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Infectious bronchitis virus in different avian physiological systems—
A field study in Brazilian poultry flocks

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ABSTRACT Avian infectious bronchitis is a highly contagious viral disease with economic effects on poultry agribusiness. The disease presents multi-systemic clinical signs (respiratory, renal, enteric, and reproductive) and is caused by one coronavirus (infectious bronchitis virus, IBV). Infectious bronchitis virus is classified into different serotypes and genotypes (vaccine strains and field variants). This study aimed to evaluate the occurrence of IBV in commercial poultry flocks from 3 important producing regions in Brazil and to determine the tropism of the main circulating genotypes to 3 different avian physiological systems (respiratory, digestive, urinary/reproductive). Clinical samples with suggestive signs of IBV infection were collected from 432 different poultry commercial flocks (198 from broilers and 234 from breeders). The total number of biological samples consisted of organ pools from the 3 above physiological systems obtained of farms from 3 important producing regions: midwest, northeast, and south. Infectious bronchitis virus was detected by reverse-transcription,

real-time PCR of the 5' untranslated region. The results showed 179 IBV-positive flocks (41.4% of the flocks), with 107 (24.8%) from broilers and 72 (16.8%) from breeders. There were similar frequencies of IBV-positive flocks in farms from different regions of the country, most often in broilers (average 54%) compared with breeders (average 30.8%). reverse-transcription was more frequently detected in the digestive system of breeders (40%), and in the digestive (43.5%) and respiratory (37.7%) systems of broilers. Infectious bronchitis virus genotyping was performed by a reverse-transcription nested PCR and sequencing of the S1 gene from a selection of 79 IBV-positive flocks (45 from broilers and 34 from breeders). The majority of the flocks were infected with Brazilian variant genotype than with Massachusetts vaccine genotype. These results demonstrate the predominance of the Brazilian variant (mainly in the enteric tract) in commercial poultry flocks from 3 important producing regions in Brazil.

Key words: infectious bronchitis virus, poultry, avian, genotype, Brazil

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INTRODUCTION

Infectious bronchitis is a highly contagious viral disease that may be associated with multi-systemic clinical signs (respiratory, renal, enteric, and reproductive) and reduced production in poultry commercial flocks. Besides these, airsacculitis due to co-infection with *Mycoplasma* or *Escherichia coli* is responsible for the condemnation of a large number of carcasses in slaughterhouses, generating great economic impact on

the poultry industry (Cavanagh and Gelb, 2008). Infectious bronchitis virus (IBV) is a member of the *Coronaviridae* family and has a single-strand positive sense RNA genome with approximately 27 kb. The IBV has 4 structural proteins: nucleocapsid, membrane, envelope, and spike (S), and this last protein is cleaved in the S1 and S2 subunits after translation (Cavanagh, 2007). The S gene is highly polymorphic and is the main responsible for the antigenic variation of the virus (Jackwood et al., 2012).

Infectious bronchitis virus has tropism for several avian cell types and presents different clinical signs and pathogenic forms according to the affected tissue (Di Fábio and Buitrago, 2009; Fan et al., 2012). Initial IBV replication occurs in the respiratory tract, usually

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in epithelial cells of trachea (eventually also in nasal turbinates, lungs, and air sacs) and the main clinical signs are of a cold. Afterward, the virus replicates in other parts of the body, as the oviduct of the female reproductive tract, urinary system (renal tubular epithelial cells), and several organs of the digestive tract (esophagus, proventriculus, duodenum, jejunum, cloacal bursa, cecal tonsils, rectum, and cloaca). According to the physiological system affected by IBV, the chickens can present evident clinical signs as respiratory disease, false layers syndrome, decreased egg production, nephritis, and enteric disorders. All these different pathogenic forms have been reported in different poultry producing regions of the world, including Brazil (Cavanagh, 2007; Villarreal et al., 2007; Cavanagh and Gelb, 2008; Di Fábio and Buitrago, 2009; Cook et al., 2012).

Confirmation of the IBV infection must be carried out by laboratory testing. Classical methodology includes virus isolation in embryonated SPF eggs and subsequent identification by serum neutralization. The whole process is lengthy and laborious because field isolates usually require more passages to cultivate the virus in embryonated eggs (De Wit, 2000; Cavanagh and Gelb, 2008). Molecular techniques, such as reverse transcription (**RT**) followed by PCR, do not require prior isolation and therefore have been widely used for detection and genotyping the virus directly from clinical samples (Cavanagh and Gelb, 2008). The main clinical specimen for this analysis is traditionally the trachea, considered the first replication site in the beginning of the infection. However, the cloaca and cecal tonsils are also recommended because IBV can replicate in the digestive tract in later stages of the infection (De Wit, 2000; Cavanagh and Gelb, 2008).

Infectious bronchitis virus control is usually performed with the use of live attenuated or inactivated vaccines (or both) in commercial poultry flocks. In Brazil, only Massachusetts (**Mass**) derived strains are allowed in commercial vaccine production by the Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento). However, this approach may not be effective when the field strains are different from the vaccine (Cavanagh, 2007). Further, new serotypes/genotypes (antigenic variants) are continuously emerging in the field due to genetic events (recombination or point mutations) within field and vaccine strains (Jackwood et al., 2012).

The first IBV isolate in Brazil was from the Mass serotype (Hipólito, 1957). Three decades later, IBV isolates of at least 5 different antigenic types were found in commercial chickens throughout Brazil (Di Fábio et al., 2000). More recent molecular epidemiological studies revealed the predominance of local field variants (identified as **BR** genotypes) in the majority of the important poultry-producing regions in Brazil (Villarreal et al., 2007; Felipe et al., 2010; Villarreal et al., 2010; Chacón et al., 2011; Fraga et al., 2013). In this period,

an IBV strain similar to genotype 4/91 was also described in southeastern Brazil (Villarreal et al., 2010). Despite these previous works, information about the frequency and distribution of IBV in geographic regions with an increase in poultry production (such as north-east and midwest) as well as the spread of the different genotypes in the physiological systems of the chickens is still scarce and not available, hindering the control and prevention of disease. This field study aimed to evaluate the occurrence of IBV in commercial poultry flocks from 3 important producing regions in Brazil and to determine the tropism of this virus (and the main circulating genotypes) to 3 different avian physiological systems (respiratory, digestive, urinary/reproductive) in commercial poultry flocks (broilers and breeders).

MATERIALS AND METHODS

Clinical Samples

Samples were collected from poultry flocks with clinical signs of infectious bronchitis (234 breeders and 198 broilers) in the period of January of 2010 to December of 2011. These flocks were from farms located in cities of 7 different states in the south (235 flocks), midwest (94 flocks), and northeast (103 flocks) regions from Brazil. The breeders age ranged from 1 to 90 wk (average = 20 wk) and broiler age ranged from 0.5 to 8.8 wk (average = 5.1 wk). All the samples were analyzed in pools (set of organs collected from 3 to 5 birds). Each pool contained organs from specific physiological systems: respiratory (lung, trachea, tracheal swabs), digestive (cecal tonsil and intestine), and urinary/reproductive (kidney, ovary, oviduct).

RNA Extraction

Viral RNA extraction was performed with commercial kits NewGene (Preamp and Prep) according to the protocol of the supplier (Symbios Biotechnology, Cachoeirinha, RS, Brazil). Briefly, swabs and macerated organs from each pool were placed in 1 mL of lysis solution (Preamp) and incubated for 10 min at 60°C. After centrifugation for 1 min ($8,609 \times g$ at room temperature), 0.5 mL was removed and added to a new tube containing 20 μ L of silica suspension. The tube was again centrifuged for 1 min ($8,609 \times g$ at room temperature). The supernatant was discarded and the pellet washed successively with 150 μ L of wash solutions A, B, and C (Prep). After the last wash, the silica was dried at 60°C and total RNA was eluted with 50 μ L of elution buffer.

IBV Detection

Infectious bronchitis virus was detected by reverse transcription followed by real-time TaqMan PCR (**RT-qPCR**) as previously described (Callison et al., 2006).

All the amplification reactions were performed in a StepOnePlus Real Time PCR System (Applied Biosystems, Norwalk, CT) under the following conditions: 1 cycle of 37°C for 30 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min.

IBV Genotyping

Some RT-qPCR-positive samples were randomly selected for IBV genotyping. The RNA from these samples was subjected to RT-nested-PCR amplification and sequencing of the S1 IBV gene as previously described (Fraga et al., 2013). Phylogenetic analysis was performed based on the nucleotide sequence, and the samples were classified in genotypes Mass and BR, the latter including the Brazilian variant genotypes BR-I and BR-II (Fraga et al., 2013).

Statistical Analysis

Chi-squared test was used to compare the frequencies of IBV-positive flocks (and also the main genotypes) in the different geographic regions, age categories, and physiological systems. Poultry flocks were classified according to the geographic origin in 3 groups: midwest (centro-oeste), northeast (nordeste), and south (sul). They were also classified into 4 categories for age comparison analysis: young broilers (0 to 4.5 wk), old broilers (4.6 to 9.0 wk), young breeders (0 to 33 wk), and old breeders (33.1 to 66 wk). The clinical samples were finally divided in 3 groups according to the analyzed specimen: respiratory (lung, trachea, tracheal swabs), digestive (cecal tonsils and intestines), and urinary/reproductive (kidney, ovary, oviduct). Differences were considered significant when $P < 0.05$.

RESULTS

IBV in Different Brazilian Regions

In this study, 1,294 organ pools were analyzed with a total of 382 broiler pools (from 198 different flocks) and 912 breeder pools (from 234 flocks). Breeders were usually more sampled (average 3.9 samples / flock) than broilers (average 1.9 samples/flock). The flock was considered IBV positive when at least one pool of the different systems (respiratory, digestive, urinary/reproductive) presented a positive amplification result by RT-qPCR. A total of 179 flocks (41.4%) presented positive result for IBV, 107 (24.8%) from broilers and 72 (16.8%) from breeders. There was no significant difference in the IBV frequency in breeder ($30.8 \pm 5.4\%$) and broiler ($54\% \pm 8.4\%$) flocks from different geographical regions, but the higher IBV frequency in broiler than in breeder flocks was observed in the 3 geographic regions (Figure 1).

Infectious bronchitis virus from 79 randomly selected flocks (45 broilers and 34 breeders) was genotyped by

performing S1 gene partial sequencing. Sequence analysis showed the occurrence of 3 genotypes, 23 samples (29.1%) of the Mass vaccine, 52 samples (65.8%) of the field variant BR-I (Chacón et al., 2011; Fraga et al., 2013), and 4 samples (5.1%) of the field variant BR-II (Fraga et al., 2013). The 2 Brazilian genotypes (BR-I and BR-II) were grouped as BR variants for the subsequent analyses. Genotypes Mass and BR were detected in broiler and breeder flocks from the 3 regions. Mass genotype was identified in 20 broiler flocks and in 3 breeder flocks. The BR was more frequent and widespread than Mass genotype, being detected in 25 broiler and 31 breeder flocks. The proportion of Mass genotype in broilers was significantly higher than that observed in breeders ($P < 0.05$).

IBV in Flocks with Different Ages

Age information was available for 174 broiler and 227 breeder flocks (these data were missing in 24 broiler and 5 breeder flocks). Infectious bronchitis virus was detected in 101 (58%) broiler flocks, 36 (35.6%) between 0 and 4.5 wk (younger broilers) and 65 (64.4%) between 4.6 and 9.0 wk (older broilers). In breeders, 70 (30.8%) were IBV positive, 43 (61.4%) between 0 and 33 wk (younger breeders) and 27 (38.6%) between 33.1 and 66 wk of age (older breeders; difference not significant). In addition, age and genotype (Mass or BR) were available for 74 flocks (41 broilers and 33 breeders). In broilers, the Mass genotype was detected more often in younger ages (0.0 to 4.5 wk) than BR strains ($62.5 \times 37.5\%$), whereas BR genotypes were largely predominant (88.2%) in the older ages (4.6 to 9 wk; $P < 0.05$). In breeders, BR strains were predominant (90.9%) in all ages (Table 1).

IBV in Different Avian Physiological Systems

Broiler and breeder flocks were classified into 7 groups according to the availability of the IBV results from the different physiological systems: digestive, respiratory, and urinary/reproductive (these last 2 were grouped together; Table 2). The results from 73 flocks (59 broiler and 14 breeder flocks) could not be compared because the organs from different systems were mixed in a unique pool for the analysis. Available analysis results from the remaining flocks were mostly obtained from the respiratory system (131 broiler, 200 breeder), followed by the digestive (62 broiler, 82 breeder) and urinary/reproductive (37 broiler, 109 breeder) systems (Table 3).

Infectious bronchitis virus-positive samples were mostly found in samples of the digestive (59/144, 40.9%), followed by respiratory (82/331, 24.7%) and urinary/reproductive (24/146, 16.4%) systems, these last 2 without a significant difference ($P < 0.05$). In the separate analysis of broiler and breeder flocks results,

Table 1. Infectious bronchitis virus detection and genotyping in broiler and breeder flocks classified by age range

Item	Age range (wk)	Detection (flocks)		Genotyping (flocks)		
		Total	Positive (%)	Total	Mass (%)	BR (%)
Broilers	0.0 to 4.5	67	36 (35.6)	24	15 (62.5)	9 (37.5)
	4.6 to 9.0	107	65 (64.4)	17	2 (11.8)	15 (88.2) ^a
Breeders	0.0 to 33.0	124	43 (61.4)	22	2 (9.1)	20 (90.9)
	33.1 to 66.0	103	27 (38.6)	11	1 (9.1)	10 (90.9)

^aStatistical difference of the proportion the Massachusetts (Mass)/Brazilian (BR) genotypes between “young” and “old” broilers (χ^2 test with $P < 0.05$).

this finding was confirmed only for breeders, because 32 of 82 (39%) were IBV positive in the digestive system, in comparison with 33 of 200 (16.5%) in the respira-

tory and 18 of 109 (16.5%) in the urinary/reproductive systems ($P < 0.05$). In broilers, the frequency of IBV detection was similar in the digestive (27 of 62, 43.5%)

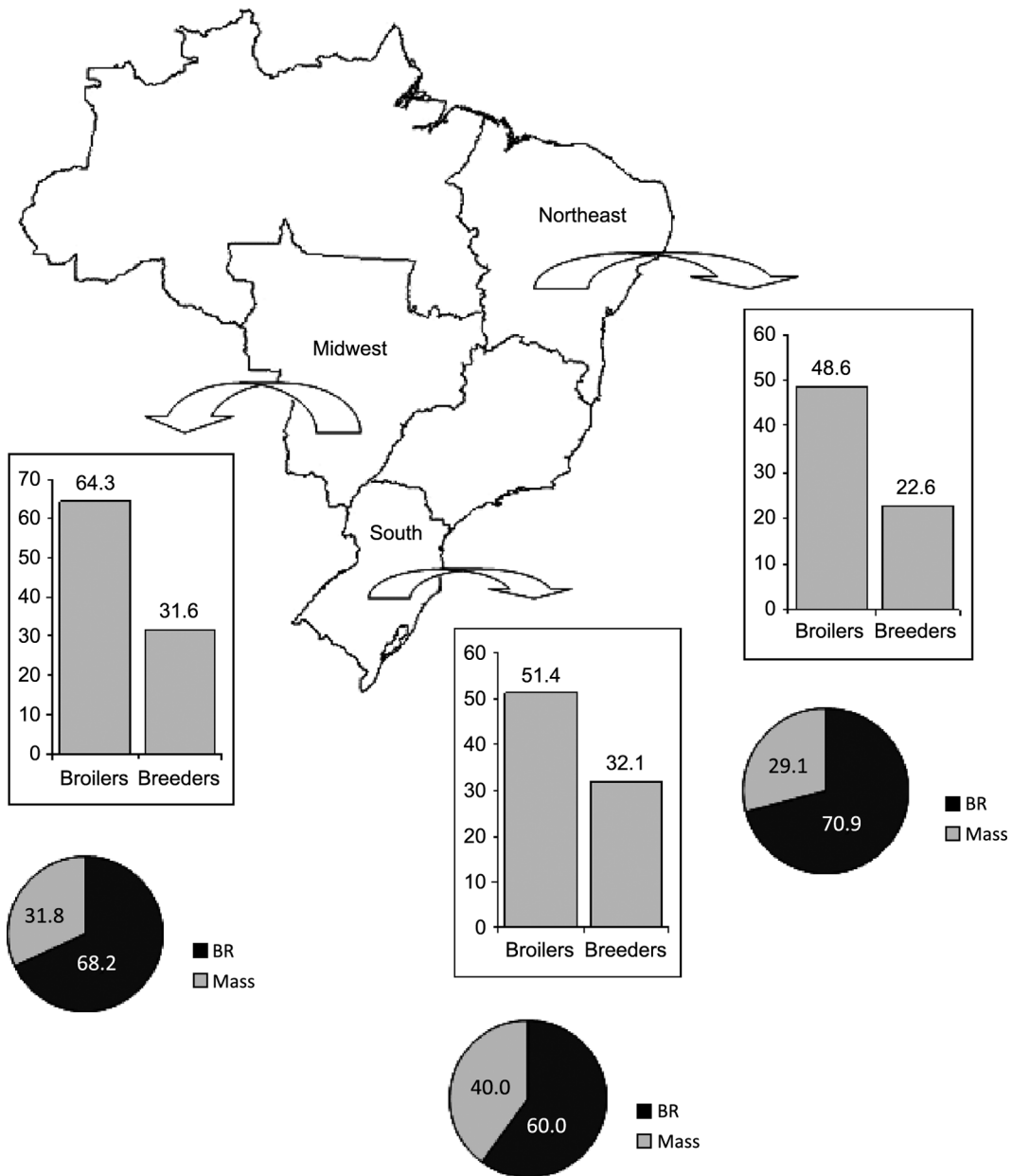


Figure 1. Infectious bronchitis virus (IBV) detection and genotyping. Bar graphics demonstrate IBV frequency (%) in broilers and breeders flocks suspect to have infectious bronchitis from the 3 different Brazilian geographic regions. Pie charts demonstrate the genotype frequency in each region. BR = Brazilian; Mass = Massachusetts.

Table 2. Total number (and percentage) of the available samples for each physiological system in broiler and breeder flocks

Group	Digestive	Respiratory	Urinary/reproductive	Broiler flocks		Breeder flocks	
				Total	%	Total	%
1	NA ¹	+	NA	67	(48.2)	100	(45.5)
2	+	+	+	27	(19.4)	60	(27.3)
3	NA	+	+	8	(5.8)	31	(14.1)
4	NA	NA	+	2	(1.4)	9	(4.1)
5	+	NA	NA	7	(5.0)	8	(3.6)
6	+	+	NA	28	(20.1)	6	(2.7)
7	+	NA	+	0	(0.0)	6	(2.7)
Total				139	(100)	220	(100)

¹Not available (samples of these systems were not collected).

and respiratory (49 of 131, 37.4%) systems, which were significantly higher than in samples of the urinary/reproductive systems (6 of 37, 16.2%; $P < 0.05$; Table 3).

These results were confirmed in the comparative analysis of 87 flocks (27 broiler and 60 breeder), which had PCR results for the 3 organ groups (digestive, respiratory, and urinary/reproductive). In broilers, IBV was commonly detected in the samples of the digestive and respiratory tracts (9 out of 12, 75% for both groups), but rarely in samples of the urinary/reproductive systems (2 out of 12, 16.7%; $P < 0.01$). In breeders, IBV was detected mainly in the samples of the digestive system (22 out of 26, 84.6%), whereas virus frequency detection was significantly lower in the samples of the respiratory (6 out of 26, 23.1%) and urinary/reproductive (11 out of 26, 42.3%) systems ($P < 0.01$; Table 4).

Genotypes Mass and BR were found in the different physiological systems investigated. The BR genotypes were found in similar frequency in the 3 systems, whereas the Mass genotype was significantly more frequent in the respiratory system than in the 2 other systems ($P < 0.01$; Table 5).

DISCUSSION

In a total of 432 flocks (broilers and breeders) with suggestive signs of infectious bronchitis, IBV was detected in 41.4% of them in 3 important poultry-producing regions of Brazil. There was no difference in IBV frequency in these regions, even between the south, traditional poultry-producing zone, and the other 2 regions (midwest and northeast) that started commercial poultry production more recently. Other previous studies have also reported IBV prevalence in different regions

of the country, showing that infectious bronchitis is a disseminated disease in Brazilian poultry flocks (Villarreal et al., 2010; Chacón et al., 2011). The present study reinforces the high frequency of this disease in the country, even in the 2 more recent and growing producing regions. It was also further demonstrated that IBV is detected in a significantly higher proportion of broiler (average 54.8%) than in breeder flocks (28.8%), probably because the biosecurity programs applied to breeders are more rigorous than to broilers.

This study also observed the occurrence of local field variant genotypes (BR-I and-II) and Mass vaccine strains in Brazilian broiler and breeder commercial poultry flocks. The BR genotype was found in a higher frequency than Mass genotype in all the 3 regions. Further, BR genotype sequences had a higher identity with other Brazilian field variants previously described (Villarreal et al., 2007; Felipe et al., 2010; Chacón et al., 2011; Fraga et al., 2013). These data reaffirm the high prevalence of BR genotypes in Brazilian commercial poultry flocks in 2 recent years (2010 to 2011), a similar scenario for the period between 2003 and 2009 (Felipe et al., 2010; Villarreal et al., 2010; Chacón et al., 2011). An important new finding is the high frequency (90%) of BR genotypes in breeder flocks in all ages (Table 1).

On the other hand, the Mass genotype was found in a significant number of poultry flocks from the 3 geographic regions, mainly in broiler flocks. The samples identified as Mass displayed a high identity with Massachusetts reference sequences (H120, M41), demonstrating these flocks were probably infected with vaccine-derived strains. Although there was no information about the IBV immunization status of the flocks, the higher frequency of Mass genotype in young broiler

Table 3. Infectious bronchitis virus (IBV) detection in the different physiological systems

Physiological system	All flocks		Broiler flocks		Breeder flocks	
	Total	IBV+ ¹ (%)	Total	IBV+ (%)	Total	IBV+ (%)
Digestive	144	59 (40.9) ^a	62	27 (43.5) ^a	82	32 (39) ^a
Respiratory	331	82 (24.7) ^b	131	49 (37.4) ^a	200	33 (16.5) ^b
Urinary/reproductive	146	24 (16.4) ^b	37	6 (16.2) ^b	109	18 (16.5) ^b

^{a,b}Different letters indicate statistical significant differences in the IBV detection from the respective physiological systems by χ^2 test ($P < 0.05$).

¹IBV+ = IBV positive.

Table 4. Comparison among the results of the 3 physiological systems in broilers and breeders (the 3 systems were investigated in all these flocks)¹

Item	Physiological system								
	Digestive			Respiratory			Urinary/reproductive		
	IBV+	IBV-	Total	IBV+	IBV-	Total	IBV+	IBV-	Total
Broiler flocks									
IBV+	9	3	12	9	3	12	2	10	12
IBV-	0	15	15	0	15	15	0	15	15
Total	9	18	27	9	18	27	2	25	27
Breeder flocks									
IBV+	22	4	26	6	20	26	11	15	26
IBV-	0	34	34	0	34	34	0	34	34
Total	22	38	60	6	54	60	11	49	60

¹Flock status was defined with at least one infectious bronchitis virus positive (IBV+) result in any clinical sample. IBV- = IBV negative.

flocks is probably related to the use of commercial Mass vaccines (usually held in the hatchery in the first day of life; Cavanagh and Gelb, 2008; Maclachlan and Dubovi, 2011).

The comparative analysis of samples from different physiological systems demonstrated a higher frequency of IBV-positive samples (mostly BR genotype, but even Mass vaccine strains) in the digestive tract than in the other physiological systems. Coronaviruses support the harsh conditions of the gastrointestinal tract (Flint et al., 2000). Further IBV strains have tropism and grow in cells of the digestive system (esophagus, proventriculus, duodenum, jejunum, cloacal bursa, cecal tonsils, rectum, and cloaca) and may establish persistent infections in birds of different ages without any pathobiological clinical effect (Cavanagh, 2003). Different strains have been isolated from cecal tonsils, cloacal swabs, and feces, many of them persisting for long periods (Raj and Jones, 1997; Cook et al., 2012). Other studies have already shown that intensive virus replication occurs in the cecal tonsils, ileum, and rectum, but not in the duodenum or proventriculus (Raj and Jones, 1996; Dolz et al., 2012). These viruses are probably being excreted in the feces, where they can still remain for a long period and be a potential source of infection (Cavanagh and Gelb, 2008; Cook et al., 2012). This is a likely explanation for the IBV persistence in the commercial flocks (mainly BR genotypes) and consequently the high prevalence of these field variants in studies performed previously in Brazil (Villarreal et al., 2010; Chacón et al., 2011; Fraga et al., 2013). Further studies should be conducted to assess the occurrence and

viability of the different genotypes (BR-I, BR-II, and Mass) in feces under field conditions.

Although less frequent, IBV was also detected in respiratory samples. The respiratory tract is the main site for the multiplication of the IBV strains, especially during the first 3 to 5 d after infection (De Wit, 2000). The viral titer decreases rapidly in the second week after infection; however, IBV can persist for a period up to 77 d in the trachea in some special situations (Naqi et al., 2003). In the present study, Mass and field variant genotypes were detected in the trachea of birds with different ages. Mass genotypes were detected primarily in younger broilers (between 0 and 4.5 wk of age, corresponding to the probable multiplication period after vaccination), although they were also observed in 3 additional breeder flocks with older ages (24, 28, and 49 wk). However, strains from the BR genotype were detected in a different number of ages in breeder and broiler flocks. Infectious bronchitis virus-positive breeder flocks had a minimum of 13 wk and a maximum of 61 wk of age, whereas positive broiler flocks had a minimum of 1.4 and a maximum of 6.5 wk (data not shown). This wide age variation is probably related to the different episodes of initial infection with BR strains that are circulating in the flocks (Cavanagh and Gelb, 2008). However, the possibility should not be ruled out that strains from the BR genotype have a longer persistence in the trachea, as found with the Arkansas strain (Cavanagh and Gelb, 2008; Jackwood et al., 2009).

Infectious bronchitis virus was rarely detected in samples (such as kidneys and oviducts) of the urinary

Table 5. Infectious bronchitis virus genotyping in the different physiological systems in broiler and breeder flocks¹

System	All flocks			Broiler flocks			Breeder flocks		
	Total	Mass (%)	BR (%)	Total	Mass (%)	BR (%)	Total	Mass (%)	BR (%)
Digestive	18	1 (5.5)	17 (94.5)	11	1 (9.1)	10 (90.9)	7	0 (0)	7 (100)
Respiratory	30	11 (36.7)	19 (63.3)	16	8 (50)	8 (50)	14	3 (21.4)	11 (78.6)
Urinary-reproductive	13	1 (7.7)	12 (92.3)	1	1 (100)	0 (0)	12	0 (0)	12 (100)
Total	61	13 (21.3)	48 (78.7)	28	10 (35.7)	18 (64.3)	33	3 (9.1)	30 (90.9)

¹Mass = Massachusetts; BR = Brazilian.

and reproductive system of broilers and breeders. The only positive sample of these system in broilers belonged to the Mass genotype, whereas all the positive samples found in the oviducts ($n = 3$) and kidney ($n = 9$) of breeders belonged to the BR genotype. The Mass genotype was detected in a sample of broilers at 4.1 wk of age (probably related to the dissemination of the Mass vaccine strain in immunized flock), whereas the BR genotype was found in breeder flocks with different ages (13 to 30 wk). Infectious bronchitis virus replication in the kidney or oviduct is not directly correlated with evident renal disease, but some IBV strains are intrinsically nephropathogenic (Cavanagh, 2007). Specifically, strains from the BR genotype have already been detected in poultry flocks with nephritis and reproductive disorders (Villarreal et al., 2010; Chacón et al., 2011). Furthermore, Dolz et al. (2012) detected IBV in epithelial cells of the ureters by immunohistochemistry, suggesting that retrograde tubular peristalsis, a physiological characteristic of poultry (Laverty et al., 2006), can contribute to the successful replication of IBV in this system.

In Brazil, veterinary laboratories usually recommend collecting and analyzing samples of the respiratory system for IBV detection. In the present study, trachea and tracheal swabs were the only samples collected and evaluated by RT-qPCR in approximately half of the commercial poultry flocks (45.5% breeders and 48.2% broilers; Table 2). Although infectious bronchitis is initially a respiratory disease, viral titers are often lower in the trachea than in the enteric organs (Cavanagh and Gelb, 2008). Previous studies also reported a higher probability to detect IBV with the use of digestive tract samples than with respiratory samples (El-Houadfi et al., 1986; Almeida et al., 2012; Boroomand et al., 2012; Dolz et al., 2012). Therefore, an important recommendation would be to collect samples of at least these 2 systems (respiratory and digestive) to a more conclusive diagnosis of the IBV status in the flocks.

In conclusion, this study demonstrates IBV detection in broiler and breeder flocks from different geographic regions of Brazil. The BR variant genotype was more prevalent than Mass vaccine strains, mostly in breeders. Moreover, it was possible to detect strains from the BR and Mass genotype in different physiological systems, mainly in the organs of the digestive system.

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