



Epidemiological study on circulation of Infectious Bronchitis Virus strains in North Eastern Italy

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ABSTRACT

Infectious Bronchitis (IB) is still a major health problem in the poultry industry, as it is endemic in probably all countries which raise chickens. Previous investigations have shown that several IB variants are present in the Italian poultry industry. In order to establish which serotypes are circulating in the Veneto and Lombardia regions an investigation was carried out during 2004 in broiler and egg-layer farms. A total of 101 samples were collected from 29 farms with 16 IBV isolations. Of these, seven isolates were identified as being of the IT-02 serotype and six as 793-B on the basis of nucleotide sequencing.

Key Words: Chicken, Infectious Bronchitis, Broiler, North Italy, Epidemiology.

RIASSUNTO

INDAGINE EPIDEMIOLOGICA SULLA CIRCOLAZIONE DEI VIRUS DELLA BRONCHITE INFETTIVA AVIARIA IN VENETO

La Bronchite Infettiva (IB) rappresenta ancora oggi uno dei principali problemi sanitari per il pollame laddove l'allevamento ha carattere intensivo. Si è dato inizio ad un'indagine conoscitiva sulla epidemiologia della IB in Veneto, successivamente allargata ad alcune province della Lombardia. Dal gennaio 2004 sono stati monitorati 29 allevamenti, per un totale di 101 campioni esaminati, di cui 16 sono risultati positivi per IBV. Sette isolati sono stati identificati come sierotipo IT-02 e sei come 793-B.

Parole chiave: Pollo, Bronchite infettiva, Broiler, Nord Italia, Epidemiologia.

Introduction

Infectious Bronchitis (IB) is still a major health problem in the poultry industry, as it is endemic in probably all countries which raise chickens. IB is a severe systemic disease resulting in a variable morbidity depending on the virulence and tissue tropism of the strain. The clinical disease, is generally characterized by respiratory signs and nephrosis in broilers and drop in egg production in egg layers and broiler breeders. IB is caused by avian

Coronavirus, an enveloped single stranded, positive-sense RNA with a large spike glycoprotein (S) that is responsible of the host antibody response. IBV may be classified in serotypes that can be characterized by serological and molecular biology techniques. Previous investigations have shown that several IB variants are present in the Italian poultry industry. Among these, some have been isolated primarily in Italy, such as variant 624-I (Capua *et al.*, 1999), while others originate from other European countries such as 793-B and B-

1648. Between 2000 and 2003 no surveys on IB were performed in the densely populated poultry areas of north-eastern Italy, although clinical signs indicative of this infection were observed. In order to establish which serotypes are circulating in the Veneto and Lombardia regions an investigation was carried out during 2004 in broiler and egg-layer farms in collaboration with veterinarians of farms where the study has been carried out.

Material and methods

The study has had as target the broiler and egg layer farms located in North-eastern Italy (Veneto and Lombardia regions). In order to improve isolation rates, SPF (Specific Pathogen Free) chickens were introduced in the farms for a period of 7-10 days as sentinels. Tissue samples, collected from sentinels (trachea, lung, kidney and cecal tonsils) were submitted for virological investigations. The tissue homogenates were inoculated into the allantoic cavity of eggs from 9- to 11-day-old embryonated SPF fowl (Gelb *et al.*, 1998), following an overnight incubation at 4°C with PBS antibiotic solution. Following a minimum of two and a maximum of four blind passages, embryos were observed to detect typical IBV lesions (dwarfing, incomplete feathering pattern and stunting) and the allantoic fluid was harvested and examined by negative contrast electron microscopy for the presence of coronavirus particles (Hyatt, 1986). In order to characterise the isolates, viral RNA was extracted from the infectious allantoic fluid. The isolates were analysed in RT-PCR, with specific primers for the S1-gene (Adzhar *et al.*, 1996), in order to generate a complementary DNA (cDNA). This cDNA, was sequenced (Keeler *et al.*, 1998), and isolates were typed on the basis of the sequence. A total of 101 samples were collected from 29 farms.

Results and discussion

The results of the investigation indicate that IBV strains are actively circulating in broiler and layer farms of North-eastern Italy. In fact sixteen IBV were isolated from 29 farms: namely serotypes IT-02 and 793-B as shown in (Table 1). In farms where serotype 793-B prevailed respiratory forms were seen more often. In one of these,

the respiratory form was associated to nephrosis. Enteric disorders were observed in farms where serotype IT-02 circulated. For three isolates obtained on three different farms, molecular characterisation is in progress.

The results presented above indicate that regardless of the geographical origin of the isolate strains, 793-B and IT-02 are present in Veneto and Lombardia regions. The findings of the present study indicate that strain 793-B is still present in Italy (Catelli and Lavazza, 2000; Tosi, 2001). Although evidence of the presence of this virus has been collected throughout the years, it is not possible to establish how many isolates are pathogenic field strains and how many are vaccine strains. In fact vaccination against this variant has been practised extensively with live attenuated vaccines, and currently there is no tool available to differentiate field from vaccine strains.

What appears of great interest is the significant number of isolations of strain IT-02. This strain appears to be widespread in broiler farms. Since at present there is no information on the pathogenicity and antigenicity of this novel variant, challenge experiments and cross protection studies should be performed in order to evaluate on one hand the pathogenicity of this variant, and on the other the possibility of using licensed products to protect birds against the clinical condition caused by this variant.

Conclusions

The results of this investigation indicate that a comprehensive knowledge on the serotypes of Infectious Bronchitis Virus circulating in particular areas is essential to plan and possibly update an appropriate vaccination strategy against this disease.

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Table 1. Province of isolation (in Italy), strains isolated lesions and clinical signs observed in the field.

Province	Type of farm and age of birds	Molecular characterization of isolate	Lesions and clinical signs
Verona	Broiler - 36 days	IBV serotype 793-B*	Mild respiratory form
Rovigo	Broiler - 36 days	IBV serotype IT-02	Not reported
Cremona	Broiler - 45 days	IBV serotype IT-02	Not reported
Mantova	Broiler - 60 days	IBV serotype 793-B	Respiratory form
Verona	Broiler - 53 days	IBV	Not reported
Padova	Broiler - 60 days	IBV	Respiratory form
Pordenone	Layers - 20 weeks	IBV	Respiratory form, depigmentation of the eggs' shell
Brescia	Broiler - 42 days	IBV serotype 793-B	Respiratory form, kidney lesions, growth problem, mortality
Verona	Broiler - 35 days	IBV serotype IT-02	Kidney lesions, enteric disorders, mortality
Verona	Broiler - 45 days	IBV serotype IT-02	Enteric forms
Cremona	Broiler - 53 days	IBV serotype IT-02	Respiratory and enteric forms
Padova	Broiler - 50 days	IBV serotype 793-B	Mild respiratory form
Padova	Broiler - 43 days	IBV serotype IT-02	Mild respiratory form
Vicenza	Broiler - 51 days	IBV serotype IT-02	Mild respiratory form
Brescia	Broiler - 45 days	IBV serotype 793B*	Not reported
Mantova	Broiler - 50 days	IBV serotype 793-B*	Mild respiratory form

* Farms where a live attenuated vaccine serotype 793/B was administered

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