Experimental infection of IS/885/00-like infectious bronchitis virus causes cystic oviduct in SPF and head swelling in commercial broiler chicks, in addition to respiratory and renal diseases

Faez Awad[a,b], Rajesh Chhabra[a], Anne Forrester[a], Julian Chantrey[a], Matthew Baylis[a,c], Stephane Lemiere[d], Hussein Aly Hussein[e] & Kannan Ganapathy[a]*

[a] University of Liverpool, Leahurst Campus Neston, South Wirral, CH64 7TE, UK

[b] University of Omar Al-Mukhtar, Faculty of Veterinary Medicine, Al-Bayda, Libya

[c] NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, UK

[d] Merial S.A.S., 29 avenue Tony Garnier, Lyon cedex 07, France

[e] Cairo University, Department of Virology, Faculty of Veterinary Medicine, Cairo, Egypt

*Corresponding author Tel.: +44 151 7946019; fax: +44 151 7946005.

E-mail address: gana@liv.ac.uk

ABSTRACT

Pathogenesis of IS/885/00-like (IS/885) strain of variant infectious bronchitis virus (IBV) was examined in one day old specific pathogen-free (SPF) and commercial broiler chicks. Chicks were humanely euthanized at 3, 6, 9, 12, 15, 21 and 28 days post infection (dpi) for necropsy examination, and tissues were collected for histopathology and virus detection by reverse transcription polymerase chain reaction (RT-PCR). Respiratory signs, gross lesions comprising tracheal caseous exudate and plugs, and swollen kidneys (with or without) urate deposits were observed in both SPF and broiler chicks. The onset of disease was delayed and of lesser severity in broiler compared to the SPF chicks, reflecting the inhibitory effects of the IBV maternal-antibodies in the broiler chicks or genetic/strain susceptibility, or both. Head swelling was observed in one infected broiler chick at 15 dpi and the virus was recovered by RT-PCR and isolation. In the IS/885-infected SPF chicks, cystic oviducts were found in two female chicks. IS/885 was isolated from the cystic fluid. Using ELISA, only low to moderate levels of the antibodies to IBV was detected in the SPF compared to broiler infected chicks.

Keywords: IBV IS/885/00-like, cystic oviduct, head swelling, chicks

1. Introduction

Avian infectious bronchitis is a common, highly contagious and economically important viral disease of chicken caused by a coronavirus (Cavanagh, 2007). A large number of serotypes and genotypes of IBV strains have been reported worldwide (de Wit et al., 2011a; Jackwood, 2012). This appears to be due to constant changes in the spike-protein of IBV strains resulting in the emergence of new IBV variants (Cavanagh, 2007). In the winter of 2000, a severe outbreak of renal disease occurred in several broiler farms in Israel. An IBV, designated IS/885/00, was isolated from the kidneys of these flocks (Meir et al., 2004).

In the Middle East and North Africa, the circulation of Massachusetts, 793B, QX, Dutch strains (Abdel-Moneim et al., 2006; Bourogaa et al., 2009; Amin et al., 2012; Boroomand et al., 2012) and Q1 (Ganapathy et al., 2015) have been reported. In addition, increasing number strains closely related to IS/885/00 have been reported in Israel (Meir et al, 2004), Egypt (Abdel-Moneim et al., 2012), Iraq (Mahmood et al., 2011), Libya (Awad et al., 2014a) and other Middle East countries (Ganapathy et al., 2015). To date, through our diagnostic services, we have detected this strain in samples received from France, Ukraine and Pakistan. These strains were detected from broiler and layer flocks experiencing respiratory distress, renal lesions and high mortality (Meir et al., 2004; Selim et al., 2013; Awad et al., 2014a).

The pathogenesis and host immune responses to few of IBV strains are known, such as those of M41 (Crinion and Hofstad, 1972; Butcher et al., 1990), Beaudette (Geilhausen et al., 1973), Australian T-strain (Chong and Apostolov, 1982; Ignjatovic et al., 2002), Moroccan G strain (El-Houadfi et al., 1986; Ambali and Jones, 1990), 793B (Dhinakar Raj and Jones, 1996; Boroomand et al., 2012), QX (Wang et al., 1998; Terregino et al., 2008; Ganapathy et al., 2012) , It-02 (Dolz et al., 2012), Q1 (Yu et al., 2001; Toffan et al., 2013) and more recently Brazilian IBV variant (USP-10 and USP-50) (Chacón et al., 2014).

Despite high circulation of IS/885/00-like strains in the Middle East and North Africa, and further detection in France, Ukraine and Pakistan, to date, there is no published information on pathogenesis of this increasingly important variant IBV. To better understand the IS/885 strain, series of experiment were performed to investigate the pathogenesis in SPF and commercial broiler chicks. Clinical signs, gross and microscopic lesions, virus detection and humoral antibody responses were evaluated.

2. Materials and methods

2.1. Virus

The virus used throughout this study (referred here as IS/885) was received as third passage allantoic fluid (AF) from the Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy. The virus had been isolated from a recent outbreak of high mortality and respiratory disease complex in broiler flocks in Egypt. Initial isolation was carried out in the virology laboratory at Cairo University, Egypt, and the AF was submitted to the Italian laboratory. There, the AF went through three further passages in embryonated chicken eggs (ECEs) and was shown to be negative for avian influenza virus (AIV) and Newcastle disease virus (NDV) and positive for IBV by RT-PCR. DNA sequences of the part-S1 gene showed 99% nucleotide identity to the Israeli strain IS/885/00 (Meir et al., 2004).

At the University of Liverpool, the virus received a further passage in 9 to 11 day-old SPF ECE. The viral titre was determined by titration in ECE and calculated as previously described (Reed and Muench, 1938) to provide $10^{4.66}$ EID_{50}/ml. The AF was free of, NDV, AIV, avian metapneumovirus (aMPV), infectious laryngotracheitis virus (ILTV), infectious bursal disease virus (IBDV) and fowl adenovirus (FAdV). The inoculum was also free of bacterial contamination when tested using blood and MacConkey agars, and no mycoplasmas was detected either by culture or PCR.

2.2. Eggs and chicks

Fertile eggs from SPF White Leghorn chickens (Lohmann Animal Health, Cuxhaven, Germany) were incubated and hatched in our facilities. Day-old commercial broiler chicks with IBV maternally-derived antibodies (MDA) were obtained from a commercial hatchery. The parent flock of the broiler chicks had been vaccinated with a live IBV H120+D274 vaccine at 3 weeks old and an inactivated IBV M41 vaccine was administered four weeks before transfer to laying farms. Chicks were kept in an isolation unit (University of Liverpool) throughout the experiment and reared on deep litter with water and feed provided *ad libitum.* The chicks were raised on wood shavings as being practiced in the commercial farms. Feed and water was free of any antibiotics. All of the experimental procedures were undertaken after the approval of the University of Liverpool ethical review committee and according to the UK legislation on the use of animals for experiments, as permitted under the project license PPL 112 40/3723.

3. Experimental design

3.1. Experiment 1 (Expt 1): Infection of SPF chicks

Seventy one-day-old SPF chicks were randomly divided into two groups, consisting of 45 and 25 chicks in the infected and control group respectively. The chicks in the infected group were inoculated oculonasally with 0.1 ml of the virus and those in the control group with virus-free AF.

3.2. Experiment 2 (Expt 2): Infection of commercial broiler chicks

Seventy one-day-old commercial broiler chicks were divided into two groups and inoculated as per Experiment 1 described above.

For both experiments, clinical signs were observed daily throughout the experimental period. At 3, 6, 9, 12, 15, 21 and 28 dpi, five infected and three control chicks were randomly selected and euthanised to evaluate the gross lesions. Tissue samples of trachea, lung, caecal tonsils and kidney were collected individually and frozen at -70^{o}C for virus detection by RT-PCR. In addition, pieces of trachea and kidney were fixed in 10% buffered formalin for histopathology. Blood samples were collected from 8 randomly selected chicks at 0, 15, 21and 28 dpi from the SPF and at 0, 3, 6, 9, 15, 21 and 28 dpi from the broiler chicks to monitor antibody responses.

3.3. Gross and microscopic examinations

All euthanised and found dead chicks were necropsied and examined for gross lesions. The upper part of the trachea and kidney tissues were fixed in 10% buffered formalin, embedded in paraffin and sections were cut for hematoxylin and eosin (H&E) staining. Trachea and kidney lesions were examined and scored as follows: 0 = no change, 1= mild, 2 = moderate, 3 = severe (Chen et al., 1996).

3.4. Processing tissue samples

Individual tissue samples of the trachea (Tr), lung (L), caecal tonsil (CT) and kidney (Kid) were collected from five chicks in each group. Each tissue was dipped in a sterile bijou containing 1.5 ml of Eagles serum-free minimum essential medium with glutamine, streptomycin [50 mg/ml] and penicillin [50 IU/ml] and stored at -70^{o}C until required.

Tissues were homogenized with sterile sand and 1.5 ml of TOC medium using a pestle and mortar. Homogenates were subjected to freeze-thaw three times and clarified by centrifugation at 3000 g for 15 minutes.

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154 (Awad et al., 2014b). Supernatant from the tissues was stored at -70 ^{o}C until required. RNA
155 was extracted from tissue samples using the QIAamp viral RNA Mini Kit following
156 manufacturer's instructions (Qiagen, UK). RT-PCR was carried out as previously described
157 (Worthington et al., 2008). Briefly, detection of the IBV genome was achieved by identifying
158 (380) base pairs of the S1 region of the S protein gene and the oligonucleotide primer used
159 were common for most of the known strains of IBV. These had been previously validated
160 using reference IBV genotypes (Worthington et al., 2008). The initial PCR used primers
161 SX1+ and SX2- . The amplicon was further amplified in a second internal PCR that used
162 primers SX3+, SX4-. The amplified DNA product from positive IBV swelling head swab
163 and cystic oviduct were the treated with 0.15 µl Exonuclease 1 (EXO) and 0.99 µl shrimp
164 alkaline phosphatase (SAP) at 37^{o}C for 30 min followed by 80^{o}C for 10 min to remove any
165 extraneous material. The Purified product together with positive sense primer (forward
166 direction using primer SX3+) were submitted to external laboratory (Source bioscience
167 sequencing, Nottingham, UK) for analysis of the partial S1 gene sequences.

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169 3.5. Serological Assay

170 Sera were tested using commercial enzyme-linked immunosorbent assay (ELISA) kits
171 (IDEXX, Hoofddorp, The Netherlands) according to the manufacturer's instructions.
172 Haemagglutination inhibition (HI) testing was performed using four haemagglutination units
173 (HAU) as previously described (Alexander and Chettle, 1977). The virus strains used as
174 antigens for the HI test were IBV M41 and 793B (Animal Health Service, Deventer, The
175 Netherlands). Haemagglutination antigens of IS/885 was prepared in our laboratory based on
176 methods previously described (King and Hopkins, 1983; Alexander and Chettle, 1977). IBV

HI titres were expressed as $\log_2$ values of the highest reciprocal of the dilution that showed HI. Titres equal to or greater than 3 $\log_2$ were considered positive.

3.6. Statistical Analysis

Data for histopathological lesions were analysed using the Mann-Whitney *U* test. The mean ELISA and HI antibody titres of infected SPF and broiler chicks sera tested with different IBV antigens were compared and analysed using one way analysis of variance (ANOVA) to test for a significant overall effect, followed by Tukey's test to identify which means were significantly different from each other. All analyses were conducted using GraphPad Prism 6.0 (http://www.graphpad.com/scientific-software/prism/).

4. Results

4.1. Clinical signs

No clinical signs or mortalities were observed in the control groups of either experiment.

In the SPF chicks, clinical signs were first observed at 1 dpi, which included mild tracheal râles, sneezing, coughing, head shaking and eye scratching. Gasping, wheezing and open mouth breathing were seen in 4-5 birds between 4 to 13 dpi. After 13 dpi, birds with mild respiratory signs and wet droppings (white or milky faeces) were observed. The clinical signs resolved by 18 dpi. Of 45 chicks, one bird died at 8 dpi (2.2%).

For the commercial broiler chicks, the clinical signs were similar to those observed in SPF chicks but with a lower severity and lasted for a longer period. Mild clinical signs of tracheal râles, sneezing, coughing and head shacking were first observed from 3 dpi and it lasted until 22 dpi. Of the 45 birds, three died; one at 12 dpi and two at 19 dpi (6.6%). Apart from

202 respiratory signs, one bird showed swelling of the head, with a foamy ocular and nasal
203 discharge at 14 dpi. The swelling increased by 15 dpi and the periocular tissues were also
204 affected (Fig 1). For welfare reasons, this bird was euthanized for necropsy and sampling was
205 carried out. A sterile dissection was performed to assess any gross lesions underneath the
206 skin. Subcutaneous swab was taken for virus detection by RT-PCR, VI and for bacterial
207 culture. Virus detection was performed in our laboratory while the bacterial culture, isolation
208 and identification was carried out by bacteriology diagnostic laboratory (School of Veterinary
209 Science, University of Liverpool).
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211
212 4.2. Gross necropsy findings

213 No detectable gross lesions were witnessed in the two control groups. In the SPF chicks
214 (Expt 1), tracheal congestion and excess mucus were found in all the chicks that were
215 necropsied at 3 dpi and such lesions disappeared by 12 dpi. Kidney lesions were first
216 observed at 6 dpi and all the necropsied chicks, showed large swelling, pale with tubules
217 and/or ureters distended with urates, and these lesions were observed until 12dpi...At 28 dpi,
218 (during the post mortem examination) serous fluid accumulation was found in the left oviduct
219 of two female SPF chicks (Fig 2). Samples of the fluid and tissues of the cystic oviduct were
220 collected for virus detection and the oviduct was also collected for histopathology. The cystic
221 fluid was positive for IBV by RT-PCR and VI, while the tissue was negative. Part-sequencing
222 of the hypervariable region of the S1 gene of this isolate revealed 99% homology with the
223 challenge virus (IS/885). Necropsy of the bird that died at 8 dpi showed large brown swollen,
224 pale kidneys with tubules and ureters distended with urates.

225 In the broiler chicks (Expt 2), the gross lesions of the trachea and kidney were similar to
226 those observed in the SPF chicks but were less severe. However, the lesions persisted for a
227 longer time than in the SPF chicks. Tracheal lesions such as yellowish caseous plugs were
228 observed at 15 dpi. Paleness and swelling of kidneys were observed from 6 to 21 dpi.
229 For the chick with the head swelling, a swab was positive for IBV and was negative for
230 bacteria. On sequencing, the virus detected was 100 % identical to the IS/885 strain (the
231 inoculum). The virus was successfully isolated in SPF ECEs and typical IBV-lesion was
232 found in the embryo (eg. dwarfing and curling of the embryos). Necropsy of birds that died
233 showed emaciation, dehydration, caseous exudate plugs in the upper trachea and pale brown,
234 swollen kidneys.
235 4.3. Histopathology
236 No significant histological abnormalities were observed in the trachea, kidneys or oviduct of
237 the control groups in either experiment.
238
239 4.3.1. Trachea of infected chicks
240 Details of histological lesions in the tracheas for SPF and broiler chicks are summarised in
241 Table 1and Table 2 respectively. In both lines of chicks (SPF and broiler), lesions were
242 similar but the severity differed throughout the study period.
243 In the SPF chicks, severe changes in the trachea were observed at 3 dpi. The most consistent
244 lesions in the SPF chick were loss of cilia and heterophil infiltration, decreased mucous cells
245 and an occasional heterophilic exudate in the tracheal lumen (Fig 3b). Mild to moderate
246 lesions were witnessed until 15 dpi. Ciliated epithelium repaired by 21 dpi. Areas of severe
247 lymphoid infiltration in the lamina propria or submucosa persisted and were found until the
248 end of the experiment (Table 1).

In the broilers chicks, at an early stage of infection, each lesion type was less severe than those observed in the SPF chicks but persisted for longer period (Fig 3c). At 21 dpi, the histopathological changes were significantly greater ($p < 0.05$) than those observed in the SPF chicks (Table 1).

4.3.2. Kidney of the infected chicks

A summary of the histopathological changes in the kidneys in SPF and broiler experiments are given in Table 3 and 4 respectively. In the SPF chicks, kidney lesions developed by 3 dpi, which included ducto-tubular dilation, interstitial heterophilic infiltration and epithelial hyperplasia. The main histological lesions consisted of interstitial lymphoid infiltration with mild lymphoid nodules observed throughout the study (Fig 3e). Most of the kidney lesions had cleared by 21 dpi, apart from mild lymphoid infiltration and lymphoid nodules that were present until the end of the experiment (Table 3).

In the broiler chicks, kidney lesions were first observed at 6 dpi, which included tubular degeneration and hyperplasia of the epithelium. Lymphoid infiltration was the main lesion throughout the observation period (Fig 3f).. No significant differences were observed among the histological scores of the lesions between the SPF and broiler chicks (Table 4).

4.3.3. Oviduct of infected SPF chicks

The oviduct lumen was multifocally partially lined by compact epithelial cells devoid of cilia (Fig 4). Many epithelial cells are shed into the lumen leaving sections without mucosa. The underlying submucosa was multifocally infiltrated by both moderate numbers of degenerate and viable lymphocytes and plasma cells with lesser numbers of macrophages.

4.4. IBV RT-PCR

No virus was detected in the control groups of either experiment throughout the study.

In the SPF chicks, the virus was detected in the trachea, lung, caecal tonsil and kidney in most of the tested samples for up to 15 dpi. Beyond that, the virus was infrequently detected in tissues (Table 5). In the broiler chicks, detection of the virus was lower than observed in the SPF chicks throughout the study. IBV was found most frequently in the caecal tonsil, followed by the trachea (Table 5).

4.5. Antibody detection

4.5.1. Enzyme-linked immunosorbent assay

Analysis of the antibody titres of both experiments using ELISA is summarised in Table 6. In the SPF chicks, sera collected prior to experimental infection were free of IBV antibodies. Chicks which received virus-free AF had ELISA titres lower than the detectable level throughout the experimental period. In the IS/885-infected chicks, an increase of antibody titres occurred at 21 dpi which decreased by 28 dpi (Table 6). In broiler chicks, high levels of IBV MDA were detected in all birds at 1-day-old. By 3 dpi, the MDA had declined in the control and infected groups and showed no significant differences (Table 6). At 9 and 15 dpi, neither the infected nor control group had antibody titres above the cut-off point. However, at 21 and 28 dpi, highly significant antibody titres were observed in the infected group compared to the control group (Table 6).

4.5.2. Haemagglutination inhibition test

Analysis of the HI antibody titres of both experiments using different IBV antigens are summarised in Tables 7 and 8. In the SPF chicks, all sera collected prior to experimental infection and from the control group were free of IBV HI antibodies (Table 7). At 15 dpi, the HI titres against the heterologous antigens of M41 and 793B were low (<3 $\log_2$) compared to

the homologous antigen (3.8 $\log_2$). At 21 dpi, chicks showed high antibody titres (≥3 $\log_2$) compared to control birds. The birds showed high significant level ($p < 0.05$) of IBV HI antibody titres when IS/885 was used as antigen throughout the sampling time (Table 7). In the broiler chicks, sera collected at one day old showed a high, significant antibody titre when either M41 or 793B were used as the antigen (Table 8). Titres had started to decline significantly by 3 dpi in both the infected and control groups. At 6 and 9 dpi, there were no detectable titres in either (<3 $\log_2$). When all IBV antigens were compared, higher levels of IBV antibody titres were seen when IS/885 used as the antigen from 15 dpi onwards.

5. Discussion

The results presented in this study demonstrate that IS/885/00 is a virulent IBV, as extensive disease was produced following infection in both types of chicks. In addition to the respiratory signs, gross lesions comprising tracheal caseous exudate and plugs, and swollen

kidneys (with or without) urate deposits were observed. Such clinical signs have been observed following infection with virulent IBV M41(Butcher et al., 1990), Moroccan G strain (Ambali and Jones, 1990),793B (Boroomand et al., 2012), It-02 (Dolz et al., 2012) and QX (Ganapathy et al., 2012). These clinical signs and lesions were more severe in SPF than in the broiler chicks, indicating differences in the susceptibility to IBV infection in these different types of birds. This could be due to the genetic line of the birds or the IBV MDAs in the broiler chicks which probably neutralized up to a certain level IBV (Ignjatovic et al., 2003). Previous studies using virulent IBV M41 demonstrated a considerable difference between two white leghorn chick lines in term of the severity and duration of respiratory signs (Otsuki et al., 1990).

It has been reported that chicks with high antibody titres had a better protection against IBV M41 infection at one day old but not at seven days old (Mondal and Naqi, 2001) and that MDA does not prevent the viral infection but does reduce the pathogenic effects of the IBV infection in young chicks (Klieve and Cumming, 1988). Our findings suggest that MDA is an important factor in determining the severity of the disease caused by IS/885 in young chicks.

Following IS/885 infection of the broiler chicks, clear periorbital swelling was noted in one bird at 15 dpi. The swab collected from this site was positive for IBV by RT-PCR and VI, and sequencing demonstrated 100% nucleotide level identity to IS/885. Neither aMPV nor bacteria (*E. coli or Mycoplasma*) were detected by PCR or cultured from swabbing, suggesting that the swelling was due to the IBV infection. This finding is similar to the first report of SHS where a untyped coronavirus and *E.coli* were isolated from a broiler chickens in Southern Africa (Morley and Thomson, 1984). IBV M41 and *E.coli* were isolated from broiler flock in the USA that experienced SHS (Droual and Woolcock, 1994). aMPV (Picault et al., 1987; Aung et al., 2008), FAdV (Droual and Woolcock, 1994; Georgiades et al., 2001)

351 and secondary infection (such as *E.coli*) have been implicated as the cause of SHS in chicken
352 (Nakamura et al., 1997; Nakamura et al., 1998).

353 In the IS/885-infected SPF chicks, necropsy examination at 28 dpi revealed dilation of the
354 oviduct with fluid content (cystic oviduct) in two female chicks. Cystic oviduct formation
355 following infection of virulent IBVs at a young age has been reported for IBV variants M41
356 (Crinion et al., 1971; Crinion and Hofstad, 1972; Jones and Jordan, 1972) and recently, QX
357 (Benyeda et al., 2009; de Wit et al., 2011b; Ganapathy et al., 2012). We report, for the first
358 time, similar pathological development in SPF chicks that received the IS/885 virus at 1-day
359 old. In addition, the virus with 99% part-S1 sequence identity to IS/885 was isolated from the
360 cystic fluid. The detection of IBV IS/885 in the oviduct fluid demonstrates both active
361 replication of the virus in the epithelium and its abundance in the fluid and were shed by
362 epithelial cells. The epithelial cells of the oviduct are the primary cells attacked by the IBV
363 (Crinion and Hofstad, 1972). The IS/885 infection has caused a diffuse loss in the cilia,
364 epithelial necrosis and infiltration of lymphocytes and plasma cells into the oviduct which
365 suggests that the virus has replicated in the epithelial cells lining the oviduct.

366 The pathogenicity of IBV strain for the oviduct varies as some do not replicate in the oviduct
367 neither causing lesions nor showing significant amounts of viral antigen in the epithelial cells
368 (Crinion and Hofstad, 1972). IBV Conn and Iowa failed to produce any gross or
369 histopathological change in the oviduct, in contrast, IBV M41 produces greatest number of
370 changes, followed by Australian T (Crinion and Hofstad, 1972). Benyeda et al. (2009) also
371 did not detect any abnormalities in the oviducts of 793B infected 1-day-old SPF chicks,
372 whereas a variable percentage of oviduct dilatations were detected following inoculation with
373 five different QX-like strains. It appears that along with M41 and QX, IS/885 should be
374 considered as a potential cause of cystic oviducts in female chickens.

375 The microscopic findings in the SPF and broiler chicks following IS/885 infection were
376 similar to other virulent IBVs as reported (Albassam et al., 1986; Nakamura et al., 1991;
377 Chen et al., 1996). In this work, the SPF chicks had greater necrosis in the trachea, indicating
378 a more extensive virus replication than broiler chicks, which may have been due to
379 extravasation of circulating maternal antibody in the latter. In both types of birds, severe and
380 early onset of histopathological lesions was seen in the trachea, compared to mild relatively
381 lesions in the kidney. Nephropathogenic IBVs have shown tropism for respiratory and
382 kidney tissues although kidney lesions are more apparent (Albassam et al., 1986; Butcher et
383 al., 1989). Based on these findings, the IS/885 strain appears to have an affinity for both
384 respiratory and renal tissues.

385 In this study, persistence of the viral genome was observed in the selected tissues until the
386 end of all experiments (28 dpi). Though, no isolation was attempted, the results showed
387 marked reduction in the detection in the trachea, lungs and kidneys of broilers in comparison
388 to the SPF chicks. This again shows the advantage of having IBV maternal-antibodies in
389 young chicks, which appears to inhibit the invasion and colonisation IS/885 in visceral tissues
390 (Mondal and Naqi, 2001).

391 The immune response after infection with IBV was measured by ELISA and HI. With
392 ELISA, a maximum mean antibody titre was found at 21 days post infection in SPF and
393 broilers. For HI, sera were assayed against M41 and 793B-since most producers use live and
394 inactivated vaccines related to these serotypes. IS/885 antigen was also used as a
395 homologous comparison. With HI, distinctive patterns were seen in broiler chicks; higher
396 mean HI titres against M41 and 793B at day-old reflects the maternal antibodies derived from
397 parent birds that were previously vaccinated using Massachusetts, 793B or D274 vaccines.
398 For 3, 6 and 9 days post infection, though there were no significant differences between the

IS/885-infected broiler chicks, the titres declined against all three HI antigens. From 15 days post infection, the mean HI titres against M41, 793B and IS/885 peaked by 21 days post infection and then declined by 28 days post infection. In contrast to day-old sera, now the mean titre is highest for the IS/885 antigen, reflecting a better reactivity with the homologous antigen. These significant levels of HI antibodies against the IS/885 antigen were witnessed when compared to the level of antibody response against M41 and 793B antigens in IS/885-infected birds. This finding support the use of the homologous virus as an antigen in the HI test, as has been reported that the HI test is strain specific (Monreal et al., 1985; de Wit et al., 1997).

6. Conclusions

This study showed that under experimental condition, the IBV IS/885 is pathogenic for both SPF and broiler chicks, with lesser disease severity in the latter, and. The IS/885 showed tropism for both respiratory and renal tissues. In addition, this virus causes head-swelling and cystic oviduct development in young chicks.

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587 **Fig. 1.** Periocular swelling of an IS/885-infected broiler chick at 15 dpi.

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592 **Fig. 2**. Cystic dilation of the oviduct of an IS/885-SPF female chick at 28 dpi.

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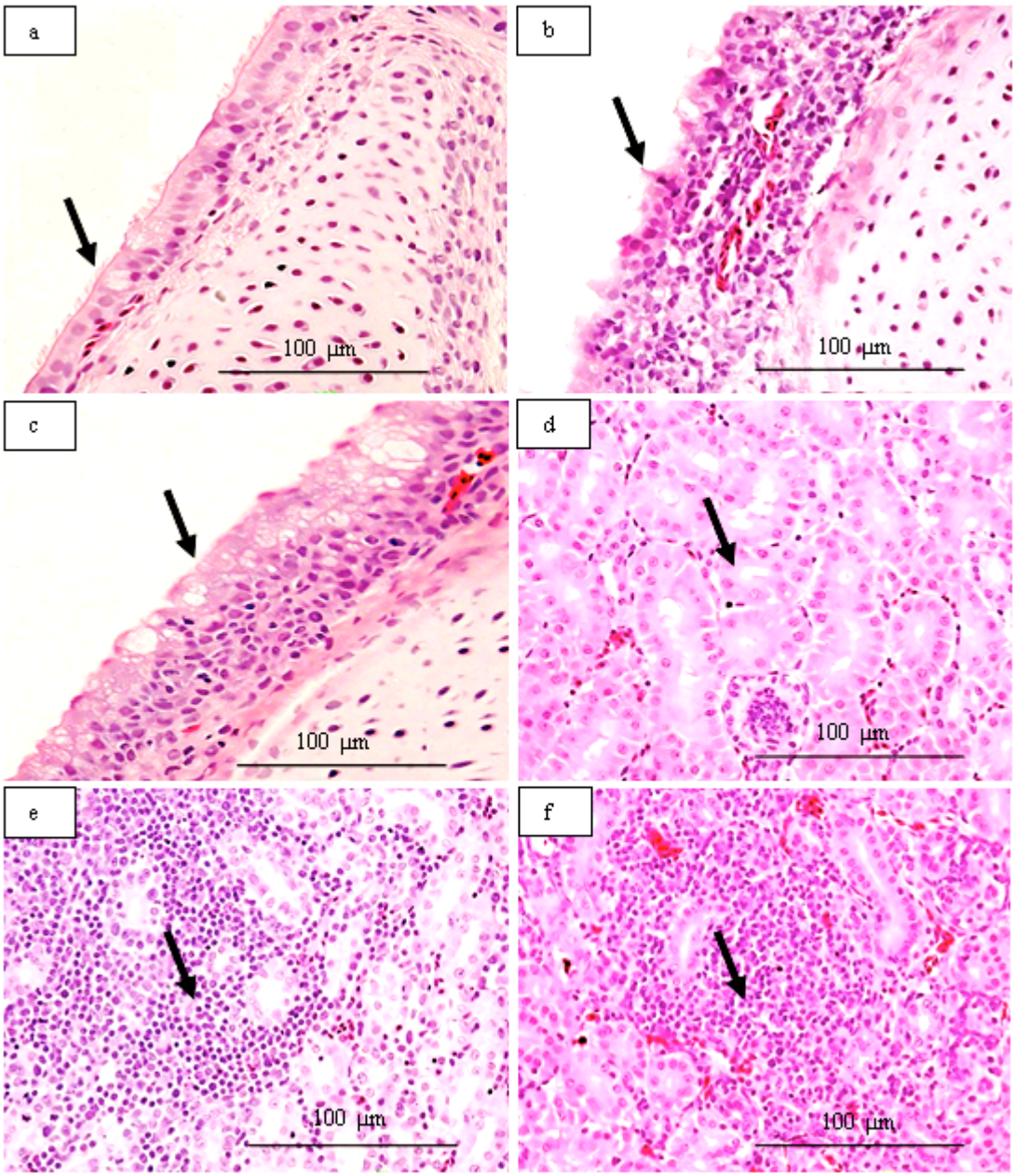

Fig. 3. Hematoxylin and eosin staining of the trachea and kidney (magnification at 400x). Panels (a) -trachea- and (d) kidney correspond to control chick tissues taken at 3 and 9 days of age respectively. (b), Extensive epithelial deciliation, with severe lymphocyte and heterophil infiltration of trachea of SPF chicks at 3 dpi. (c), Mild epithelial deciliation, with moderate lymphocyte and heterophil infiltrate and mild epithelial hyperplasia of broiler chicks at 3 dpi. (e), Moderate to severe lymphocyte and mild heterophil interstitial infiltration in kidney of SPF chicks at 9 dpi. (f), Mild to moderate lymphocyte and heterophil interstitial infiltration in kidney of broiler chick at 9 dpi

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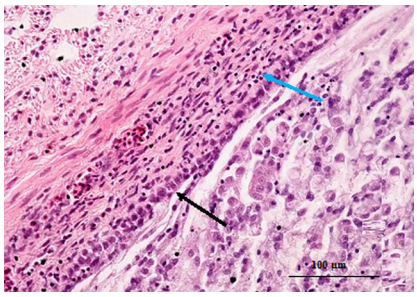

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607 **Fig. 4.** Hematoxylin and eosin stains of the cystic oviduct of IS/885-infected female SPF
608 chicks at 28 dpi (magnification at 400x). Multifocal moderate chronic lymphocytic salpingitis
609 with cystic dilation. Diffuse loss in the cilia (black arrow) and infiltration of lymphocytes and
610 plasma cells in submucosa (Blue arrow).

611

612 **Table 1**

613 Histopathology scores of the tracheal lesions in SPF chicks infected with IBV IS/885 strain.

Tracheal lesions	SPF (Days post infection)						
	3	**6**	**9**	**12**	**15**	**21**	**28**
Epithelial deciliation	3.0±0.0[a]	ND	3.0±0.0	2.8±0.2	0.5±0.2	0.0±0.0[a]	0.0±0.0
Epithelial degeneration	0.5±0.2	ND	1.2±0.2	1.0±0.3	1.0±0.4	0.5±0.2	0.5±0.5
Decrease mucous cells	1.2±0.2[a]	ND	2.2±0.2	2.6±0.2	2.0±0.4	1.2±0.5	1.5±0.5
Heterophil infiltration	2.0±0.0 [a]	ND	0.5±0.2	0.8±0.3	0.0±0.0	0.0±0.0 [a]	0.0±0.0
Epithelial hyperplasia	1.7±0.2	ND	2.7±0.2[a]	2.6±0.2[a]	1.2±0.2	1.0±0.0 [a]	1.5±0.5
Lymphoid infiltration	2.0±0.0 [a]	ND	1.5±0.2	1.4±0.2	2.0±0.0	1.7±0.2	2.0±0.0

ND: not done; Data are expressed as the mean histopathological lesion score ± SEM (n = 5). Tracheal lesions scores as follows: no change (0), mild (1), moderate (2) or severe (3). For each time point and lesions, different superscripts for infected SPF and broiler chicks indicate significant differences between them ($p < 0.05$). Absence of a letter indicates that there were no significant differences ($p > 0.05$).

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Table 2

Histopathology scores of the tracheal lesions in broiler chicks infected with IBV IS/885 strain.

Tracheal lesions	Broiler (Days post infection)						
	3	6	9	12	15	21	28
Epithelial deciliation	1.6±0.4[b]	2.8±0.2	2.4±0.2	2.4±0.4	2.2±0.4	2.0±0.3[b]	0.0±0.0
Epithelial degeneration	0.8±0.2	1.2±0.2	1.4±0.2	1.8±0.3	1.0±0.0	1.4±0.2	0.2±0.2
Decrease mucous cells	0.2±0.2[b]	1.8±0.5	1.8±0.2	2.0±0.3	2.8±0.2	2.2±0.3	0.5±0.2
Heterophil infiltration	0.8±0.3[b]	0.8±0.3	1.6±0.5	1.0±0.3	0.6±0.2	1.2±0.3[b]	0.2±0.2
Epithelial hyperplasia	1.2±0.2	1.4±0.2	1.6±0.2 [b]	1.0±0.0[b]	1.0±0.4	2.0±0.0[b]	1.5±0.2
Lymphoid infiltration	1.0±0.0[b]	1.2±0.2	1.8±0.2	1.6±0.2	1.6±0.4	2.2±0.2	1.5±0.2

ND: not done; Data are expressed as the mean histopathological lesion score ± SEM (n = 5). Tracheal lesions scores as follows: no change (0), mild (1), moderate (2) or severe (3). For each time point and lesions, different superscripts for infected SPF and broiler chicks indicate significant differences between them ($p < 0.05$). Absence of a letter indicates that there were no significant differences ($p > 0.05$).

623 **Table 3**

624 Histopathology scores of the kidney lesions in SPF chicks infected with IBV IS/885 strain.

Kidney lesions	SPF (Days post infection)						
	3	6	9	12	15	21	28
Epithelial degeneration	0.0±0.0	0.0±0.0	0.6±0.2	0.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0
Ducto-tubular dilation	0.4±0.2	0.4±0.2	0.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Heterophil infiltration	0.2±0.2	1.0±0.0[a]	0.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Lymphoid infiltration	0.0±0.0	0.0±0.0	1.0±0.0	1.6±0.2	1.0±0.0	0.6±0.2	0.4±0.2
Epithelial regeneration	0.0±0.0	0.0±0.0	0.2±0.2	0.2±0.2	0.8±0.2	0.0±0.0	0.0±0.0
Epithelial hyperplasia	0.2±.02	0.0±0.0	0.6±0.2	0.2±0.2	1.0±0.0[a]	0.0±0.0	0.0±0.0
Lymphoid nodules	0.0±0.0	0.0±0.0	0.6±0.2	0.8±0.2	1.0±0.0[a]	0.4±0.2	0.8±0.2

Data are expressed as the mean histopathological lesion score ± SEM (n = 5). Kidney lesions scores as follows: no change (0), mild (1), moderate (2) or severe (3). For each time point and lesions, different superscripts for infected SPF and broiler chicks indicate significant differences between them ($p < 0.05$). Absence of a letter indicates that there were no significant differences ($p > 0.05$).

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628 **Table 4**

629 Histopathology scores of the kidney lesions in broiler chicks infected with IBV IS/885 strain.

Kidney lesions	Broiler (Days post infection)						
	3	6	9	12	15	21	28
Epithelial degeneration	0.0±0.0	0.4±0.2	0.0±0.0	0.0±0.0	0.2±0.2	0.2±0.2	0.0±0.0
Ducto-tubular dilation	0.0±0.0	0.0±0.0	0.2±0.2	0.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0
Heterophil infiltration	0.0±0.0	0.0±0.0[a]	0.2±0.2	0.0±0.0	0.2±0.2	0.0±0.0	0.4±0.2
Lymphoid infiltration	0.0±0.0	0.0±0.0	1.0±0.0	1.2±0.2	0.6±0.2	0.6±0.2	0.0±0.0
Epithelial regeneration	0.0±0.0	0.8±0.3	0.2±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Epithelial hyperplasia	0.0±0.0	0.2±0.2	0.0±0.0	0.0±0.0	0.2±0.2[b]	0.2±0.2	0.0±0.0
Lymphoid nodules	0.0±0.0	0.0±0.0	0.0±0.0	0.6±0.4	0.0±0.0[b]	0.4±0.4	1.0±0.0

Data are expressed as the mean histopathological lesion score ± SEM (n = 5). Kidney lesions scores as follows: no change (0), mild (1), moderate (2) or severe (3). For each time point and lesions, different superscripts for infected SPF and broiler chicks indicate significant differences between them ($p < 0.05$). Absence of a letter indicates that there were no significant differences ($p > 0.05$).

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633 **Table 5**

634 Virus detection by RT-PCR in tissue of SPF or broiler chicks infected with IBV IS/885

635 strain.

Days post infection	SPF				Broiler			
	Tr	L	CT	Kid	Tr	L	CT	Kid
3	5*	5	4	4	3	1	5	5
6	5	5	5	5	5	2	5	2
9	5	5	5	5	2	0	4	0
12	5	5	4	5	4	1	2	1
15	5	5	5	5	4	2	4	1
21	2	3	4	3	0	0	3	1
28	1	3	3	0	1	0	5	2
Total≠	28	31	30	27	19	6	28	12

* Values presented are number of IBV-positive cases out of five birds examined. Tr= Trachea, L= lung, CT= caecal tonsil, Kid= kidney. ≠ Total number of tissues that was positive for IBV.

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639 **Table 6**

640 Mean IBV ELISA antibody titres in the SPF and broiler chicks infected with IBV IS/885
641 strain.

Days post infection	SPF		Broiler	
	Infected	**Control**	**Infected**	**Control**
0	21±9.0	21±9.0	2867±229	2867±229
3	ND	ND	1813±343	1809±544
6	ND	ND	531±153	501±64
9	ND	ND	311±85	282±75
15	160±64[a]	4.0±2.0[b]	378±175	75±19
21	551±146[a]	12±5.0 [b]	1116±129[b]	19±8.0[b]
28	481±194[a]	7.0±4.0[b]	623±35[b]	10±8.0[b]

ND: test not done. Data are expressed as the mean values ± SEM (n = 8). Data in the same row with different superscript letters are significantly different in antibody titres ($p < 0.05$). Absence of a letter indicates that there were no significant differences ($p > 0.05$) between any of the time points. The cut-off values for ELISA = 396.

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645 **Table 7**

646 Mean HI antibody titres in the SPF chicks infected with IS/885 strain using homologous and

647 heterologous antigens.

Days post infection	Infected			Control		
	M41	**793B**	**IS/885**	**M41**	**793B**	**IS/885**
0	0.6±0.2	1.1±0.2	0.7±0.2	0.6±0.2	1.1±0.2	0.6±0.2
3	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND
9	ND	ND	ND	ND	ND	ND
15	1.5 ± 0.3^{ab}	0.6 ± 0.1^{a}	$3.8\pm0.2^{c*}$	1.1±0.1	0.6±0.1	0.6±0.1
21	$4.0\pm0.2^{a*}$	$3.6\pm0.3^{a*}$	$5.1\pm0.3^{b*}$	0.8±0.3	0.7±0.2	0.3±0.1
28	$2.5\pm0.4^{a*}$	$2.6\pm0.1^{a*}$	$3.9\pm0.2^{b*}$	0.6±0.1	0.2±0.1	0.3±0.2

ND: test not done. Data are expressed as the mean values ± SEM (n = 8). Data in the same row across with different superscript letters are significantly different ($p < 0.05$). Absence of a letter indicates that there were no significant differences ($p > 0.05$) between the IBV antigens at any of the time points. *Values differed significantly from each representative control group. The cut-off point $=>3\log_2$

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651 **Table 8**

652 Mean IBV HI antibody titres in the broiler chicks infected with IS/885 strain using IBV
653 homologous and heterologous antigens.

Days post infection	Infected group			Control		
	M41	**793B**	**IS/885**	**M41**	**793B**	**IS/885**
0	6.2±0.6^{a}	5.8±0.5^{a}	3.5±0.2^{b}	6.2±0.6^{a}	5.8±0.5^{a}	3.5±0.2^{b}
3	5.1±0.7^{a}	5.0±0.2^{a}	2.6±0.1^{a}	5.1±1.1^{a}	5.5±0.8^{a}	3.1±0.1^{b}
6	2.4±0.6	1.3±0.3	2.1±0.2	3.0±0.3^{a}	3.3±0.5^{b}	1.7±0.3^{a}
9	1.6±0.8	1.4±0.2	1.8±0.2	1.6±0.8	2.3±0.6	1.1±0.2
15	3.5±0.1^{a*}	3.3±0.1^{a*}	4.0±0.2^{a*}	1.3±0.4	0.7±0.1	1.0 ±0.0
21	5.0±0.0^{a*}	4.2±0.4^{ab*}	5.8±0.2^{c*}	1.1±0.3	0.6±0.1	0.3±0.2
28	3.7±0.1^{a*}	3.5±0.1^{a*}	4.5±0.1^{c*}	0.7±0.2	0.4±0.1	0.1±0.1

Data are expressed as the mean values ± SEM (n = 8). Data in the same row across with different superscript letters are significantly different ($p < 0.05$). Absence of a letter indicates that there were no significant differences ($p > 0.05$) between the IBV antigens at any of the time points. *Values differed significantly from each representative control group. The cut-off point $=>3\log_2$

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