https://doi.org/10.17221/109/2017-VETMED

Field occurrence of avian infectious bronchitis virus in the Czech Republic and Slovakia

M. Klimcik[1]*, R. Currie[2]

[1]Xaverov a.s., Kostelec nad Cernymi lesy, Czech Republic
[2]x-OvO Limited, Dunfermline, United Kingdom

*Corresponding author: klimcik@xaverov.com

ABSTRACT: The epidemiological situation regarding the infectious bronchitis virus (IBV) population in Europe as well as the presence of predominant IBV strains is well described. The aim of this epidemiological study was to describe the real field situation in the Czech Republic and Slovakia, as no data are available for the last ten years. The study was also focused on differentiation between field IBV strains and vaccine/vaccine origin IBV strains in different poultry segments including backyard flocks. Between July 2013 and July 2016, cloacal, tracheal and/or visceral swab samples were collected from 145 Czech and Slovak chicken broiler, breeder and layer flocks. The majority of flocks was kept for production purposes, but to enable a more complete picture of the situation in the field backyard flocks with more than 50 birds were also included. As in other cases which were reported worldwide and based on collaboration with x-Ovo laboratories, samples were analysed using the real-time polymerase chain reaction (RT-qPCR) to detect the presence of the RNA of IBV. When positive, approximately 400 base pairs encoding the hypervariable region of the IBV S1 protein were sequenced. Sequencing results, cycle threshold values and vaccination history were used as criteria to try and distinguish vaccine strains from field strains. A significant percentage of all flocks presented clinical signs suggestive of IBV infection. From the total number of samples examined, 16.5% were negative. In 12.4% of the samples that did contain RNA from IBV, the genotype could not be determined. In most cases, this was due to the recovery of RNA quantities below the lower limit of detection of the sequencing PCR. The remaining positive samples predominantly contained RNA from IBV strains that belonged to the 4/91 – 793B – CR88 (44.7%), Massachusetts (30%), D274 – D207 (11.6%) and D388 – QX (8.7%) genotypes. Estimations indicated that approximately 23.9%, 48.4%, 58.3% and 0% of these detections, respectively, were vaccine strains. Infections with types UKR/27/2011, CK/CH/Guandong/Xindadi/0903 and K33/09 were observed sporadically. The results confirm that IBV infections are highly prevalent in Czech and Slovak chickens and that at least seven different IBV types were circulating during the monitored period. This underlines the necessity of providing flocks with a strong and broad protective immunity against IBV.

Keywords: genotyping; poultry; chicken; broiler; breeder; layer; field strain

Depending on the relationship between the strain and the host immunological response, infectious bronchitis virus (IBV) can result in avian disease of major economic importance. Although observed under laboratory conditions, unambiguous clinical cases are rare in practice. On the other hand, IBV is a serious concurrent infection in various disease entities. It assumes increasing importance in terms of profitability when farm and integration sizes are large. Backyard flocks, even those with 100% IBV field strain positivity, are not regarded as a problem from the production point of view by owners.

IBV infection in young birds is mostly associated with respiratory problems. Some viral strains affect the kidney. Mortality in young poultry categories is much higher than in older birds and can reach

Supported by the Intervet Ltd. a Czech subsidiary of Merck and Co., Inc. Kenilworth, USA.

https://doi.org/10.17221/109/2017-VETMED

30%. In long-lived birds (commercial layers, broiler breeders and layer breeders), infection results in decreased egg production and egg quality.

IBV was first recorded in 1931 in Massachusetts (Schalk and Hawn 1931). This original IBV was therefore called the Massachusetts type. As a single stranded RNA virus, IBV appeared highly susceptible to mutation. Variations in the S1 spike protein, which is localised on the surface of the virus particles, have led to the emergence of numerous variants worldwide (Jackwood and de Wit 2014). Vaccines, especially those containing genetically very different IBV strains, may provide different levels of protection against variants, which influences the control of the disease by vaccination. In the past, such variants were identified on the basis of virus neutralisation assays (serotyping) but, nowadays, these tests have been replaced by molecular techniques (genotyping).

The situation with respect to IB prevalence and IBV types involved in infection may easily change over time (de Wit et al. 2011a). Also, the IBV population including the predominant IBV strains is very well known in EU countries, and the number of predominant strains is not high (de Wit et al. 2011a; de Herdt et al. 2016). No epidemiological surveys have been carried out in the Czech and Slovak poultry sectors to show the IBV population structure and the predominant IBV strains present. Therefore, the aim of the present study was to investigate the current prevalence of infectious bronchitis in Czech and Slovak chicken flocks and to identify the IBV types involved.

MATERIALS AND METHODS

Flock data and sampling. From July 2013 to July 2016, veterinary practitioners submitted samples from 15 broiler, 70 broiler breeder, six layer breeder and 54 commercial layer chicken flocks. All flocks were kept for production purposes. In the case of the final layer segment, 13 backyard flocks numbering more than 50 birds were also included, without the integrations. The samples were collected as part of routine monitoring, mostly in the course of IBV vaccine population control in flocks without clinical signs related to infectious bronchitis infection, and also in flocks exhibiting signs that might indicate an IBV infection, such as respiratory disorders, wet litter, drops in egg production and eggshell deformities. Submissions were always accompanied by a complete questionnaire that gathered detailed information on the date and site of sampling, the type and age of sampled birds, the clinical signs and lesions observed and the vaccination history (products used, age and route of administration and dosing).

For each flock, ten dry swabs were taken from the cloaca, visceral organs and/or trachea of the birds, put in individual plastic tubes or smeared onto FTA card and sent to the diagnostic laboratory. The interval between the collection of samples and laboratory examination was approximately two weeks. Apart from during transport, the samples were stored between 2 and 8 °C.

Molecular analyses. In order to confirm the presence of RNA from IBV (regardless of genotype) in the submitted samples, the real-time polymerase chain reaction (RT-qPCR) method described by Callison et al. (2006) was applied to ten swab sample pools. Up to 35 cycles were run. The cycle threshold values were noted in positive samples, which were then further analysed using universal primers specific for the S1 region of the IBV genome as well as an additional primer pair appropriate for the detection of the D1466 genotype (Cavanagh et al. 1999; Worthington et al. 2008). This generated a PCR product for sequencing of approximately 400 base pairs encoding the hypervariable region of the S1 protein. Sequencing was conducted according to Worthington et al. (2008), with slight modifications. Obtained sequences were compared with those available in the NCBI Genbank nucleotide database in order to determine the IBV genotype involved.

Distinction between field strains and vaccine strains. There is currently no available *in vitro* method with the ability to clearly distinguish field viruses and vaccine viruses of the same genotypes. The live vaccine strains that were predominantly used during the described period of sampling in Czech and Slovak poultry belong to the Massachusetts, 4/91 – 793B – CR88 (Gough et al. 1992; Cook et al. 1996) and D274 – D207 (Davelaar et al. 1984; Jordi et al. 1989) genotypes. At the time of the current survey, commercial vaccines containing the D388 – QX strains (Yu et al. 2001; Beato et al. 2005; Landman et al. 2005; de Wit et al. 2011b; Toffan et al. 2011) were not available. Multiple criteria were used to estimate the proportion of field viruses in samples that con-

https://doi.org/10.17221/109/2017-VETMED

tained RNA from these genotypes, similarly as in the case of other epidemiological surveys that have been carried out in collaboration with the x-OvO laboratory (de Herdt et al. 2016): "First, the sequence of the detected viruses was compared to corresponding sequences in an internal database built by examining vials of all common commercial vaccines through next generation sequencing" (x-OvO Limited, unpublished). Sequences were also compared to those obtained in vaccinated flocks in other countries where no field strains equivalent to the vaccine genotype were known to circulate. The criteria proposed by Worthington et al. (2008) were used for evaluation of the results. For viruses with 100% sequence homology to the vaccine strains, it was considered that these were most probably indeed vaccine strains. Less than 99% homology may have indicated a field challenge, while the intermediate range was considered questionable.

As a second criterion, obtained cycle threshold values were compared to cycle threshold values established during serial monitoring of vaccinated and infected flocks under field and experimental conditions. When the measured cycle threshold value was significantly lower than expected, this could constitute an additional argument for the involvement of a field virus. Especially cycle threshold values below 20 – indicating a large amount of viral RNA in the samples – were considered indicative of a field virus infection.

The vaccination history was also taken into account; the identity of the vaccines used as well as the eventual interval between their administration and the sampling of the birds contributed to the evaluation. Finding the sequence of a genotype that was not included in the vaccination schedule or finding a sequence in vaccinated flocks at a much later moment than could be expected from experiments and field experiences, were considered possible indicators of a field infection.

After evaluating the weight of evidence provided by the outcome of the three criteria, the detected viruses were classified as presumed field strains or presumed vaccine strains.

RESULTS

The results of the molecular screening in 145 Czech and Slovak chicken flocks from July 2013 to July 2016 are summarised in Table 1, Table 2 and Table 3.

Overall, 16.6% of the 145 examined flocks did not reveal any RNA from IBV and were therefore considered negative. The remaining 83.4% of the samples were IBV-positive (Table 1).

In 14.9% of the positive samples, it was impossible to classify the detected viruses into a specific

Table 1. Results of screening for the presence of infectious bronchitis viruses (IBV) from July 2013 to July 2016 in Czech and Slovak chicken flocks, using RT-qPCR and sequencing analysis

	Complete collection of samples	Samples obtained from flocks of		
		broilers	breeders	layers
Number of flocks tested	145	15	76	54
Result of RT-qPCRanalysis				
IBV-positive (%)	83.4	60	88.2	83.3
IBV-negative (%)	16.6	40	11.8	16.7
Genotype distribution (%) of positive samples				
4/91 – 793B	38.0	33.3	35.8	42.3
Massachusetts	25.6	11.1	37.3	11.1
D274 – D207	9.9	0	10.4	11.1
D388 – QX	7.4	44.4	0	11.1
CK/CH/Guandong-Xindadi/0903	1.7	0	0	4.4
UKR/27/2011	1.7	0	1.5	2.2
K33/09	0.8	0	0	2.2
Untypeable	14.9	11.1	15.0	15.6

Table 2. Proportion (%) of RT-qPCR positive samples that showed either 100%, between 99% and 100% or less than 99% identity with commercially available vaccine strains, on the basis of sequencing analyses of the hypervariable region of the gene encoding the infectious bronchitis virus (IBV) S1 protein

IBV genotype	Sequencing identity to homologous vaccine strains		
	100%	99–100%	< 99%
4/91 – 793B	19.6	4.3	76.1
D388 – QX	0	0	100.0
D274 – D207	8.3	50.0	41.7
Massachusetts	21.9	25.0	53.1

Homology of less than 99% in the S1 hypervariable region probably indicates a very low efficacy of homologous vaccination. From all results, no more than 10% showed homology lower than 95%

https://doi.org/10.17221/109/2017-VETMED

Table 3. Overview of presumed field strains of infectious bronchitis virus (IBV) detected in Czech and Slovak chicken flocks between July 2013 and July 2016*

IBV genotype	%	Numbers of isolates obtained from		
		broilers	breeders	layers
4/91 – 793B – CR88	50	0	21	14
Massachusetts	22.9	0	11	5
D388 – QX	12.9	4	0	5
D274 – D207	7.0	0	2	3
CK/CH/Guandong/ Xindadi/0903	2.9	0	0	2
UKR/27/2011	2.9	0	1	1
K33/09	1.4	0	0	1

% = percentage of total number of isolates

*Presumed to be field strains on the basis of their S1 sequence homology, PCR Ct values and vaccination history

Table 4. Overview of number of chicken flocks showing clinical signs of infectious bronchitis virus (IBV) detected in Czech and Slovak chicken flocks between July 2013 and July 2016

IBV genotype	%	S/V	Numbers of isolates obtained from		
			broilers	breeders	layers
4/91 – 793B – CR88	40.3	19/4	3	16	4
Massachusetts	12.3	3/4	0	6	1
D388 – QX	10.5	6/0	4	0	2
D274 – D207	8.8	2/3	0	2	3
CK/CH/Guandong/ Xindadi/0903	1.8	1/0	0	0	1
UKR/27/2011	0	0/0	0	0	0
K33/09	1.8	1/0	0	0	1
Untypeable	24.5	N/A	0	9	5

% = percentage of flocks showing clinical signs, S/V = probable field strain/vaccine ratio

genotype. Untypeable strains were found mostly in samples with a very high cycle threshold, indicating that they contained only a very small quantity of RNA, insufficient for appropriate sequencing. In the remaining 85.1% of IBV-positive samples, the detected viruses could be classified into seven different genotypes (Table 1).

Strains belonging to the 4/91 – 793B – CR 88, Massachusetts, D274 – D207 and D388 – QX genotypes were most prevalent, accounting for approximately 95% of all typeable detections. For these genotypes, 19.6%, 21.9%, 8.3% and 0% of the strains, respectively, showed 100% identity with homologous vaccine strains (Table 2). The result of D388 – QX was influenced by the lack of a commercially available vaccine on the market at the time.

The 4/91 – 793B – CR 88 variant type was found in 50% of the IBV-positive samples. It was very likely that this type was involved in approximately 40% of the IBV field infections. Presumed field strains were found most often in breeder and in layer flocks (Table 4).

Classical Massachusetts strains were found in 22.9% of the IBV-positive flocks. It was very likely that this type was present in approximately 12% of cases showing clinical signs, but less than half of these samples were field strains. Presumed field infections were observed, especially in breeder flocks and sporadically in layer birds (Table 4).

D388 – QX strains of IBV represented 12.9% of the IBV-positive cases. They were considered responsible for approximately 10% of IBV disorders, predominantly in broiler flocks (Table 4).

A variant type of the D274 – D207 strain accounted for 7% of the positive samples, approximately 58% of which included a vaccine. Presumed field strains were detected almost exclusively in breeder flocks (Table 4).

Other genotypes were found sporadically. Two strains obtained from backyard layers belonged to the CK/CH/Guandong/Xindadi/0903 variant type (Ji et al. 2011). The UKR/27/2011 variant type (Ovchinnikova et al. 2012) was found once in a broiler breeder flock (roosters) and once in a backyard layer flock. Variant K33/09 was also found just once, in commercial layers.

Thirteen backyard layer flocks were also included in this epidemiological study, representing approximately 9% of all samples. Backyard flocks showed 100% IBV positivity (Table 5); 77% of these strains were IBV field strains, 15% vaccine strains and 8% of samples were untypeable.

DISCUSSION

Molecular techniques enable the gathering of informative data in epidemiological studies which focus on IBV infections in chickens, and are especially suitable for identifying the IBV types involved (Worthington et al. 2008). From the practical point of view, molecular techniques allow more conveni-

https://doi.org/10.17221/109/2017-VETMED

Table 5. Results of screening for the presence of infectious bronchitis viruses (IBV) from July 2013 to July 2016 in Czech and Slovak backyard layer flocks

IBV genotype	%	S/V
4/91 – 793B – CR88	38.5	5/0
Massachusetts	0	0/0
D388 – QX	15.4	2/0
D274 – D207	15.4	0/2
CK/CH/Guandong/Xindadi/0903	15.4	2/0
UKR/27/2011	7.7	1/0
K33/09	0	0/0
Untypeable	7.6	N/A

% = percentage of total number of isolates, S/V = probable field strain/vaccine ratio

ent sampling by enabling the taking of samples as dry swabs and/or FTA cards from live birds using non-invasive techniques. Also, transport to the laboratory does not require special precautions during storage and/or shipment (Cavanagh et al. 1999; Worthington et al. 2008). These techniques were also used in the case of the present study.

To properly describe an epidemiological situation, it must be determined whether the strains are field or live homologous vaccine in origin (de Herdt et al. 2016). No standardised method to achieve this differentiation of strains currently exists (Worthington et al. 2008; Jackwood and de Wit 2014). Because the simple comparison of the S1 protein sample sequence and vaccine vial sequences does not give unambiguous results, the cycle threshold values of the samples in RT-qPCR and the vaccination history of the birds was also considered in this study. Although it cannot be excluded that in some cases, vaccine strains and field strains have been confused, this approach was suitable for providing insights into the overall IBV situation of chickens in the Czech Republic and Slovakia.

Because no previous results regarding the field situation in the Czech and Slovak poultry sectors are available, this epidemiological survey should be considered as a pilot study. Future field trials should focus on each of the different poultry segments, i.e., broiler, breeder and layer integrations, and also backyard flocks, which represent almost one half of the final layer number in both countries. The backyard flocks investigated between 2013 and 2016 represent a constant reservoir of IBV field strains that is characterised by very high diversity. This, together with the lack of regular vaccination, increases the importance of monitoring due to the potential of new IBV strain development and the resulting high risk for big poultry integrations.

The clinical signs related to IB infection and which were considered in this study are frequently observed but not pathognomonic. Within the flocks showing infectious bronchitis clinical signs, 12.3% of samples gave negative results, 16.9% of samples were confirmed to be vaccine strains and 21.6% of results were untypeable. On the other hand, in the group without clinical signs, 28.8% of samples were confirmed as field strains. Finally, this study could supply only a snapshot of the current IBV population structure in the studied birds. The interaction between the host and the virus should be more closely studied in, e.g., virulence factor studies or by immune system response monitoring.

The results of the present study indicate that IBV infection is highly prevalent in Czech and Slovak commercial chicken flocks with 100% prevalence in backyard layer flocks in both countries. At least seven types of IBV appear to have been circulating in the past three years. This underlines the necessity of providing chickens with a strong vaccinal immunity against IBV. According to the protectotype concept (Cook et al. 1999), broad protection against a wide range of IBV types can be obtained by applying vaccines.

REFERENCES

Beato MS, De Battisti C, Terregino C, Drago A, Capua I, Ortali G (2005): Evidence of circulation of a Chinese strain of infectious bronchitis virus (QXIBV) in Italy. Veterinary Record 156, 720.

Callison SA, Hilt DA, Boynton TO, Sample BF, Robison R, Swayne DE, Jackwood MW (2006): Development and evaluation of a real-time Taqman RT-PCR assay for the detection of infectious bronchitis virus from infected chickens. Journal of Virological Methods 138, 60–65.

Cavanagh D, Mawditt K, Britton P, Naylor CJ (1999): Longitudinal field studies of infectious bronchitis virus and avian pneumovirus in broilers using type-specific polymerase chain reactions. Avian Pathology 28, 593–605.

Cook JKA, Orbell SJ, Woods MA, Huggins MB (1996): A survey of the presence of a new infectious bronchitis virus designated 4/91 (793B). Veterinary Record 138, 178–180.

https://doi.org/10.17221/109/2017-VETMED

Cook JKA, Orbell SJ, Woods MA, Huggins MB (1999): Breadth of protection of the respiratory tract provided by different live-attenuated IB vaccines against challenge with IB viruses of heterologous serotypes. Avian Pathology 28, 477–485.

Davelaar FG, Kouwenhoven B, Burger AG (1984): Occurrence and significance of infectious bronchitis virus variant strains in egg and broiler production in the Netherlands. The Veterinary Quarterly 6, 114–120.

de Herdt P, de Gussem M, van Gorp S, Currie R (2016): Infectious bronchitis virus infections of chickens in Belgium: an epidemiological survey. Vlaams Diergeneeskundig Tijdschrift 85, 285–290.

de Wit JJ, Cook JKA, van der Heijden HM (2011a): Infectious bronchitis virus variants: a review of the history, current situation and control measures. Avian Pathology 40, 223–235.

de Wit JJ, Nieuwenhuisen-van Wilgen J, Hoogkamer A, van de Sande H, Zuidam GJ, Fabri THF (2011b): Induction of cystic oviducts and protection against early challenge with infectious bronchitis virus serotype D388 (genotype QX) by maternally derived antibodies and by early vaccination. Avian Pathology 40, 463–471.

Gough RE, Randall CJ, Dagless M, Alexander DJ, Cox WJ, Pearson D (1992): A 'new' strain of infectious bronchitis virus infecting domestic fowl in Great Britain. Veterinary Record 130, 493–494.

Jackwood MW, de Wit S (2014): Infectious bronchitis. In: Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, Nair V (eds): Diseases of Poultry. 13th edn. Wiley-Blackwell, Ames. 139–159.

Ji J, Xie J, Chen F, Shu D, Zuo K, Xue C, Qin J, Li H, Bi Y, Ma J, Xie Q (2011): Phylogenetic distribution and predominant genotype of the avian infectious bronchitis virus in China during 2008–2009. Virology Journal 8, 184.

Jordi BJAM, Kremers DAWM, Kusters HG, van der Zeijst BAM (1989): Nucleotide sequence of the gene coding for the peplomer protein (= spike protein) of infectious bronchitis virus, strain D274. Nucleic Acids Research 17, 6726.

Landman WJM, Dwars RM, de Wit JJ (2005): High incidence of false layers in (re)production hens supposedly attributed to a juvenile infectious bronchitis infection. In: Proceedings of the XIV. International Congress of the World Veterinary Poultry Association, Turkey, Istanbul. 135 pp.

Ovchinnikova EV, Zinyakov NG, Drygin VV (2012): Molecular characterization of infectious bronchitis viruses isolated in Russia and CIS countries. EMBL/GenBank/DDBJ databases, GenBank JX233491.1.

Schalk AF, Hawn MC (1931): An apparently new respiratory disease of baby chicks. Journal of the American Veterinary Medical Association 78, 413–422.

Toffan A, Terregino C, Mazzacan E, Castaldello I, Capua I, Bonci M (2011): Detection of Chinese Q1 strain of infectious bronchitis virus in Europe. Veterinary Record 169, 212–213.

Worthington KJ, Currie RJ, Jones RC (2008): A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathology 37, 247–257.

Yu L, Jiang Y, Low S, Nam SJ, Liu W, Kwangac J (2001): Characterization of three infectious bronchitis virus isolates from China associated with proventriculus in vaccinated chickens. Avian Diseases 45, 416–424.

Received: August 22, 2017
Accepted after corrections: September 20, 2017