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Emergence of a Nephropathogenic Avian Infectious Bronchitis Virus with a Novel Genotype in India

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We describe the emergence of a nephropathogenic avian infectious bronchitis virus (IBV) with a novel genotype in India. The Indian IBV isolate exhibited a relatively high degree of sequence divergence with reference strains. The highest homology was observed with strain 6/82 (68%) and the least homology with strain Mex/1765/99 (34.3%).

Infectious bronchitis virus (IBV) is prevalent in all countries with an intensive poultry industry, with the incidence of infection approaching 100% in most locations. The virus is belong to group 3 coronavirus (4). IBV has always been something of a moving target for many reasons, such as wide variations in the serotypes and virulence of strains that have developed from time to time, a highly contagious nature, and the evolution of specific tissue tropism and recombinants due to simultaneous infection of multiple virus types and use of live vaccines. Vaccination is only partially successful due to continual emergence of antigenic variants and requires the application of multiple vaccines at many sites due to the simultaneous presence of multiple antigenic types. Although many countries share some common antigenic types, IBV strains within a geographic region are unique and distinct; examples of this include Europe, the United States, and Australia (1, 2, 6–9, 21).

IBV genome consists of ca. 27 kb and codes for three structural proteins: the spike (S) glycoprotein, the membrane (M) glycoprotein, and the nucleocapsid (N) phosphoprotein. The S glycoprotein is composed of two glycopolypeptides: S1 and S2 (3). Neutralizing and serotype-specific antibodies are directed against the S1 glycoprotein, and the greatest divergence in the amino acid sequence is concentrated between residues 53 and 148 of S1 (17).

Until recently, the Indian subcontinent was free of variant forms of IBV. The most prevalent form of IB was only the respiratory form related to the Massachusetts strain (13, 15). However, genotyping and phylogenic analysis of Indian isolates were previously not been done. We investigated outbreaks of visceral gout and nephrosis in commercial broiler chicks in the western parts of India for evidence of IBV. The disease was reported mostly in 1- to 2-week-old broiler chicks in unvaccinated flocks. The clinical signs observed are typical of IB: gasping, upward respiration, and tracheal rales. Grossly, the birds presented with distended ureters filled with uric acid and visceral gout. Kidney lesions were principally those of an interstitial nephritis: granular degeneration, vacuolation, and desquamation of tubular epithelium.

The IBV was isolated by intra-allantoic passage of clinical material (kidneys) from affected chicks. After the third passage in specific-pathogen-free embryonated eggs, we observed lesions in the specific-pathogen-free embryos: mortality of embryos, stunting, curling, and uric acid deposition in the kidneys and ureter. The allantoic fluid of inoculated eggs were found to be negative for Newcastle disease virus and avian influenza virus by spot hemagglutination assay. The presence of coronavirus-like particles in allantoic fluid was confirmed by electron microscopic examination of infectious allantoic fluid.

Direct automated cycle sequencing of a reverse transcription-PCR product of the S-1 subunit of the spike peplomer gene was used to identify IBV genome with degenerate primers CK4 and CK2 (10, 11). The S-1 subunit nucleotide sequences generated by direct automated cycle sequencing were aligned and analyzed with commercial software to determine their relationship to the S-1 nucleotide sequences of IBV strains on deposit in the GenBank and EMBL databases. The sequence reported here has been submitted to the GenBank nucleotide database and has the accession number AY091551.

Indian IBV isolate PDRC/Pune/Ind/1/00 was found to have a unique S-1 sequence compared to selected reference strains from different countries and continents (Fig. 1). The reference IBV strains included in the analysis were from the United States: Massachusetts, Arkansas, Connecticut, Delaware O72, JMK, PA/Wolgemuth/98, MD/106/00, CV-56B, and SE-17. The European strains included were UK/7/93, 6/82, D207, H120, B1648, and D1466. The Mexican IBV strains included were Mex/7483/98 and Mex/1765/99. The Australian strains were V5/90, Vic S, N1/62, and Q3/88. The sequence analysis of the S1 gene demonstrates that Indian isolate PDRC/Pune/Ind/1/00 possesses a unique genotype compared to other reference

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Percent similarity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		IBV strains
1		60.0	62.9	39.4	35.4	34.3	68.0	62.3	61.1	60.6	67.4	66.9	64.0	60.6	60.6	64.0	53.7	64.6	61.1	60.0	62.9	62.9	66.3	1	PDRC/Pune/Ind/1/00
2	35.8		82.2	34.6	32.8	35.7	72.2	70.6	68.9	70.6	71.7	71.7	71.7	68.3	70.0	74.4	54.4	67.8	68.3	67.2	61.0	59.4	58.9	2	Mex/7483/98
3	34.3	15.0		37.4	32.8	34.3	76.9	72.7	69.9	71.4	76.9	76.9	74.3	69.4	69.2	75.9	55.1	71.0	74.7	73.7	63.3	65.2	65.2	3	JMK
4	54.2	58.0	58.0		62.0	65.7	40.2	38.5	37.4	34.6	40.2	39.7	34.6	32.4	34.6	37.4	31.8	35.8	35.2	34.6	39.0	39.1	39.7	4	DE/072/92
5	59.8	63.5	63.3	36.3		66.4	35.0	37.8	35.6	30.6	33.9	33.9	35.6	30.6	32.8	33.9	31.1	36.7	33.3	32.8	36.7	36.1	37.2	5	D1466
6	59.0	60.1	59.3	31.7	33.6		35.0	33.6	32.2	29.4	34.3	34.3	30.8	28.7	29.4	32.9	28.7	30.8	28.7	28.7	32.9	34.3	35.0	6	Mex/1765/99
7	31.6	22.7	20.9	52.9	60.0	57.2		76.4	73.6	76.9	97.3	98.4	75.8	71.7	72.5	72.5	59.9	71.4	77.5	76.4	68.9	68.0	69.1	7	6/82
8	34.3	26.1	26.2	55.9	57.9	57.4	20.3		73.7	75.1	76.4	75.8	77.7	75.0	74.1	73.9	55.1	68.3	74.7	73.7	63.8	65.2	65.2	8	ARK DPI
9	35.4	28.1	28.1	55.9	59.6	60.3	23.6	24.7		69.7	74.7	73.1	76.3	69.4	67.6	70.4	55.9	69.9	74.2	73.7	63.3	65.7	66.3	9	B1648
10	36.4	26.0	25.0	61.0	66.3	65.2	19.0	23.8	26.8		76.9	76.4	81.1	82.8	85.4	69.7	55.7	66.1	75.1	75.1	63.8	61.9	61.9	10	CV-56B
11	32.2	23.3	20.9	53.4	61.1	58.0	2.7	20.3	22.5	19.0		95.6	76.4	71.7	72.0	72.0	59.3	70.3	78.6	77.5	68.4	66.9	68.0	11	UK/142/86 CK2/CK4 P
12	32.8	23.3	20.9	53.4	61.1	58.0	1.6	20.9	24.2	19.6	4.4		75.3	71.1	72.0	72.0	59.9	70.9	77.5	76.4	68.4	67.4	68.5	12	D207
13	32.6	26.1	24.6	60.5	61.2	62.4	21.4	22.3	22.0	17.3	20.9	22.0		78.3	77.8	72.9	56.7	68.9	76.9	75.8	65.5	68.5	68.0	13	N1/62
14	34.9	27.8	26.8	61.6	65.3	64.3	22.4	23.3	27.5	15.6	22.4	23.0	20.6		84.4	70.0	55.6	65.0	72.2	71.7	62.1	58.9	59.4	14	MD/106/00
15	35.3	26.6	27.2	60.5	64.6	64.5	24.6	24.3	28.4	14.6	24.6	25.1	21.1	13.9		70.3	53.0	63.9	71.9	70.3	62.1	59.7	59.7	15	PA/Wolg/98
16	33.1	22.8	23.0	56.5	62.9	58.9	24.7	26.1	28.0	29.2	25.3	25.3	27.1	28.3	28.6		54.5	67.8	73.1	72.0	64.4	64.6	65.2	16	SE17
17	41.9	39.4	36.8	62.6	63.4	65.9	35.4	36.8	36.7	36.1	36.0	35.4	35.7	37.7	40.0	37.9		57.9	57.5	57.0	53.1	49.7	51.4	17	Q3/88
18	34.3	28.4	27.3	61.7	62.5	66.9	26.9	30.1	28.4	30.0	28.0	27.5	29.5	30.3	32.8	30.6	32.8		66.1	65.6	65.0	67.4	66.9	18	UK/7/93 CK2/CK4 P
19	33.5	27.0	22.3	57.7	62.5	62.6	17.2	24.2	22.3	21.3	16.1	17.2	22.0	24.2	24.6	25.8	32.8	29.8		97.8	66.7	68.0	69.1	19	V5/90 CK2/CK4 P
20	34.7	27.5	23.2	57.7	62.5	62.6	18.3	24.7	22.8	21.3	17.2	18.3	23.1	24.2	25.7	26.3	32.8	30.4	2.2		66.7	68.5	69.1	20	Vic S
21	32.0	31.4	31.2	54.9	57.8	58.8	27.4	31.6	31.6	31.1	28.6	27.4	29.4	33.5	32.8	30.5	39.3	31.2	29.1	29.1		81.9	84.2	21	Conn
22	33.1	32.9	31.1	54.0	55.7	56.2	28.5	31.5	30.9	33.1	29.6	29.1	28.2	34.7	34.3	32.0	42.6	30.6	29.6	29.6	16.4		93.4	22	Mass 41
23	30.2	33.5	31.1	52.3	55.2	55.5	27.9	31.5	30.4	32.6	29.1	27.9	28.7	34.1	34.3	31.5	40.3	31.1	28.5	28.5	14.1	6.6		23	H120
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		

Percent Divergence

FIG. 1. Percent S1 protein similarity values for PDRC/Pune/Ind/1/00 versus selected avian IBV reference strains from different countries.

strains of various countries and is unrelated to North American, European, and Australian strains (Fig. 2). The Indian isolate exhibited <40% similarity in S1 protein sequence to strains D1466, Mex/1765/99, and DE/072/92 but shared 53 to 68% relatedness in S1 protein sequence with rest of the reference strains. The highest homology was observed with strain 6/82 (68%) and the least homology with strain Mex/1765/99 (34.3%).

Our data, together with those of previous publications, document several outbreaks of emerging IBV virus infection in the regions where intense poultry farming is practiced (2, 6–9, 20, 21). Because widespread vaccination has been conducted to control IBV, this immune selection pressure, together with the high mutation rate of the genome, may explain the existence of many serotypes and variants. The differences of as little as 5% between S1 sequences of IBV can result in poor cross-protection offered by currently used vaccines (4). Several variants, as well as isolates, related to European or American strains have previously been reported in China and other parts of Asia, with which India either shares political borders or trade exchange (14, 16, 18, 19). Thus, the Indian isolate PDRC/Pune/Ind/1/00 could have emerged from recombination of these variants with the local circulating virus or vaccine strains. Although not confirmed by virus isolation and sequencing of genome, the presence of European 793/B related virus in India has been reported by serological methods (5). The measures to restrict the introduction of exotic IBV strains should therefore be considered since the disease has a significant economic impact; in broilers, these production losses are due to poor weight gains, condemnation at processing, and mortality, whereas in laying birds losses are due to suboptimal egg production and downgrading of eggs. Although previous vaccines in India were mainly imported from the United States, several vaccines from Europe were also used in the past. In view of the emergence of novel variants due to

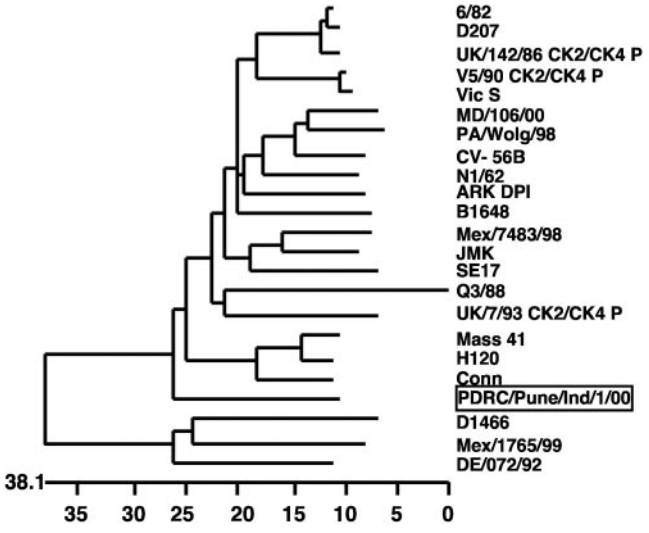

FIG. 2. Phylogenetic tree for PDRC/Pune/Ind/1/00 based on nucleic sequence data of S1 glycoprotein gene. Sequencing of genome was performed by direct automated cycle sequencing of a reverse transcription-PCR.

recombination during mixed infection of IBV (12), the policy of importing live attenuated IBV vaccines for domestic vaccination purposes in India also needs to be redefined.

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