULAR ARTICLES

Detection of enteric pathogens in Turkey flocks affected with severe enteritis, in Brazil

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Abstract Twenty-two flocks of turkeys affected by enteric problems, with ages between 10 and 104 days and located in the Southern region of Brazil, were surveyed for turkey by PCR for turkey astrovirus type 2 (TAstV-2), turkey coronavirus (TCoV), hemorrhagic enteritis virus (HEV), rotavirus, reovirus, *Salmonella* spp., and *Lawsonia intracellularis* (Li) infections. Eleven profiles of pathogen combination were observed. The most frequently encountered pathogen combinations were TCoV-Li, followed by TCoV-TAstV-2-Li, TCoV-TastV-2. Only TCoV was detected as the sole pathogen in three flocks. Eight and 19 flocks of the 22 were positive for TAstV-2 and TCoV, respectively. Six were positive for S*almonella* spp. and *L. intracellularis* was detected in 12 turkey flocks. Reovirus and HEV were not detected in this survey. These results throw new light on the multiple etiology of enteritis in turkeys. The implications of these findings and their correlation with the clinical signs are comprehensively discussed, illustrating the complexity of the enteric diseases.

Keywords Enteric virus · Bacteria · Enteritis · Turkey

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Introduction

Enteric disorders in turkeys are considered a multifactorial disease associated with infection caused by enteropathogenic viruses and bacteria. Most research has focused on turkey astroviruses (TAstV), turkey coronaviruses (TCoV), and *Escherichia coli* (Barnes and Guy 2003; Mor et al. 2013). Nevertheless, other viruses such as adenovirus, rotavirus, and reovirus and bacteria including *Salmonella* spp. are enterotropic agents associated with enteric problems. These enteric disorders are responsible for increasing mortality, growth depression, and immune dysfunctions (1). For this reason, TAstV and TCoV coinfections are suspected to predispose poults to infections by other "opportunistic" pathogens, such as *E. coli* and other agents (Barnes and Guy 2003; Qureshi et al. 2000).

TAstV has been detected in Brazil (Villarreal et al. 2006; Bunger et al. 2009; Silva et al. 2009). Infection usually occurs during the first 4 weeks of age (Reynolds et al. 1987a), and the virus is more prevalent than any other enteritis-causing agent in poults (Pantin-Jackwood et al. 2007; Pantin-Jackwood et al. 2008; Reynolds and Saif 1986; Reynolds et al. 1987b; Saif et al. 1985), leading to an increase in mortality (Koci and Schultz-Cherry 2002).

TCoV causes a disease of significant economic importance to the turkey industry, called transmissible enteritis or "bluecomb" disease, which affects turkeys of all ages. However, clinical signs are more common in the first 2 weeks of life, usually appearing suddenly, with a high rate of morbidity, depression, anorexia, diarrhea, dehydration, and weight loss (Bunger et al. 2009; Reynolds and Saif 1986; Reynolds et al. 1987a). TAstV and TCoV coinfections are common in turkeys and cause a highly negative impact on intestinal absorptive functions (Ismail et al. 2003). These coinfections have been suggested as one of the causative factors of PEMS.

Avian reoviruses have been detected in enteric diseases, including runting syndrome and PEMS (Heggen-Peay et al.

2002; Pantin-Jackwood et al. 2008) where their role is often uncertain, but they can cause increase in pathogenicity of other infectious agents, including coccidia (Ruff and Rosenberger 1985), *Cryptosporidium* spp. (Guy et al. 1987), and *E. coli* (Rosenberger et al. 1986).

Rotaviruses infections have been found in several domesticated bird species including chickens, pheasants, and ducks (Estes 1990). Recently, a high incidence of rotavirus has been reported in chickens and turkeys in the United States (Day et al. 2007; Jindal et al. 2009; Pantin-Jackwood et al. 2006) but was found not only in feces of poults showing enteritis but also in health ones (McNulty 2003; Pantin-Jackwood et al. 2008).

Hemorrhagic enteritis (HE) is an acute disease caused by a *Siadenovirus* that affects turkeys of 4 weeks of age or more, characterized by acute depression, bloody droppings, and death (Pierson and Domermuth 1997; Pierson and Fitzgerald 2008). The immunosuppressive nature of HE can extend the course of the disease and predispose birds to secondary bacterial infection with, for example, *E. coli* (Pantin-Jackwood et al. 2008).

The genus *Salmonella* includes more than 2,500 serotypes found in many different habitats (Grimont and Weill 2007) and the isolation of *Salmonella* spp. reported in poultry and poultry products is more frequent than in any other species (Gast 2003). Although the role of *Salmonella* spp. in PEMS is not well understood, it has been reported in turkey flocks affected by diarrhea, depression, and lethargy (Jindal et al. 2009).

Lawsonia intracellularis is an obligate intracellular bacterium involved in enteritis outbreaks in different animal species. The agent has been described in deer, rats, hamsters, guinea pigs, rabbits, sheep, horses, foxes, ferrets, swine, non-human primates, emus, and ostriches (Cooper et al. 1997). In swine, a species where the disease has been studied in detail, the chronic form of the disease shows diarrhea with soft, brow feces, rough coat hair, decreased feed consumption, and reduced weight gain. The acute form of the disease usually affects finishing pigs and gilts (4–12 months of age) and is manifested as severe intestinal hemorrhage. Sudden death is common and postmortem examination reveals a pale (anemic) carcass, with hemorrhagic intestinal contents (Jacobson et al. 2010).

The aim of this survey was to detect viruses and bacteria-related with enteric problems in turkeys in Brazilian commercial flocks.

Materials and methods

Clinical history

From January to March 2004, turkey flocks from different farms located in the South region of Brazil (States of Parana, Santa Catarina, and Rio Grande do Sul) showed 70 % morbidity and 30 % mortality in a disease that began during the first week of life. It was characterized by severe diarrhea, weight loss, ruffled feathers, prostration, drooping wings, and nervousness. The signs persisted up to at least 13 weeks of age. At postmortem examination, atrophy of the bursa of Fabricius, loss of intestinal mucosa, enteritis, and gas in the gut were evident. Samples from 22 turkey flocks affected in this way and aged between 10 and 104 days were received at the Laboratory of Avian Pathology in the University of São Paulo. Each sample consisted of the whole enteric tract of five birds per flock, randomly selected (Table 2). The samples were prepared as 20 % suspensions of enteric contents from the entire length in 0.01 M PBS pH 7.4 and clarified at 12,000×*g* for 30 min at 4°C.

PCR detection of viral agents

Total RNA or DNA were extracted with TRIzol reagent (Invitrogen Corporation Carlsbad, CA, USA) according to the manufacturer's instructions for fecal suspensions and so was done to negative (ultra-pure water) and positive controls.

Astrovirus

Astroviruses type 2 were examined by an RT-PCR with specific primers and reaction conditions described by Koci et al. (2000) (Table 1), with some modifications. For positive control was used a field strain of turkey astrovirus (Swamy et al. 1996). Next, 7 μl of RNA were resuspended in DEPC-treated water and denatured at 95 °C for 5 min and added to the reverse transcription mix containing 1 × First Strand Buffer, 1 mM of dNTP, DTT 10 mM, 1 μM of each primer (MKPol10 e MKPol11), and 200U of M-MLV Reverse Transcriptase (Invitrogen™) to a final reaction of 20 μl. The reverse transcription was carried out at 45°C/60', followed by 72°C/10'. The PCR was performed with the addition of 4 μl of complementary DNA (cDNA) to the PCR mix (1 × PCR Buffer (Invitrogen™), 0.2 mM of each dNTP, 0.5 μM of each primer (MKPol10 and MKPol11), 1.5 mM MgCl2, 28.25 μl of ultra-pure water, and 2.5U of *Taq* DNA polymerase (Invitrogen™) to a final reaction of 50 μl and submitted to an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C/30", 56°C/1', and 72°C/2', with a final extension at 72°C/10'.

Coronavirus

Primers and reaction conditions described by Cavanagh et al. (2002) (Table 1), were used with some modifications. An infectious bronchitis virus strain H120 was used as positive control. Next, 7 μl of each RNA were resuspended in DEPC water, denatured at 95°C for 5 min and added to the reverse

Table 1 Primer sequences, genes, agents, and amplicon sizes for the RT-PCR and PCR assays used in this study

Target agents	Target gene	Primer name	Primer sequence	Amplicon size—bp	Reference
Astrovirus type2	Rd Rp	MKPol10 MKPol11	5′ TGG CGG CGA ACT CCT CAA CA 3′ 5′ AAT AAG GTC TGC ACA GGT CG 3′	802 bp	Koci et al. 2000.
Coronaviruses	UTR	UTR 41 UTR 11 UTR 31	5′ ATG TCT ATC GCC AGG GAA ATG TC 3′ 5′ GCT CTA ACT CTA TAC TAG CCT A 3′ 5′ GGG CGT CCA AGT GCT GTA CCC 3′	179 bp	Cavanagh et al. 2002.
Hemorrhagic enteritis virus	Hexon	HEV1F HEV2R	5′ TACTGCTGCTATTTGTTGTG-3′ 5′ TCATTAACTCCAGCAATTGG 3′	1647 bp	Hess et al. 1999.
Avian Rotavirus	NSP4	NSP4-F30 NSP4-R660	5′ GTG CGG AAA GAT GGA GAA C 3′ 5′ GTT GGG GTA CCA GGG ATT AA 3′	1120 bp	Patin-Jackwood et al. 2008.
Avian Reovirus	S4	S4-F13 S4-R1133	5′ GTG CGT GTT GGA GTT TCC CG 3′ 5′ TAC GCC ATC CTA GCT GGA 3′	630 bp	Patin-Jackwood et al. 2008.
Salmonella	InvA	InvAF InvAR	5′ TTG TTA CGG CTA TTT TGA CCA 3′ 5′ CTG ACT GCT ACC TTG CTG ATG 3′	521 bp	Swamy et al. 1996.
Lawsonia intracellularis	16S	A B C D	5′ TAT GGC TGT CAA ACA CTC CG 3′ 5′ TGA AGG TAT TGG TAT TCT CC 3′ 5′ TTA CAG GTG AAG TTA TTG GG 3′ 5′ CTT TCT CAT GTC CCA TAA GC 3′	182 bp	Jones et al. 1993.

transcription mix containing 1 × First Strand Buffer, 1 mM of each of dNTP, DTT 10 mM, 1 μM of each primer (UTR 41 and UTR 11), and 200U of M-MLV reverse transcriptase (InvitrogenTM) to a final reaction of 20 μl. The reverse transcription was carried out at 45°C/60', followed by 72°C/10'. The PCR was performed with the addition of 5 μl of c-DNA to a PCR mix containing 1 × PCR Buffer (Invitrogen™), 0.2 mM of each dNTP, 0.5 μM of each primer (UTR 41 and UTR 11), 1.5 mM MgCl2, 28.25 μl of ultra-pure water and 2.5 U of Taq DNA polymerase (Invitrogen™) to a final reaction of 50 μl and submitted to 94°C/3' for initial denaturation, followed by 35 cycles of 94°C/1', 48 and 72°C/1'30" and a final extension at 72°C/10'.

The nested step was performed with the addition of 5 μl of the PCR product to the nested mix (1 × PCR Buffer (Invitrogen™), 0.2 mM of each dNTP, 0.5 μM of each primer (UTR 41 and UTR 31), 1.5 mM MgCl2, 28.25 μl of ultra-pure water, and 2.5U of 164 Taq DNA polymerase (Invitrogen™) to a final reaction of 50 μl and submitted to the same cycles of the PCR step.

Hemorrhagic enteritis virus

Hemorrhagic enteritis virus (HEV) was surveyed by a PCR. DNA extracted was used in the PCR with the specific primers and reaction conditions described by Hess et al. (1999) (Table 1). HEV commercial vaccine was used as positive control. The PCR was performed with the addition of 5 μl of extracted DNA to a PCR mix containing 1 × PCR Buffer (InvitrogenTM), 0.2 mM of each dNTP, 0.5μM of each primer (HEV1F and HEV2R), 2 mM MgCl2, 24.6 μl of ultra-pure water, and 4U of Taq DNA polymerase (InvitrogenTM) to a final reaction of 50 μl and submitted to 94°C/3' for initial denaturation, followed by 40 cycles of 94°C/1', 55°C/1', and 72°C/1'30" and a final extension at 72°C/10'.

Rotavirus

cDNA was obtained from RT reaction, in the same condition described previously for other RNA viruses. A PCR reaction was conducted as report previously (Pantin-Jackwood et al. 2008) (Table 1). The reaction contained 2 μl of the cDNA, 2.5 μl of 10 × PCR Buffer (InvitrogenTM), 4 μl of 1.25 mM of a dNTP pool, 1.5 μl of each 10 pmol/μl primer (sense NSP4 F30 and antisense NSP4 R660), 0.75 μl of 50mM MgCl2, 12.5 μl of ultra-pure water, and 0.25 μl of 1.25U Taq DNA polymerase (Invitrogen™) to a final reaction of 25 μl and submitted to 95°C/5' for initial denaturation, followed by 35 cycles of 94°C/30", 58°C/30", and 72°C/1' and a final extension at 72°C/10'.

Reovirus

cDNA was obtained from RT reaction, in the same condition described previously for other RNA viruses. A PCR reaction was conducted as described (Pantin-Jackwood et al. 2008) in Table 1. The reaction contained 2 μl of the cDNA, 2.5 μl of 10 × PCR Buffer (Invitrogen™), 4 μl of 1.25 mM of a dNTP pool, 1.5 μl of each 10 pmol/ul primer (sense NSP4 F30 and antisense NSP4 R660), 0.75μl of 50mM MgCl2, 12.5 μl of ultra-pure water, and 0.25 μl of 1.25U Taq DNA polymerase (InvitrogenTM) to a final reaction of 25 μl and submitted to 95°C/5' for initial denaturation, followed by 35 cycles of 94°C/30", 58°C/30", and 72°C/1' and a final extension at 72°C/10'.

Detection of bacterial agents

Salmonella

Salmonella spp. were detected by a PCR. DNA extraction was carried out as described by Boom et al. (1990), and the specific primers and reaction conditions described by Swamy et al. (1996) (Table 1). *Salmonella* enteritidis strain SA 135 from our laboratory was used as a positive control and ultra-pure water as the negative control. The PCR was performed with the addition of 0.75 μl of DNA to the PCR mix 1 × PCR Buffer (Invitrogen™), 0.2 mM of each dNTP, 0.5 μM of each primer (MKPol10 and MKPol11), 1.5mM MgCl2, 14.4 μl of ultra-pure water, and 2.0U of *Taq* DNA polymerase (Invitrogen™) to a final reaction of 25 μl and submitted to an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94, 60, and 72 °C/1' with a final extension at 72 °C/10'.

Lawsonia intracellularis

The detection of *L. intracellularis* nested PCR and reaction conditions were carried out as previously described by Jones et al. (1993) (Table 1). The DNA extraction was carried out as described by Boom et al. (1990) from fecal suspensions and from negative (ultra-pure water) and positive (Enterisol® Ileitis, vaccine strain of *L. intracellularis* obtained from the Boehringer Ingelheim, São Paulo, SP, Brazil) controls.

The PCR was performed with the addition of 5 μl of extracted DNA to a PCR mix containing 1 × PCR Buffer (Invitrogen™), 0.2 mM of each dNTP, 10 μM of each primer (A and B), 1.5 mM $MgCl_2$ and 1U of Taq DNA polymerase (Invitrogen™) and ultra-pure water to a final reaction of 25 μl and submitted to 94 °C/5', followed by 35 cycles of 94, 55, and 72 °C/1'. Nested PCR was performed on 2 μl of each amplification product by using internal primers C and D. The reaction conditions, step times and temperatures, and number of cycles were the same used in the first amplification. The PCR and nested products were submitted to electrophoresis in 1.5 % agarose gel, stained with 0.5 μg/ml ethidium bromide and observed under UV light. Each step (RNA/DNA extraction, reverse transcription, PCR, nested, and electrophoresis) was carried out in different rooms with exclusive pipettes, gloves, and aprons. In the nested step, a tube with ultra-pure water was added every three samples, also added mix and taken to the thermocycler to monitor amplicon contamination.

Page detection for rotavirus

The samples were examined for rotavirus 11-segmented of dsRNAs with PAGE (polyacrylamide gel electrophoresis) according to the method of Herring et al. (1982). Total RNA was extracted with phenol/chloroform, precipitated with ethanol and resolved in 3.5/7.5 % discontinuous polyacrylamide gel under 20 mA for 2 h and stained with silver. NCDV Rotavirus strain (White et al. 1970) was included as positive controls and PBS 0.01 M/BSA 0.1 % pH 7.2 as negative control.

Results

The results of this survey revealed that turkey flocks were affected by several of the pathogens under study, in combinations or alone, which were likely to be compromising their performance (Table 2). TCoV, TAstV-2, Rotavirus, *Salmonella* spp. and *L. intracellularis* coinfections were detected in flocks of turkeys aged 10 to 104 days in a Brazilian turkey-producing region which were suffering from severe enteritis and high mortality. Out of the 22 turkey flocks, 19 were affected with TCoV; eight with TAstV-2; and six with rotavirus by PCR, although when the PAGE technique was employed, all samples were negative. Interestingly, *Salmonella* ssp. and *Lawsonia intracellularis* were detected in 6 and 12 flocks, respectively. All flocks were negative for hemorrhagic enteritis virus (HEV) and reovirus by PCR. The pattern of most frequent combinations, in number of pathogens associated, was TCoV-Li, followed by TCoV-TAstV-2-Li, TCoV-TastV-2. In three flocks, TCoV was detected as the only agent. Some minor combinations such as Sal-Li, TCoV-Sal, and TCoV-Rota-Li also were observed in the affected flocks. According to all pathogens combination shown, in Table 2, there was not prevalent that, supposedly, could indicate an important infectious agent in enteric problems in Brazil.

Discussion

Clinical findings of affected turkey flocks are in agreement with the enteritis and the PEM description (Nagaraja and Pomeroy 1997; Jindal et al. 2012). Our study demonstrated that TCoV, TAstV-2, Rotavirus, *Salmonella* spp., and *L. intracellularis* were identified in turkey flocks aged between 10 and 104 days in Brazilian turkey-producing regions associated with severe enteritis and high mortality. Coinfections were detected in most of 22 flocks presenting clinical signs. The most frequently detected combination was TCoV-Li, followed by TCoV-TAstV-2-Li, and TCoV-TastV-2, demonstrating wide range of multifactorial enteric problems. In the most coinfections, TCoV was involved.

TCoV infection in turkeys leads to virus replication in the apical portion of the intestinal villi, causing malabsorption, poor digestion, and diarrhea and changing of the intestinal environment (Naqi et al. 1971). In contrast, TAstV replicates in the basal portion of the villi and, more rarely, in the crypts, causing osmotic diarrhea (Behling-Kelly et al. 2002; Reynolds and Schultz-Cherry 2003). Furthermore, infections

Table 2 Detection of infectious agents in poultry enteritis and mortality syndrome (PEMS) in turkeys in Brazil (all samples were negative for HEV and reovirus)

Flock designation	Age (days)	Clinical signs	Detection of infectious agents						Pathogen patterns
			Viral agents				Bacterial agents		
			TCoV	TAstV	Rotavirus		*Salmonella*	*Lawsonia intracellularis*	
					PAGE silver staining	PCR			
1	19	Severe diarrhea	−	−	−	−	+	+	Sal, Li
2	19	Severe diarrhea	+	−	−	−	−	+	TCoV, Li
3	14	Severe diarrhea	+	+	−	−	−	+	TCoV, TAstV, Li
4	14	Severe diarrhea	−	−	−	+	+	−	Rota, Sal
5	49	Diarrhea, weight loss, ruffled feathers, prostration, nervousness	+	+	−	+	+	−	TCoV, TAstV, Rota, Sal
6	49	Diarrhea, weight loss, ruffled feathers, prostration, nervousness	+	+	−	−	+	+	TCoV, TAstV, Sal, Li
7	49	Diarrhea, weight loss, ruffled feathers, prostration, nervousness	+	+	−	−	−	−	TCoV, TAstV
8	51	Diarrhea	−	−	−	+	+	+	Rota, Sal, Li
9	13	Diarrhea, nervousness	+	+	−	−	−	+	TCoV, TAstV, Li
10	19	Diarrhea, weight loss, ruffled feathers, prostration, nervousness	+	−	−	+	−	+	TCoV, Rota, Li
11	91	Diarrhea, weight loss, ruffled feathers, prostration, nervousness	+	+	−	−	−	−	TCoV, TAstV
12	29	Diarrhea, weight loss, ruffled feathers, prostration, nervousness	+	−	−	−	+	−	TCoV, Sal
13	10	Diarrhea, weight loss, ruffled feathers, prostration, nervousness	+	−	−	+	−	−	TCoV, Sal
14	91	Severe diarrhea, weight loss	+	+	−	−	−	−	TCoV, TAstV
15	19	Severe diarrhea, weight loss	+	−	−	−	−	+	TCoV, Li
16	56	Severe diarrhea, weight loss, prostration	+	−	−	−	−	−	TCoV
17	56	Severe diarrhea, weight loss, prostration	+	−	−	−	−	−	TCoV
18	99	No information on health status	+	−	−	−	−	−	TCoV
19	104	No information on health status	+	−	−	−	−	+	TCoV, Li
20	104	Diarrhea, weight loss, prostration	+	−	−	−	−	+	TCoV, Li
21	100	Severe diarrhea	+	−	−	+	−	+	TCoV, Rota, Li
22	80	Diarrhea, prostration, arthritis	+	+	−	−	−	+	TCoV, TAstV, Li
Total			19/22	8/22	0/22	6/22	6/22	12/22	11

with these viruses make the enteric tract susceptible to secondary infections by pathogenic bacteria. This characteristic of pathogenesis allows understanding various clinical signs and the diarrhea observed in the animals of this survey.

The results showed that TCoV was detected in all samples from flocks affected and all ages with more than one clinical signs reported, suggesting that it could be the main causal agent involved with the syndrome and the other agents could be considered as secondary.

TCoV infection has been reported in the USA (Pantin-Jackwood et al. 2008), UK (Cavanagh et al. 2002), Canada (Dea et al. 1986), Australia (Nagaraja and Pomeroy 1997), and Brazil (Villarreal et al. 2006; Bunger et al. 2009; Silva et al. 2009) as the causative agent of enteric diseases similar to those reported in this study. TAstV in commercial poultry has a worldwide distribution and causes the most prevalent viral infection in turkeys aged 1 to 5 weeks. This virus often occurs in association with other viruses, mainly rotavirus D, playing a role in enteric problems (Reynolds et al. 1987). Nevertheless, in the present study, birds of several older ages (up to 104 days, i.e., almost 15 weeks) were found positive to TAstV-2, suggesting that the age range of susceptible animals is wider than previously reported. However, in our study, only one flock has this coinfection aged 49 days.

Experimental infection of turkeys using TAstV associated with TCoV led to a more severe clinical response and a high mortality rate when compared to single-inoculations (Yu et al. 2000). In our survey, increase in the severity of clinical manifestation was not described. In cases of PEMS and enteric problems, mortality is usually high, and it is possible that outbreaks of this disease are caused by coinfections with two or more viruses, such as TAstV and TCoV (Xu et al. 1990; Barnes and Guy 2003; Jindal et al. 2012). In our study, we detected a concomitance of four of the seven agents studied and the synergism between coronaviruses and other enterotropic viruses, such as Astroviruses, *Salmonella*, and *L. intracellularis* could have been responsible for the severity of the enteric disease observed and for the high mortality rates in the flocks surveyed. Jindal et al. (2009) reported the inoculation of a suspension positive for rotavirus, astrovirus, and *Salmonella*, resulted in significant lower body weights that controls. TAstV and TCoV can be detected in intestinal contents of poults prior to the onset of clinical disease and gross pathologic changes; in the same way, poults in the later stages of astrovirus infection may display clinical signs even without detectable astrovirus particles in their intestinal tract (Reynolds and Schultz-Cherry 2003). This may explain why some apparently normal flocks or without information, such as those in the present study, were positive to both TCoV and Li and while flocks exhibiting typical signs of TCoV infection were negative to these viruses. Avian reoviruses are frequently identified in enteric diseases, including runting syndrome, growth impairment, and PEMS (Heggen-Peay et al. 2002). In our survey, reovirus was not detected, although some clinical manifestation could be associated with this virus (for ex. flocks 1, 2, and 8). The same result was obtained with HEV. According to turkey industry, in Brazil, since 2002, flocks are not vaccinated due to absence of clinical manifestation or diagnosis of hemorrhagic enteritis. The presence of rotavirus detected by PCR is worth noting, because the commonly used PAGE detection has been shown to be 100,000 times less sensitive than the revere transcription of the polymerase chain reaction (Gouvea et al. 1994; Bezerra et al. 2012). Pantin-Jackwood et al. (2007) also found out a higher prevalence of astrovirus and rotavirus in healthy flocks when compared the same results in those previously studied using PAGE and electron microscopy techniques. Besides, PCR is able to detect non-group A rotaviruses, in the case of mixed infections (Pantin-Jackwood et al. 2007; Bezerra et al. 2012). Remarkably, all six positive results were found to be in association with another agent and five of them showed signs that indicate relevant damage into the intestinal mucosa, probably due to destruction of enterocytes by rotavirus in association with *Salmonella* spp. Plus damage of crypts cells by TAstV and of cells from the apical portion of villi by TCoV exacerbated the clinical status of birds.

This study presents the first description of *Lawsonia intracellularis* in turkeys and their possible relation with enteric problems. Jones et al. (1993) observed that 21 clinically normal pigs showed microscopic lesions suggestive of proliferative enteritis. The clinical findings, age of the flocks, and agents detected illustrated the complexity of the enteric diseases, and an interpretation of the role of each agent involved in enteric problems remains a challenge for future studies.

In conclusion, as turkey production in Brazil has grown significantly in different regions of the country, surveillance on the prevalence and distribution of TAstV, TCoV, rotavirus, *Salmonella* spp., and *L. intracellularis* must be carried out in order to determine risk assessment for turkey industry. Molecular techniques as PCR and RT-PCR are more sensitive and sensible in the diagnosis giving the opportunity to detect many agents and determinate their presence in the enteric diseases. Furthermore, experimental studies using SPF birds are needed to appraise the synergism among these enteropathogens and others such as protozoa and bacteria, such as *E. coli* and *Brachyspira* spp. A comprehensive molecular characterization of the identified viruses and bacteria will help understand genetic diversity and assist in establishing preventive and control measures for turkey enteric disease.

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