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REVIEW ARTICLE

Infectious bronchitis virus: immunopathogenesis of infection in the chicken

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SUMMARY

The immunopathogenesis of infectious bronchitis virus (IBV) infection in the chicken is reviewed. While infectious bronchitis (IB) is considered primarily a disease of the respiratory system, different IBV strains may show variable tissue tropisms and also affect the oviduct and the kidneys, with serious consequences. Some strains replicate in the intestine but apparently without pathological changes. Pectoral myopathy has been associated with an important recent variant. Several factors can influence the course of infection with IBV, including the age, breed and nutrition of the chicken, the environment and intercurrent infection with other infectious agents. Immunogenic components of the virus include the S (spike) proteins and the N nucleoprotein. The humoral, local and cellular responses of the chicken to IBV are reviewed, together with genetic resistance of the chicken. In long-term persistence of IBV, the caecal tonsil or kidney have been proposed as the sites of persistence. Antigenic variation among IBV strains is related to relatively small differences in amino acid sequences in the S1 spike protein. However, antigenic studies alone do not adequately define immunological relationships between strains and cross-immunisation studies have been used to classify IBV isolates into 'protectotypes'. It has been speculated that changes in the S1 protein may be related to differences in tissue tropisms shown by different strains. Perhaps in the future, new strains of IBV may arise which affect organs or systems not normally associated with IB.

INTRODUCTION

The study of pathogenesis was formerly regarded as a messy, unsatisfactory area for virus research—a sad state of affairs that was lamented by early enthusiasts (Mimms, 1964). That view has changed now and there is an urgent need to know how viruses cause diseases. The study of mechanisms of pathogenicity was recently placed at the top of the research objectives proposed for priority treatment by an *ad hoc* group on vaccinology (Bourne, 1996). However, pathogenesis cannot be studied without also considering immunology and mechanisms of protection. This review attempts to concisely present the current state of knowl-

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edge on the immunopathogenesis of infection in chickens with infectious bronchitis virus (IBV).

In 1931 a 'new respiratory disease of baby chicks' was identified in the USA by Schalk & Hawn and named infectious bronchitis (IB). Following infection of chickens with IBV, falls in egg production and quality were reported for the first time by Van Roekel *et al.* (1951) and Broadfoot & Smith (1954). In 1962, Cumming found that IBV was the cause of nephrosis-nephritis syndrome seen in Australia. In more recent years, IBV has also been isolated from cloacal swabs and gut tissues, but while infection has sometimes been associated with diarrhoea, no pathological changes have been described in the alimentary tract. Swollen head syndrome (SHS) in chickens has generally been associated with infection with the avian pneumovirus (APV), turkey rhinotracheitis virus (TRTV) (Picault *et al.*, 1987; Lu *et al.*, 1994). However, in the first description of SHS, Morley & Thomson (1984) isolated a coronavirus, while in other reports, Massachusetts strains of IBV have also been isolated (Shirai *et al.*, 1993; Droual & Woolcock, 1994). Recently, a variant strain of IBV, 793/B was isolated from broiler breeder flocks where the affected birds had bilateral myopathy affecting both superficial and deep pectoral muscles (Gough *et al.*, 1992).

Thus, it is clear that strains of IBV have wide and variable tissue tropisms, and the clinical manifestations of the disease can be diverse. Although the name IB does not encompass the varied clinical manifestations observed with IBV infections, for the present this name has still been retained to avoid confusion.

RESPIRATORY INFECTION

Replication of IBV in the respiratory tissues causes characteristic, but not pathognomonic signs such as gasping, coughing, tracheal rales and nasal discharge. Occasionally, puffy, inflamed eyes and swollen sinuses may be seen (Parsons *et al.*, 1992; Capua *et al.*, 1994). In uncomplicated cases these signs last for only 5 to 7 days and disappear within 10 to 14 days. The affected chickens also appear depressed, and feed consumption and weight gain are significantly reduced from 3 days after infection (Otsuki *et al.*, 1990). In uncomplicated cases, mortalities are generally low and have been attributed to asphyxiation due to blocking of the lower trachea or bronchi by plugs of mucus.

The upper respiratory tract is the main site of IBV replication, following which a viraemia occurs and the virus gets widely disseminated to other tissues (McMartin, 1993). The virus is primarily epitheliotropic and enters the epithelial cells by viropexis (Patterson & Bingham, 1976). Studies using immunofluorescence (IF) (Jones & Jordan, 1972; Yagyu & Ohta, 1990), immunoperoxidase (IP) (Nakamura *et al.*, 1991; Owen *et al.*, 1991) and electron microscopy (Purcell & Clarke, 1972; Nakamura *et al.*, 1991) have shown virus replication in ciliated epithelial and mucus-secreting cells. During the clinical phase of the disease, maximum virus titres are recorded in the trachea between 5 and 10 days post-infection (p.i.) (Ambali & Jones, 1990; Otsuki *et al.*, 1990), but occasionally

virus may be present for up to 28 days p.i. (Alexander & Gough, 1977; Cook, 1968).

Virus also replicates in the epithelial cells of lungs (Janse *et al.*, 1994) and airsacs (Nauwynck & Pensaert, 1988). High virus titres are seen in these tissues between 4 to 11 days p.i. (Nauwynck & Pensaert, 1988; Otsuki *et al.*, 1990).

Infected chickens have mucosal thickening with serous or catarrhal exudate in the nasal passages, sinuses and trachea. The incidence of nasal exudate in experimentally-infected chickens has been used to assess the severity of disease in different inbred lines (Parsons *et al.*, 1992) and in bursectomised birds (Cook *et al.*, 1991a). Small areas of pneumonia may be observed in the lungs. The air sacs may appear cloudy or contain a yellow caseous exudate (King & Cavanagh, 1991).

The progression of lesions in the trachea has been divided into three stages, degenerative, hyperplastic and recovery (Nakamura *et al.*, 1991; Purcell & McFerran, 1972). Deciliation and desquamation of epithelial and mucous-secreting cells occur in the first 1 or 2 days with a mild infiltration of heterophils and lymphocytes in the lamina propria. Heterophils are often seen infiltrating between ciliated epithelial cells and occasionally in the lumen of the trachea. During the hyperplastic stage, newly-formed epithelial cells are observed which usually have no cilia. By 4 to 6 days the reparative processes begin with complete recovery by 10 to 20 days (Chen *et al.*, 1996). In affected airsacs, epithelial cell desquamation, oedema and some fibrinous exudate may be seen (King & Cavanagh, 1991).

Variation in virulence for respiratory tract between IBV strains

Difficulty in quantifying severity of respiratory infection caused by IBV has always been a problem in studying its pathogenicity (Cook & Huggins, 1986). The virus causes stasis of tracheal cilia both *in vivo* and *in vitro*, and this parameter has been used to assess severity of respiratory infection. Otsuki *et al.* (1990) found that following infection with the M41 strain, the duration and severity of tracheal ciliary damage was longer in a susceptible line of chicken (15I) than in a resistant line (C). The authors proposed that this method could be used to compare the pathogenicity of different strains or susceptibility of different breeds to IBV. Cubillos *et al.* (1991) found that in unvaccinated chickens challenged with four IBV isolates from Chile (one belonging to the Massachusetts serotype and three variants), the tracheal damage in terms of ciliary activity was variable, suggesting differences in virulence of IBV strains for the trachea.

While tracheal organ cultures (TOC) have been used extensively for cultivation and assay of IBV, distinct differences in virulence among IBV strains have not been observed in this *in vitro* system. Cook *et al.* (1976), while standardizing TOC for the isolation and assay of IBV, compared three strains of IBV on the basis of their effect on tracheal cilia, but found no marked differences. Dhinakar Raj & Jones (1996c) also reported little difference among seven IBV strains using measurement of ciliary activity as a criterion for damage to the

tracheal epithelium. Of nine strains compared in TOC by Cubillos *et al.* (1991), the hyperplasia caused by two viruses was the only histological difference. IBV infections may not occur as a single entity in the field. With the presence of several respiratory diseases in chickens caused by bacteria and viruses, the role of these agents in increasing the severity of IBV or otherwise is an important contributory factor in influencing the pathogenesis of the disease (see later).

REPRODUCTIVE EFFECTS

Laying hens

In layers, IB can cause a severe decline in egg production, and later, a deterioration in shell and internal quality (McDougall, 1968; Sevoian & Levine, 1957). Such effects may be accompanied by mild (Muneer *et al.*, 1986, 1988) or no respiratory signs (Cook, 1984; Cook & Huggins, 1986). Some strains produce only a loss in shell colour (Cook & Huggins, 1986). The severity of production decline varies with the period of lay, the virulence of the virus involved and other non-specific factors. The response of individual hens varies greatly (McMartin, 1968). Production may start to increase after 2 to 3 weeks, but reaches only sub-optimal levels. When laying is resumed, some eggs have soft-shells, while others are mis-shapen or rough-shelled.

When laying hens were infected with IBV strain M41, viral antigen was demonstrated in the epithelium of the oviducts between 6 and 9 days p.i. (Jones & Jordan, 1971). Areas of glandular hypoplasia caused by IBV leads to reduction in the synthesis of albumen proteins especially ovomucin, lysozyme and other major proteins which constitute the structural matrix of the thick albumen (Butler *et al.*, 1972). Hence, there is a decrease in the proportion of both thick and inner thin albumen, and an increase in the amount of outer thin albumen causing 'watery-whites'. Presence of blood or meat spots in the egg albumen has also been reported (McDougall, 1968; Muneer *et al.*, 1987).

Inspissated yolk material may be seen in the abdominal cavity of infected layers. Microscopic changes in the oviduct include reduction in the height of the epithelial cells, reduction in number or complete absence of cilia, dilation of glands, lymphocytic foci and cellular infiltration in the lamina propria and inter tubular stroma (Sevoian & Levine, 1957).

The mechanisms whereby IBV infection causes egg production of some birds to cease and for varying periods of time have not been elucidated.

Young chicks

IBV infection of female chicks of less than 2 weeks of age can cause permanent damage to the developing reproductive tract, resulting in 'false layers' that do not lay normally at sexual maturity (Broadfoot *et al.*, 1956; Jones & Jordan, 1970). Following infection of day-old chicks with IBV strain M41, virus was isolated between days 5 and 11 p.i. (Jones & Jordan, 1972) and was also found to replicate

in the epithelium of the oviducts (Crinion & Hofstad, 1972a,b; Jones & Jordan, 1972).

Gross changes in the oviduct caused by early infection may vary from the presence of a continuous patent but underdeveloped structure to a blind sac projecting forward from the cloaca (Jones & Jordan, 1970, 1972). The middle third of the oviduct is the most severely affected with areas of localised hypoplasia seen between normal patent oviducts. Caudal to the hypoplastic regions, macroscopic cysts filled with a clear serous fluid may be seen (Crinion *et al.*, 1971a).

The histopathological changes in the oviduct include decreased height and loss of cilia from epithelial cells, dilation of the tubular glands, infiltration of heterophils, lymphocytes and plasma cells, and oedema and fibroplasia of the lamina propria (Crinion *et al.*, 1971a,b; Crinion & Hofstad, 1972a,b).

The effect of IBV on the male reproductive tract has not been reported.

Variation between strains

Variations in the ability of IBV strains to cause decreases in egg production and quality were reported by Guittet *et al.* (1988); D274 was the least virulent while M41 and a variant strain PL84084 had the same degree of pathogenicity. Cook & Huggins (1986) found that some variant strains of IBV caused only small decreases in egg production, but had a marked effect on egg colour. In contrast, a more recent variant (Parsons *et al.*, 1992) caused a substantial decline in egg production with little loss of egg colour in the field, although no experimental work has been done with this virus.

Differences in virulence of IBV strains for the immature chicken oviduct were reported by Crinion & Hofstad (1972a); Massachusetts and T strains were virulent, while Connecticut and Iowa 609 were not. Embryo passage of IBV strain M41 reduced its virulence for the oviduct (Crinion & Hofstad, 1972b). *In vitro*, oviduct organ cultures (OOC) were highly susceptible to the H52 strain of IBV regardless of the age of the chickens and no differences in susceptibility were seen between magnum and uterus regions (Peters *et al.*, 1979). Pradhan *et al.* (1984) showed that IBV strain M41 causes stasis of cilia in OOC prepared from precociously-induced oviducts in young chicks by oestrogen treatment. This work has been extrapolated to compare the virulence of seven strains of IBV *in vitro* using ciliostasis and a calmodulin assay to quantify the damage to oviduct epithelium (Dhinakar Raj & Jones, 1996c). An IBV isolate belonging to serotype D207 was the most virulent while an enterotropic variant strain G was the least.

NEPHRITIS

Although even those strains of IBV considered primarily to affect the respiratory tract such as M41 can occasionally cause kidney damage (Jones, 1974), nephropathogenicity has been associated only with certain strains. Greater virulence of the virus for the kidney was first reported in Australia (Cumming, 1962).

Since then, nephropathogenic IBV (NIBV) has been reported from USA and certain parts of Europe (Butcher *et al.*, 1990; Kinde *et al.*, 1991; Lambrechts *et al.*, 1993; Picault *et al.*, 1991; Zanella, 1988).

NIBV strains initially cause respiratory symptoms followed by signs due to kidney damage which include increased water consumption and wet droppings (Winterfield & Hitchner, 1962; Cumming, 1963). Mortalities occur and follow a consistent pattern (Cumming & Chubb, 1988). The first deaths usually occur around 6 days p.i., increase rapidly to a peak around 10 days with the last deaths seen around 16 days p.i. However, the mortality rates depend on several intrinsic and extrinsic factors (see below).

Virus replication in the kidneys has been shown in the proximal convoluted tubules (Chong and Apostolov, 1982), distal convoluted and collecting tubules (Owen *et al.*, 1991) and collecting ducts (Chen *et al.*, 1996). Structural alterations in the tubular epithelial cells (Condron & Marshall, 1986) caused impaired fluid and electrolyte transport leading to acute renal failure. An increase in urinary water losses in chickens infected with NIBV was found to be associated with lower urine osmolality and higher rates of urinary excretion of sodium, potassium and calcium (Afanador & Roberts, 1994; Condron & Marshall, 1985; Heath, 1970). Negative sodium balance was a direct effect of increased output of sodium in the urine, while negative potassium balance was due to decreased intake.

The kidneys of chickens infected with NIBV are swollen and pale, with tubules and ureters distended with urates (Cumming, 1963). The relative kidney weight and kidney asymmetry are increased. The haematocrit values of infected birds was decreased and plasma uric acid levels were increased (Afanador & Roberts, 1994). Despite lack of gross lesions microscopic changes of nephritis may still be present (Winterfield & Albassam, 1984).

Chen *et al.*, (1996) proposed that IBV-induced renal lesions can be considered to be a ductotubular interstitial nephritis. The virus causes granular degeneration, vacuolation and desquamation of the tubular epithelium with massive infiltration of heterophils in the interstitium in acute stages of the disease. The changes in the chronic phase were classified as being active or inactive types of interstitial lymphocytic nephritis (Albassam *et al.*, 1986). The chronic inactive form of nephritis was indicative of IBV replication in the kidneys and subsequent clearance; while the chronic active type suggested a persistent viral replication and damage to epithelial cells of the kidney tubules. Virus was isolated from 50% of the birds with chronic nephritis (Chong & Apostolov, 1982). The histopathological changes in the kidneys following NIBV infections have been described by several workers (Albassam *et al.*, 1986; Chen *et al.*, 1996; Pohl, 1974; Purcell *et al.*, 1976; Siller & Cumming, 1974).

Variation between strains

The type of kidney lesions produced by different NIBV strains were similar but their severity varied (Albassam *et al.*, 1986; Chandra, 1987). The Australian strain 'T' induced the most rapid and severe lesions following both intra-venous

(i.v.) (Chandra, 1987) or intra-ocular (Albassam *et al.*, 1986) inoculations of susceptible chickens. The effect of IBV on the trachea is apparently independent of the effect on kidney (Ratanasethakul & Cumming, 1983). A strain of NIBV, 'S' virus was less severe on the kidneys than 'T', but more severe on the tracheal mucosa. Using an i.v. inoculation model to titrate kidney infectivity, Lambrechts *et al.* (1991) found no significant differences in the infectivity among Belgian field NIBV isolates, but the infectivity of egg-passaged virus was highly reduced.

VIRUS IN THE INTESTINE

Several strains of IBV have been isolated from cloacal swabs, faeces and caecal tonsils (Alexander & Gough, 1977; Alexander *et al.*, 1978; Cook, 1984; Lucio & Fabricant, 1990). *In vitro* explants of several gut tissues have been shown to support the growth of IBV (Bhattacharjee, 1994; Bhattacharjee & Jones, 1997; Darbyshire *et al.*, 1976). In studies where virus isolation was attempted from several tissues, maximum virus isolations were obtained from the oesophagus of chickens infected with ECV2, an enteric isolate of IBV (Lucio & Fabricant, 1990) and 793/B-like virus (Dhinakar Raj & Jones, 1996a). Oesophageal swabs have also been used to identify IBV using the polymerase chain reaction (D. Cavanagh, personal communication). However, it is not clear whether the virus actually multiplies in the oesophagus or whether virus is swallowed after being expelled from the trachea. IBV has also been isolated from proventriculus, duodenum and jejunum (Ambali & Jones, 1990; Lucio & Fabricant, 1990). Darbyshire *et al.*, (1976) have shown that proventriculus was inferior only to respiratory tissues and oviduct in supporting virus multiplication *in vitro*. However, the site of virus multiplication in these tissues has not been confirmed, but presumably occurs in the epithelial cells.

In contrast, in the tissues of the lower gut, IBV replication has been described (without photographs) in cells resembling histiocytes and lymphoid cells in the caecal tonsils (Owen *et al.*, 1991) and demonstrated by IF in apical epithelial cells of the villi in ileum and rectum (Ambali & Jones, 1990; Dhinakar Raj & Jones, 1996a). Although IBV has a wide tropism for gut tissues no gross or histological changes have been reported.

A variant strain of IBV, strain G, was classified as being enterotropic by virtue of its prolonged persistence in the gut compared to the trachea (El Houadfi *et al.*, 1986). Recently, it was seen that a variant IBV strain 793/B was more enterotropic than pneumotropic and was even associated with diarrhoea in broilers (Dhinakar Raj & Jones, 1996a). It seems that studies on the replication of IBV in the gut have been neglected, probably because no IBV strain has been demonstrated to be enteropathogenic. MacDonald *et al.* (1983) reported that following crop inoculation of chicks with H52 and H120 vaccines, virus was rarely isolated from the gut or any other visceral tissues and no antibody response occurred. However, the kidneys were found to be resistant to i.v. challenge. More intensive studies on oral infection with enterotropic IBV strains or dual infections with the

virus and other enteric pathogens, such as salmonella, coccidia or rotavirus, might yield more information on the effects of IBV replication in the gut.

The ability of IBV strains to survive in the presence of low pH, digestive enzymes and bile salts may be relevant to enteric replication. Otsuki *et al.* (1979a) and Cowen & Hitchner (1975) showed that some strains suffer a greater loss of titre than others when kept at pH 3.0 for 3 or 4 h, but there was no indication that any of the more resistant strains were isolated from the gut. Ambali & Jones (1991a) compared strain M41 with an enterotropic variant (G). Both viruses had a similar sensitivity to trypsin, but the variant showed a 50-fold greater resistance to sodium tauroglycocholate, which might partly explain its ability to replicate in the gut.

PECTORAL MYOPATHY

The important variant strain of IBV, 793/B, was recently isolated from a broiler breeder flock where the affected birds had bilateral myopathy affecting both deep and superficial pectoral muscles (Gough *et al.*, 1992). There was marked swelling and pallor of deep pectoral muscles together with the presence of occasional fascial haemorrhages and a layer of gelatinous oedema over its surface. Experimental infection of 6 week old broilers with an isolate of IBV belonging to this group resulted in mild oedematous separation of muscle fibres but with no corresponding increase in serum creatine kinase concentrations (Dhinakar Raj & Jones, 1996a). It has been clearly shown that the virus was not involved directly in the pathogenesis of the muscle lesions but formation and deposition of immune complexes, such as those found in the kidneys (Dhinakar Raj & Jones, 1996a), in the capillary walls of the muscle could be a possible reason for the development of this strange lesion.

VIRUS IN OTHER TISSUES

IBV has been isolated from the Harderian gland (Dhinakar Raj & Jones, 1996a; Gelb *et al.*, 1991b; Toro *et al.*, 1996a) and IBV-positive cells have been shown in the stroma of the gland by IF staining (Toro *et al.*, 1996a). IBV vaccination by eyedrop resulted in a massive infiltration of lymphocytes, increase in plasma cell numbers (Davelaar & Kouwenhoven, 1976; Survashe *et al.*, 1979; Montgomery *et al.*, 1994; Toro *et al.*, 1996a) and desquamation of tubular epithelium in the Harderian gland with restoration from 14 days after vaccination (Toro *et al.*, 1996a). Increased numbers of plasma cells (Survashe *et al.*, 1979) and lymphoid tissue development (Montgomery *et al.*, 1994) were also seen in the lachrymal gland following IBV vaccination.

IBV has also been isolated from the bursa of Fabricius (El Houadfi *et al.*, 1986; Ambali & Jones, 1990), and gross and histopathological lesions have been shown following experimental H52 and H120 infections (MacDonald & McMartin, 1976). Although IBV has been isolated from a variety of other tissues such as liver (Alexander & Gough, 1977; Ambali & Jones, 1990) and spleen (Otsuki *et al.*,

1990) it has not been documented to be involved with any functional damage. IBV has been isolated from semen and eggs of infected chickens (Cook, 1971), but vertical transmission appears to be of little importance.

FACTORS INFLUENCING PATHOGENESIS

Several intrinsic (age and breed of chickens) and extrinsic (nutrition, environment and intercurrent infections) factors influence the pathogenesis of IBV infections.

Age

All ages are susceptible, but the clinical disease is more severe in young chicks (Animas *et al.*, 1994b) although recovery of the virus from the trachea was similar compared to 6-week old infected chickens (Animas *et al.*, 1994a). However, the duration of virus excretion in the faeces was longer in 2-week old infected chicks compared to 4- and 6-week-old chickens. As age increases chicks become more resistant to IBV-induced mortality (Smith *et al.*, 1985), nephropathic effects (Albassam *et al.*, 1986) and oviduct lesions (Crinion & Hofstad, 1972a). MacDonald *et al.* (1980) described fewer pathological changes in kidneys of chickens infected at 3 weeks of age than those infected at day-old or 10 weeks of age, although the virus was inoculated i.v., which is an unnatural route of infection.

Breed

Purchase *et al.* (1966) demonstrated variation in mortality following IBV inoculation of embryos from different inbred lines. Bumstead *et al.* (1989) compared mortalities in several inbred lines of chickens following inoculation with a pool of strains of IBV and/or *Escherichia coli* and found marked differences among them. On this basis, the lines of chickens were classified as being resistant or sensitive to IBV. However, these inbred lines did not show marked differences in susceptibility to the variant strain of IBV, 793/B (Parsons *et al.*, 1992).

Genetic differences in susceptibility to nephritis are also marked, with light breeds being more susceptible than heavy breeds (Cumming & Chubb, 1988). The mortalities in 4-week-old cockerels of 10 Australian commercial egg-laying strains varied from 11 to 59%. NIBV has been shown to cause higher mortalities in broilers than layers (Ignjatovic, 1988; Zanella, 1988) although the virus multiplies to the same extent in both types of birds (Lambrechts *et al.*, 1993). Male chicks are twice as susceptible as females to nephritis (Cumming, 1969).

Nutrition

High protein diets increase mortality from IBV-induced nephrosis; chickens fed meat meal or poultry by-product meal-based diets experience higher mortality than those fed soybean based diets (Cumming, 1969; Cumming & Chubb, 1988).

Environment

Low temperatures have a dramatic effect on mortality due to NIBV. Reduction in temperature from 20°C to 16°C increased mortality from 8 to 50% (Cumming, 1969) with greater severity of histopathological lesions in kidneys (Ratanasethakul & Cumming, 1983). Exposure to cold stress has been used to increase the severity of challenge for assessing protection afforded by IBV vaccines (Klieve & Cumming, 1990). Cold stress also increased the severity of IBV-induced tracheal lesions (Ratanasethakul & Cumming, 1983) and promoted more extensive air sacculitis after combined infections with IBV and Mycoplasma synoviae (MS) (Yoder *et al.*, 1977).

Intercurrent infections

Mycoplasmas

Adler *et al.* (1962) found that intranasal inoculation of either the Massachusetts strain of IBV or *M. gallisepticum* (MG) given alone produced little by way of clinical signs, but dual infections resulted in coryza, tracheitis and airsacculitis. The incidence of airsacculitis was highest in chickens infected with IBV 1 week after MG inoculation. In adult chickens combined infections with IBV and MG has been shown to cause a more severe effect on egg production and quality than inoculation with either agent alone (Blake, 1962).

Olson *et al.*, (1964) exposed chickens to IBV and MS but found that dual infections were not synergistic. In contrast, later work by Kleven *et al.*, (1972) found that airsacculitis resulted when chickens were infected with IB and Newcastle disease (ND) vaccines after MS infection. Hopkins & Yoder (1982), in similar experiments with MS, gave different strains of IBV 2 to 5 days before the mycoplasma. They showed that different strains of IBV had differing abilities to induce airsacculitis. Field isolates of IBV had the most severe effects. Vaccines of high passage produced very mild lesions while those of low passage caused intermediate lesions.

Bacteria

Gross (1958), in one of the first studies, infected 5-week-old chickens with IBV and gave pathogenic *E. coli* by aerosol at intervals afterwards. The most severe pericarditis was seen when the bacteria were given 11 to 22 days after the virus. Smith *et al.* (1985), using a combined inoculation of a pool of strains of IBV and *E. coli*, developed an experimental model for IBV which closely resembled natural outbreaks of the disease. The damage to tracheal epithelium caused by IBV facilitates *E. coli* invasion and multiplication leading to death or lesions in surviving chickens. Using this model, several reports have described differences in virulence of IBV strains, differences in susceptibility between genetic lines of chickens and detailed interactions between the agents (Avellanda *et al.*, 1994;

Bumstead *et al.*, 1989; Cook *et al.*, 1986, 1991b; Cubillos *et al.*, 1991; Smith *et al.*, 1985).

Raggi *et al.* (1967b) showed that when IBV and *Haemophilus paragallinarum* were given together intra-nasally, the incubation period was shorter, there was higher mortality and lesions were more severe.

There is no reported evidence of dual infections with IBV and *Pasteurella multocida*, the causative agent of fowl cholera. However, Bisgaard (1977), in a study of chickens in the field, found that where the bacterium *P. haemolytica* was present with IBV, there was no apparent exacerbation of the viral infection.

Other respiratory viruses

IBV is known to interfere with the replication of NDV in eggs and Raggi & Lee (1964) have shown that interference also occurs in chickens, but this is only one way. They found that when IBV was given to chickens with NDB1 vaccine, there was subsequently no production of haemagglutination-inhibition (HI) antibodies to NDV, but NDV did not interfere with IBV infection. Raggi *et al.* (1967a) found no synergism between IBV and infectious laryngotracheitis virus (ILTV), but Pattison *et al.* (1971) reported that aerosol vaccination with IBV increased the mortalities due to ILTV. APV, the cause of TRT and mild respiratory disease in chickens may also occur in respiratory outbreaks involving IBV, but the significance of these two agents being present together is yet to be reported.

Triple infections

Fabricant & Levine (1962) examined triple infections involving IBV, MG and pathogenic *E. coli*. They reported that with regard to disease lesions the descending order of severity was IBV, MG and *E. coli*; MG and *E. coli*; IBV and *E. coli*; and *E. coli* alone. A similar study by Nakamura *et al.* (1994) was performed with live IB and ND vaccines given to young chicks with MG and/or *E. coli* or both. Again, the most severe combination was when vaccine, *E. coli* and MG were given together.

Thus, it is clear that several respiratory pathogens interact synergistically with IBV or IB vaccines and enhance the severity and duration of the disease. In many instances, the interval between infections is important, as are the challenge doses. However, with few exceptions (e.g. Nakamura *et al.*, 1992), it is not known whether the synergisms are due to immunosuppression or simply that the epithelial damage caused by one agent permits greater penetration by others.

IBV and immunosuppressive agents

The two classical examples of immunosuppressive viruses of chickens are infectious bursal disease (IBD) which principally affects the B-cells and chicken anaemia virus (CAV) which affects the T-cells.

There is abundant evidence that IBDV infection impairs the humoral response to IBV. Giambrone *et al.* (1977) infected chicks with IBDV at day-old and IBV at 14 days. Compared to single IBV infection with both viruses, antibody titres were lower and airsac lesions greater. Rosenberger & Gelb (1978) also showed that IBDV at day-old affected the response to IB vaccines so that there was lowered resistance to challenge. Furthermore, Winterfield *et al.* (1978) showed that IBDV increased susceptibility to IBV, reduced IBV antibody levels and explained unsatisfactory immunity when replacement and broilers were vaccinated at an early age.

There are no reports of dual infection of chickens with IBV and CAV. Goodwin *et al.* (1992) reported that there were no significant differences in IBV-specific antibody titres between chicken flocks positive or negative for CAV antibodies. However, when chicks infected with IBV strain M41 were treated with the T-cell immunosuppressor drug cyclosporin A (CSA), the clinical signs in these birds were more severe and virus titres in tissues, especially kidneys, were greater than in infected but untreated birds, although the persistence of the virus was not increased (Dhinakar Raj & Jones, 1997a). IBV-induced mortality was also increased. It seems possible that infection with CAV at a critical time might induce some of these effects on IB.

IMMUNOSUPPRESSION BY IBV

Although IBV has been isolated from lymphoid tissues such as bursa of Fabricius and Harderian gland, the evidence for a direct immunosuppression is limited. It is not clear whether IBV multiplies in the lymphocytes but cultured macrophages were found to be resistant (von Bulow & Klasen, 1983). A virulent IBV has been shown to induce transitory reduction in proliferative responses of whole blood lymphocytes to T-cell mitogens, PHA (Wakenell *et al.*, 1995) and concanavalin A (Dhinakar Raj & Jones, 1997a). IBV vaccines were found to depress the Harderian gland responses to killed *Brucella abortus* (Montgomery *et al.*, 1991, 1994). However, no correlation between histological responses and HI titres to IBV were found with depression of responses to *Br. abortus*.

IMMUNE RESPONSES OF THE CHICKEN TO IBV

Immunogenic proteins of IBV

IBV is a positive-stranded RNA virus and is the prototype of the Family Coronaviradae. It has three structural proteins. The spike 'S' glycoprotein is located at the surface of the virion, and consists of two subunits, S1 and S2, with molecular weights of 92 and 84 K, respectively. The membrane 'M' glycoprotein is partially exposed at the surface of the virion with molecular weights ranging from 27 to 36 K, and the nucleocapsid 'N' protein is internally located with a molecular weight of 52 K (Wadey & Westaway, 1981; Cavanagh, 1983a,b,c).

The S1 glycoprotein of IBV induces virus neutralizing (VN) and HI antibodies

(Cavanagh *et al.*, 1984, 1986; Kant *et al.*, 1992; Koch *et al.*, 1990; Mockett *et al.*, 1984; Niesters *et al.*, 1987) and has been considered as the most likely inducer of protection (Cavanagh *et al.*, 1986; Ignjatovic & Galli, 1994), but S2 and N proteins may also be important since they carried epitopes for induction of cross-reactive antibodies (Ignjatovic & Galli, 1995). The time of appearance of S1, S2 and N ELISA-antibodies have been shown to be similar, being detected 2 weeks after live IBV vaccination (Ignjatovic & Galli, 1995). This coincides with the appearance of VN antibodies (Gough & Alexander, 1977; Mockett & Darbyshire, 1981; Darbyshire and Peters, 1984). Epitopes on N and S2 proteins that gave rise to cross-reactive antibodies showed the same degree of conservation while S1 epitopes were shown to be marginally less conserved (Ignjatovic & Galli, 1995).

A T-cell epitope has been identified in the IBV 'N' protein (Boots *et al.*, 1991) and has been shown to induce anti-viral responses (Boots *et al.*, 1992). Cellular immune responses elicited by a live IBV vaccine have also been found to be cross-reactive and the responses varied in magnitude with the serotype of IBV used for *in vitro* stimulation (Dhinakar Raj & Jones, 1997b).

Innate immunity

Immunity can be considered to be either innate or acquired. Innate immunity comprises a collection of factors which resist invasion by external agents, such as physical barriers provided by skin and mucous membranes, soluble factors like lysozyme, complement and acute phase proteins, and cells such as granulocytes, macrophages and natural killer (NK) cells. The main features of innate immunity are lack of specificity and immunological memory.

Heterophils (neutrophils) constitute the 'first line of defence' against infectious agents and are the first cells to be recruited to the site of infection, following initiation of an inflammatory response. In IBV-infected chickens, heterophils are the most numerous early inflammatory cells in respiratory lavage fluids (Fulton *et al.*, 1993). Using a heteropaenic chicken model (Kogut *et al.*, 1993) the importance of these cells in limiting IBV replication was studied (Dhinakar Raj *et al.*, 1997c). It was found that they had no effect on virus multiplication and, in fact, contributed to the damage in the tracheal epithelium.

The role of macrophages in IBV infections is unknown, while no alterations in NK cell activity has been found following IBV infection (Wakenell *et al.*, 1995). Serum levels of an acute phase protein, $\propto_1$ acid glycoprotein, have been found to peak on day 6 following IBV infection (Nakamura *et al.*, 1996).

Acquired immunity

Acquired immunity results in the activation of antigen-specific effector mechanisms including B-cells (humoral), T-cells (cellular) and macrophages, and the production of memory cells.

Humoral antibodies

Upon receiving proper stimuli, B-cells differentiate into plasma cells to secrete antibodies either in the presence (T-dependent antigen) or absence (T-independent antigen) of T-helper (T_n) cells. Chickens develop a good humoral response to IBV infections, measurable by enzyme-linked immunosorbent assay (ELISA), haemagglutiation inhibition (HI) or VN tests (De Wit *et al.*, 1992; Gough & Alexander, 1977; Monreal *et al.*, 1985; Wilcox *et al.*, 1983). However, there is a lack of correlation between titres of circulating antibodies and resistance to infection (Raggi & Lee, 1965; Winterfield & Fadly, 1972; Gough & Alexander, 1977).

Immunoglobulin G (IgG), the major circulating Ig, is the antibody detected by HI and an ELISA developed to measure it is more sensitive (Mockett & Darbyshire, 1981). Anti-IBV IgG can be detected as soon as four days pi, reaches a peak at about 21 days but can remain in high titre in the serum for many weeks (Mockett and Darbyshire, 1981). This is the antibody measured in conventional serological tests to monitor IBV infections or vaccine uptake.

Immunoglobulin M (IgM), present only transitorily after infection, reaches peak concentrations about 8 days after IBV infection and levels then decline (Mockett and Cook, 1986). Although an IgM-specific ELISA has been shown to be useful in the diagnosis of recent infections (Martins *et al.*, 1991), these antibodies needed to be separated either by sucrose density gradient centrifugation (Gillette, 1974) or column chromatography (Mockett & Cook, 1986) before performing the ELISA. The availability of an antibody-capture ELISA for IBV-specific IgM assays would facilitate IB diagnosis where demonstration of the virus is time-consuming.

The importance of B-cells in IBV infections has been studied by depletion experiments using the hormone testosterone propionate (Chubb, 1974), the chemical cyclophosphamide (Chandra, 1988; Chubb, 1974) and surgical bursectomy (Cook *et al.*, 1991a). Cyclophosphamide-treated chickens showed increased clinical signs and more severe histopathological lesions in the kidney (Chandra, 1988) attributable to the prolonged persistence of virus. IBV infection of a surgically bursectomised resistant chicken line (line C) resulted in increased severity and duration of clinical infection but not mortality (Cook *et al.*, 1991a). However, humoral antibodies seemed to protect the tracheal epithelium following secondary challenge. Presence of high titres of humoral antibodies correlate well with the absence of virus recovery from kidneys and genital tract (Gough & Alexander, 1977; MacDonald *et al.*, 1981; Yachida *et al.*, 1985) and protection against drop in egg production (Box *et al.*, 1988). IBV-specific antibodies probably prevent the spread of virus by viraemia from the trachea to other susceptible organs such as the kidneys and oviduct.

Vaccination studies with IBV have always focussed on humoral immune responses in relation to protection. Nevertheless, the lack of correlation between antibodies and resistance, discrepancies between *in vitro* strain differentiation by VN tests and *in vivo* cross-protection results (Darbyshire, 1985) and

re-excretion of virus in the presence of high titres of circulating antibodies (Jones & Ambali, 1987) all suggest that while humoral antibodies play a role in recovery from IBV infection, other immunological mechanisms are involved.

Maternal antibodies

Maternally derived antibodies (MDA) can provide protection against IBV, but they are short-lived (Darbyshire & Peters, 1985; Cook *et al.*, 1991b). Presence of MDA has no adverse effect on the efficacy of live IBV vaccines administered at one-day of age (Davelaar & Kouwenhoven, 1977, Cook *et al.*, 1981, 1991b). Maternally-derived IgG has been demonstrated in tracheal washes (Mockett *et al.*, 1987).

Local immunity

Local immunity in the respiratory tract is of fundamental importance in protection against IBV (Gomez & Raggi, 1974; Gillette, 1981; Hawkes *et al.*, 1983). This has been exemplified by the use of an *in vitro* challenge model using TOC from immunized chickens, for cross-protection studies (Lohr *et al.*, 1991). IBV-specific IgA and IgG have been demonstrated in tracheal washes of infected chickens (Hawkes *et al.*, 1983; Dhinakar Raj & Jones, 1996b) and antibody-secreting cells were shown in tracheal sections (Nakamura *et al.*, 1991).

Local immunity at the oviduct level has been shown by demonstration of virus-specific IgG and IgA in the oviduct washes of infected hens (Dhinakar Raj & Jones, 1996b). It was found that in addition to local production, antibodies also transuded from the serum later in the course of infection, but their value in protection of the oviduct has not been determined. In young chicks, local antibodies in the oviduct were found to be less protective compared to those in the trachea using *in vitro* challenge of OOC prepared from vaccinated chickens (Dhinakar Raj & Jones, 1996d).

Although IBV has been shown to multiply in the gut (Lucio & Fabricant, 1990; Ambali & Jones, 1990), Lutticken *et al.* (1988) could not detect any antibodies in gut washings following vaccination of day-old chicks with H120 vaccine and revaccination at 4 weeks of age with live H52 vaccine, inactivated M41 in an oil emulsion or inactivated M41 adjuvanted with avridine. In contrast, Dhinakar Raj & Jones (1996b) demonstrated local antibody production in the duodenum and caecal tonsils of 16-week-old hens infected with an enterotropic strain of IBV (strain G). It is not clear whether the induction of these antibodies is strain-related and their role in limiting the replication of IBV in the gut needs to be investigated.

The Harderian gland of the chicken contains a large age-dependent population of plasma cells and is the source of immunoglobulins in the lachrymal fluid (Baba *et al.*, 1988). It plays an important role in the development of vaccinal immunity since vaccines are generally given by spray or eye-drop. Davelaar & Kouwenhoven (1981) reported that the protection against IBV of day-old ocular-vaccinated

chickens was localized mainly in the oculo-nasal mucosa and removal of the Harderian gland caused a decreased level of protection (Davelaar & Kouwenhoven, 1980). IBV-specific IgA has been demonstrated in the lachrymal fluid (Cook *et al.*, 1992; Davelaar *et al.*, 1982; Toro *et al.*, 1994) and its synthesis in the Harderian gland has been shown (Davelaar *et al.*, 1982). IgG in tears was mainly serum-derived (Davelaar *et al.*, 1982; Toro *et al.*, 1993). IgA levels in tears appeared to be better correlated with resistance to IBV re-infection (Toro & Fernandez, 1994) than levels of serum antibody (Yachida *et al.*, 1985) and their measurement was recommended for antibody profiling of chicken flocks.

Cook *et al.* (1992) found more IBV-specific IgA in the lachrymal fluids of chicken lines resistant to IBV while antibody titres in tracheal washes were similar. Variation in IBV-specific IgG levels in serum and IgA levels in lachrymal fluids has also been demonstrated in different chicken lines after ocular vaccination with IBV (Toro *et al.*, 1996b). It was found that light layer (white leghorn) chickens had a significantly higher and more homogenous serum IgG response between days 5 and 9 p.i., and lachrymal IgA response between days 5 and 14 p.i. than broiler or brown-egg (heavy) layer chicks.

Cellular immunity

Reports concerning a role for cell-mediated immunity in protection against IBV are limited. Antigen-specific proliferation of T-lymphocytes in IBV-infected or vaccinated chickens has been demonstrated (Timms *et al.*, 1980; Timms & Bracewell, 1981, 1983). In some chickens, a positive correlation between lymphoproliferative responses and resistance to challenge has been shown (Timms & Bracewell, 1981).

Mouse monoclonal antibodies (Mab) that distinguish between chicken T-lymphocytes have been described (Chan *et al.*, 1988; Lillehoj *et al.*, 1988). The CD4 and CD8 antigens are found on two main populations of T-cells, T-helper (T_h) and T-cytotoxic / suppresser ($T_{c/s}$) cells, respectively.

Janse *et al.* (1994) contended that local immunity to IBV in the trachea is mediated by T-cells. CD4 and CD8 cells were shown in sections of trachea and lung of chickens infected with IBV (Janse *et al.*, 1994; Dhinakar Raj & Jones, 1996a). However, it is not clear which of these cells are more important in virus clearance, since Janse *et al.* (1994) found an increase in CD4 cells, while Dhinakar Raj & Jones (1996a) found higher proportions of CD8 cells. The differences may be related to the strains of IBV used.

When chickens were treated with CSA to suppress the T-cells, virus titres in the kidneys were 1 to 3 $\log_{10}$ median ciliostatic doses (CD_{50}) higher than in intact birds (Dhinakar Raj & Jones, 1997a). Thus, T-cells may also play an important role in limiting virus replication in the kidneys.

Chubb *et al.* (1987) demonstrated the presence of cytotoxic lymphocytes (CTL) in the spleen and peripheral blood following IBV infection using adherent cells as target cells and neutral red as indicator of lysis. However, Wakenell *et al.* (1995) could not demonstrate CTL using kidney cells as targets and the conven-

tional chromium-release assay. Delayed-type hypersensitivity (DTH) responses were induced in response to live IBV (Chubb *et al.*, 1988) and to affinity-purified S1, S2, N and M proteins (Igjnatovic & Galli, 1995).

Cytokines

Cytokines secreted in response to a general stimulus such as concanavalin A (Con A) or by specific antigen are important mediators of cellular immunity. T-cell growth factor (TCGF) or IL-2 and IFN-γ are among the most important soluble factors produced by lymphocytes. Only IFN-γ has been studied in relation to IBV but results are conflicting. Otsuki *et al.* (1988) detected variable levels of IFN-γ in chickens with various strains of IBV whereas other workers (Lomniczi, 1974; Holmes & Darbyshire, 1978) could not detect IFN-γ in serum or organ cultures of chickens infected with IBV. Furthermore, whether IBV is susceptible to anti-viral effects of IFN-γ is controversial (Holmes & Darbyshire, 1978; Otsuki *et al.*, 1979b). Otsuki *et al.* (1991) found no differences in IFN-γ levels of IBV-resistant and IBV-sensitive lines of chickens.

Resistance to IBV

The identification of resistant and sensitive inbred lines of chickens to IBV (Bumstead *et al.*, 1989) has provided an excellent model to study immune mechanisms in relation to resistance of chickens to IBV. Otsuki *et al.*, (1990) found that although a resistant line (C) and a sensitive line (151) were equally susceptible to infection initially, recovery was more rapid in the resistant line. Ultrastructural and histochemical studies showed that though the type of damage to the tracheal epithelium following IBV infection was similar in both lines of chickens, lesions were more severe and longer lasting in the sensitive line (Nakamura *et al.*, 1991). The severity and duration of clinical infection in bursectomised line C chickens were similar to those seen in the sensitive line (Cook *et al.*, 1991a). However, no increase in mortality was observed, in contrast to high mortality recorded in line 151 chickens. Comparisons of secretory antibody responses between the two lines of chickens revealed increased local antibody secretion in the saliva and lachrymal fluid of the resistant line (Cook *et al.*, 1992).

Suppression of T-cells by CSA in a line of chickens resistant to IBV (brown leghorn; BLH), induced them to behave like a sensitive line, in terms of mortality (Dhinakar Raj & Jones, 1997a). The mortality of intact BLH chickens infected with a pool of ten IBV strains was 0% compared to 43% in CSA-treated BLH chickens. The virus titres in kidneys were much higher than in intact birds. The classes of T-cells involved in these effects need to be studied in further detail by analysing the kinetic changes in T-cell subsets following IBV infections using flow cytometry or by specific depletion *in vivo* by Mabs against T-cell subsets.

VIRUS PERSISTENCE

Although IB is generally considered as an acute respiratory disease, prolonged virus excretion has been reported (Cook, 1968; Alexander *et al.*, 1978; Chong & Apostolov, 1982). When day-old chicks were infected with an enterotropic IBV, strain G, faecal excretion could not be detected beyond day 35 p.i., but when birds reached sexual maturity re-excretion occurred (Jones & Ambali, 1987). Virus re-excretion could not be induced earlier by hormone injections (Ambali & Jones, 1991b), but it could after T-cell suppression by CSA (Bhattacharjee *et al.*, 1995). Reactivation of virus was confirmed by appearance of IBV-specific IgM in the serum (Bhattacharjee *et al.*, 1995).

Mainly because of the prolonged or intermittent recovery of IBV from these tissues, 2 candidate sites have been proposed for virus persistence in the chicken, the kidneys (Chong & Apostolov, 1982) and/or caecal tonsils (Cook, 1968; Alexander *et al.*, 1978). However, recent work has suggested that the kidneys are the more likely site. When strain M41 was used to infect day-old chickens and T-cell immunosuppression by CSA treatment was given from 5 weeks p.i., virus was recovered first from the kidneys and then from the trachea and lungs but never from the caecal tonsils (Dhinakar Raj & Jones, 1997a). The target cells of the virus in the kidneys are in the tubular epithelium (Chong & Apostolov, 1982; Ambali & Jones, 1990) which provides an ideal site for virus persistence because of its immunologically privileged nature (Mimms, 1988).

Occurrence of persistence was also found to be related to the age at infection (Dhinakar Raj & Jones, 1997a). When chicks were infected at 2 weeks of age and treated with CSA from 5 weeks p.i. no virus re-excretion was observed, but when chicks were infected at day-old, virus re-excretion was successfully induced with CSA. Notably, virus re-excretion was not accompanied by clinical symptoms.

The persistence of IBV may have practical implications in the epizootiology of the disease and perhaps a role in evolution of variant strains of the virus. Furthermore, re-excretion of persistent virus may serve as an unnoticed source of infection to susceptible chickens.

ANTIGENIC VARIATION AMONG IBV STRAINS

Antigenic types (serotypes)

IBV does not constitute a single homogenous antigenic type. The prototype virus is Massachusetts M41. Since the first identification of a different serotype (named Connecticut) by Jungherr *et al.*, (1956) several new antigenic types have been reported in various countries (see Captua *et al.*, 1994; Cook, 1983, 1984; Gelb *et al.*, 1991a; Parsons *et al.*, 1992; Picault *et al.*, 1986). The major virus neutralizing antibody site of IBV, which defines serotype, resides in the S1 subunit of the spike protein (Mockett *et al.*, 1984; Cavanagh *et al.*, 1986). Differences in only a few amino acids in the S1 protein can result in different VN serotypes (Cavanagh *et al.*, 1992a) which accounts for the plethora of IBV strains which exists today.

Hence, different antigenic types identified by VN tests do not imply that the isolates have substantially different S1 proteins or overall antigenic properties or greatly different evolutionary lineages.

As in other RNA viruses, antigenic variation is probably facilitated by the high error rate during the transcription of RNA template and the absence of a proof-reading mechanism. It has been shown that point mutations may lead to the generation of IBV variants in the field (Jia *et al.*, 1995). However, both circumstantial (Cavanagh & Davis, 1988; Cavanagh *et al.*, 1992b; Jia *et al.*, 1995; Kusters *et al.*, 1989, 1990; Wang *et al.*, 1994) and experimental (Kottier *et al.*, 1995) evidence suggest that the main mechanism of generation of variant strains of IBV is by recombination. This could be promoted by the use of more than one strain of IBV for vaccination or by a mixture of vaccine and challenge viruses.

Immunogenic types (protectotypes)

Isolates of IBV shown to be distinct by the VN test can still induce partial or complete cross-immunity (Arvidson *et al.*, 1990; Darbyshire, 1980, 1985; Hitchner *et al.*, 1964; Raggi & Lee, 1965; Winterfield & Fadly, 1972; Winterfield *et al.*, 1976). For example, vaccination with H120 caused a 30,000-fold reduction in titres of the challenge virus, Australian 'T' strain (Darbyshire, 1985), while on the basis of VN tests *in vitro*, no evidence of a serological relationship between these two viruses could be demonstrated (Darbyshire *et al.*, 1979). Hence, antigenic studies alone do not adequately define immunological relationships between strains. Thus, it was suggested that cross-immunization studies could be used to classify IBV isolates into protectotypes (Lohr, 1988) as this would reduce the large number of serotypes to a smaller number of protectotypes and provide more practical information to the field.

Cross-immunisation tests have been performed in experimental chickens (Darbyshire, 1980, 1985; Lambrechts *et al.*, 1993) or in *in vitro* conditions using TOC from immunized birds (Hinze *et al.*, 1991; Dhinakar Raj & Jones, 1996d) to determine protective immunological relationships between IBV strains. Arvidson *et al.* (1990) have described a model to study immunogenic relationships between IBV strains based on the vaccinating dose required to prevent the multiplication of a standard challenge dose of a homologous strain in the lungs of chickens. These cross-immunization tests would help to determine whether an already existing vaccine could offer protection to a new variant. This information is critical before embarking on a long and cumbersome process of new vaccine production.

Variation in tropisms?

Since tissue affinities are a function of the viral peplomer-mediated attachment of virus to cells, changes in the spike protein might lead to altered tropism of the virus. Six of ten differences in amino acid sequences of Gray and JMK strains of IBV were found between residues 99 and 127; hence it was postulated that this

region may play a role in differences in tissue tropism exhibited by these viruses (Kwon & Jackwood, 1995). However, no correlation was found by Sapats *et al.* (1996) between S1 amino acid sequences and nephropathogenicity of nine Australian strains of IBV. Nevertheless, it is not unreasonable to expect new variant strains of IBV to emerge with unusual tissue tropisms and such an example of this is strain 793/B.

CONCLUSIONS

IBV has been effectively controlled by the extensive use of vaccines but it still remains a major economic problem some 65 years after it was first reported. The constant emergence of variant strains has challenged vaccination strategies. With the advent of molecular techniques much of the recent work on IBV has concentrated on improved diagnostic and strain differentiation methods. However, the appearance and spread of the economically-important variant strain 793/B has given us a reminder of the need for a more detailed understanding of the immunopathogenesis of the disease and to be prepared for emergence of variants with unusual tissue tropisms and disease manifestations.

REFERENCES

Adler, H.E., McMartin, D.A. & Ortmayer, H. (1962) The effect of infectious bronchitis virus on chickens infected with *Mycoplasma gallisepticum*. *Avian Diseases, 6*, 267–274.

Afanador, G. & Roberts, J.R. (1994) Effect of nephropathogenic infectious bronchitis virus on renal function in male broiler chickens. *British Poultry Science, 35*, 445–456.

Albassam, M.A., Winterfield, R.W. & Thacker, H.L. (1986) Comparison of the nephropathogenicity of four strains of infectious bronchitis virus. *Avian Diseases, 30*, 468–476.

Alexander, D.J. & Gough, R.E. (1977) Isolation of avian infectious bronchitis virus from experimentally infected chickens. *Research in Veterinary Science, 23*, 344–347.

Alexander, D.J., Gough, R.E. & Pattison, M. (1978) A long-term study of the pathogenesis of infection of fowls with three strains of avian infectious bronchitis virus. *Research in Veterinary Science, 24*, 227–233.

Ambali, A.G. & Jones, R.C. (1990) Early pathogenesis in chicks of infection with an enterotropic strain of infectious bronchitis virus. *Avian Diseases, 34*, 809–817.

Ambali, A.G. & Jones, R.C. (1991a) Effects of trypsin and sodium tauroglycocholate on an enterotropic variant infectious bronchitis virus. *Veterinary Record, 129*, 510–511.

Ambali, A.G. & Jones, R.C. (1991b) The effects of oestrogen and progesterone on re-excretion of infectious bronchitis virus strain G in specific-pathogen-free chickens. *Journal of Hygiene, Epidemiology, Microbiology and Immunology, 35*, 429–439.

Animas, S.B., Otsuki, K., Hanayama, M., Sanekata, T. & Tsubokura, M. (1994a) Experimental infection with avian infectious bronchitis virus (Kagoshima-34 strain) in chicks of different ages. *Journal of Veterinary Medical Science, 56*, 443–447.

Animas, S.B., Otsuki, K., Tsubokura, M. & Cook, J.K.A. (1994b) Comparison of the susceptibility of chicks of different ages to infection with nephrosis-nephritis causing strain of infectious bronchitis virus. *Journal of Veterinary Medical Science, 56*, 449–453.

Arvidson, Y., Tannock, G.A., Senthilselvan, A. & Zerbes, M. (1990) A model for determining immunogenic relationships between avian infectious bronchitis virus. *Archives of Virology, 111*, 227–238.

Avellaneda, G.A., Villegas, P., Jackwood, M.W. & King, D.J. (1994) *In vivo* evaluation of the pathogenicity of field isolates of infectious bronchitis virus. *Avian Diseases, 38*, 589–597.

Baba, T., Masumoto, K., Nishida, S., Kajikawa, T. & Mitsui, M. (1988) Harderian gland dependency of immunoglobulin A production in the lachrymal fluid of chickens. *Immunology, 65,* 67–71.

Bhattacharjee, P.S. (1994) Enterotropism and persistence of avian infectious bronchitis virus in chickens. *PhD Thesis,* University of Liverpool.

Bhattacharjee, P.S. & Jones, R.C. (1997) Susceptibility of organ cultures from chicken tissues for strains of infectious bronchitis isolated from the intestine. *Avian Pathology, 26,* 553–563.

Bhattacharjee, P.S., Carter, S.D., Savage, C.E. & Jones, R.C. (1995) Re-excretion of infectious bronchitis virus in chickens induced by cyclosporin. *Avian Pathology, 24,* 435–441.

Bisgaard, M. (1977) Incidence of *Pasteurella haemolytica* in the respiratory tract of apparently healthy chickens and chickens with infectious bronchitis. *Avian Pathology, 6,* 285–292.

Blake, J.T. (1962) Effects of experimental chronic respiratory disease and infectious bronchitis on pullets. *American Journal of Veterinary Research, 23,* 847–853.

Boots, A.M.H., Kusters, J.G., Van Noort, J.M., Zwaagstra, K.A., Rijke, E., Van der Zeijst, B.A.M. & Hensen, E.J. (1991) Localisation of a T-cell epitope within the nucleocapsid protein of the avian corona virus. *Immunology, 74,* 8–13.

Boots, A.M.H., Benaissa-Trouw, B.J., Hesselink, W., Rijke, E., Schrier, C. & Hensen, E.J. (1992) Induction of antiviral immune responses by immunisation with recombinant-DNA encoded avian coronavirus nucleocapsid protein. *Vaccine, 10,* 119–124.

Bourne, F.J. (1996) Research priorities for the future. In T. F. Davison, T. R. Morris & L. N. Payne (Eds) *Poultry Immunology,* Poultry Science Symposium Series vol. *24,* pp. 419–432. Abingdon: Carfax.

Box, P.G., Holmes, H.C., Finney, P.M. & Froymann, R. (1988) Infectious bronchitis in laying hens: the relationship between haemagglutination-inhibition antibody levels and resistance to experimental challenge. *Avian Pathology, 17,* 349–361.

Broadfoot, D.I. & Smith, W.M., Jr (1954) Effects of infectious bronchitis in laying hens on egg production, percent unsettable eggs and hatchability. *Poultry Science, 33,* 653–655.

Broadfoot, D.I., Pomeroy, B.S. & Smith, W.M., Jr (1956) Effects of infectious bronchitis in baby chicks. *Poultry Science, 35,* 757–762.

Bumstead, N., Huggins, M.B. & Cook, J.K.A. (1989) Genetic differences in susceptibility to a mixture of avian infectious bronchitis virus and *Escherichia coli. British Poultry Science, 30,* 39–48.

Butcher, G.D., Winterfield, R.W. & Shapiro, D.P. (1990) Pathogenesis of H13 nephropathogenic infectious bronchitis virus. *Avian Diseases, 34,* 916–921.

Butler, E.J., Curtis, M.J., Pearson, A.W. & McDougall, J.S. (1972) Effect of infectious bronchitis virus on the structure and composition of egg albumen. *Journal of the Science of Food and Agriculture, 23,* 359–369.

Capua, I., Gough, R.E., Mancini, M., Casaccia, C. & Weiss, C. (1994) A novel infectious bronchitis strain infecting broiler chickens in Italy. *Journal of Veterinary Medicine Series B, 41,* 83–89.

Cavanagh, D. (1983a) Coronavirus IBV glycopolypeptides: size of their polypeptide moieties and nature of their oligosaccharides. *Journal of General Virology, 64,* 1187–1191.

Cavanagh, D. (1983b) Coronavirus IBV: further evidence that the surface projections are associated with two glycopolypeptides. *Journal of General Virology, 64,* 1787–1791.

Cavanagh, D. (1983c) Coronavirus IBV: structural characterisation of the spike protein. *Journal of General Virology, 64,* 2577–2583.

Cavanagh, D. & Davis, P.J. (1988) Evolution of avian coronavirus IBV: sequence of the matrix glycoprotein gene and intergenic region of several serotypes. *Journal of General Virology, 69,* 621–629.

Cavanagh, D., Darbyshire, J.H., Davis, P. & Peters, R.W. (1984) Induction of humoral neutralising and haemagglutination-inhibiting antibody by the spike protein of infectious bronchitis virus. *Avian Pathology, 13,* 573–583.

Cavanagh, D., Davis, P.J., Darbyshire, J.H. & Peters, R.W. (1986) Coronavirus IBV: virus retaining spike glycopolypeptide S2 but not S1 is unable to induce virus-neutralising of hamagglutination-inhibiting antibody or induce chicken tracheal protection. *Journal of General Virology, 67,* 1435–1442.

Cavanagh, D., Davis, P.J., Cook, J.K.A., Li, D., Kant, A. & Koch, G. (1992a) Location of amino acid differences in the S1 spike glycoprotein subunitof closely related serotypes of infectious bronchitis virus. *Avian Pathology, 21,* 33–43.

Cavanagh, D., Davis, P.J. & Cook, J.K.A. (1992b) Infectious bronchitis virus: evidence for recombination within the Massachusetts serotype. *Avian Pathology, 21,* 401–408.

Chan, M.M., Chen, C.L.H., Ager, L.L. & Cooper, M.D. (1988) Identification of the avian homologue of mammalian CD4 and CD8 antigens. *Journal of Immunology, 140,* 2133–2138.

Chandra, M. (1987) Comparative nephropathogenicity of different strains of infectious bronchitis virus in chickens. *Poultry Science, 66,* 954–959.

Chandra, M. (1988) Comparative nephropathogenicity of infectious bronchitis virus in bursectomised and non-bursectomised chickens. *American Journal of Veterinary Research, 49*, 831–834.

Chen, B.Y., Hosi, S., Nunoya, T. & Itakura, C. (1996) Histopathology and immunochemistry of renal lesions due to infectious bronchitis virus in chickens. *Avian Pathology, 25*, 269–283.

Chong, K.T. & Apostolov, K. (1982) The pathogenesis of nephritis in chickens induced by infectious bronchitis virus. *Journal of Comparative Pathology, 92*, 199–211.

Chubb, R.C. (1974) The effect of suppression of circulating antibody on resistance to the Australian avian infectious bronchitis virus. *Research in Veterinary Science, 17*, 169–173.

Chubb, R.C., Huynh, V. & Law, R. (1987) The detection of cytotoxic lymphocyte activity in chickens infected with infectious bronchitis virus or fowlpox virus. *Avian Pathology, 16*, 395–405.

Chubb, R.C., Huynh, V. & Bradley, R. (1988) The induction and control of delayed type hypersensitivity reactions induced in chickens by infectious bronchitis virus. *Avian Pathology, 17*, 371–383.

Condron, R.J. & Marshall, A.T. (1985) Pathogenesis of infectious bronchitis nephritis. 2. Studies of water and electrolyte balance in colostomised chickens. *Avian Pathology, 14*, 509–520.

Condron, R.J. & Marshall, A.T. (1986) Pathogenesis of infectious bronchitis nephritis 1. Morphometric analysis of kidney proximal tubular epithelium in chickens. *Journal of Comparative Pathology, 96*, 47–61.

Cook, J.K.A. (1968) Duration of experimental infectious bronchitis in chickens. *Research in Veterinary Science, 9*, 506–514.

Cook, J.K.A. (1971) Recovery of infectious bronchitis virus from eggs and chicks produced by experimentally inoculated hens. *Journal of Comparative Pathology, 81*, 203–211.

Cook, J.K.A. (1983) Isolation of a new serotype of infectious bronchitis-like virus from chickens in England. *Veterinary Record, 112*, 104–105.

Cook, J.K.A. (1984) The classification of new serotypes of infectious bronchitis virus isolated from poultry flocks in Britain between 1981 and 1983. *Avian Pathology, 13*, 733–741.

Cook, J.K.A. & Huggins, M.B. (1986) Newly isolated serotypes of infectious bronchitis virus: their role in disease. *Avian Pathology, 15*, 129–138.

Cook, J.K.A., Darbyshire, J.H. & Peters, R.W. (1976) The use of chicken tracheal organ cultures for the isolation and assay of avian infectious bronchitis virus. *Archives of Virology, 50*, 109–118.

Cook, J.K.A., Smith, H.W. & Huggins, M.B. (1986) Infectious bronchitis immunity: its study in chickens experimentally infected with mixtures of infectious bronchitis virus and *Escherichia coli*. *Journal of General Virology, 67*, 1427–1434.

Cook, J.K.A., Davison, T.F., Huggins, M.B. & McLaughlan, P. (1991a) Effect of *in-ovo* bursectomy on the course of an infectious bronchitis virus infection in line C white leghorn chickens. *Archives of Virology, 118*, 225–234.

Cook, J.K.A., Huggins, M.B. & Ellis, M.M. (1991b) Use of an infectious bronchitis virus and *Escherichia coli* model infection to assess the ability to vaccinate successfully against infectious bronchitis in the presence of maternally-derived immunity. *Avian Pathology, 20*, 619–626.

Cook, J.K.A., Otsuki, K., Da Silva Martins, N.R., Ellis, M.M. & Huggins, M.B. (1992) The secretory antibody response in inbred lines of chickens to avian infectious bronchitis virus infection. *Avian Pathology, 21*, 681–692.

Cowen, B.S. & Hitchner, S.B. (1975) pH stability studies with avian infectious bronchitis virus (Coronavirus) strains. *Journal of Virology, 15*, 430–432.

Crinion, R.A.P. & Hofstad, M.S. (1972a) Pathogenicity of four serotypes of avian infectious bronchitis virus for the oviduct of young chickens of various ages. *Avian Diseases, 16*, 351–363.

Crinion, R.A.P. & Hofstad, M.S. (1972b) Pathogenicity of two embryo-passage levels of avian infectious bronchitis virus for the oviduct of young chickens of various ages. *Avian Diseases, 16*, 967–973.

Crinion, R.A.P., Ball, R.A. & Hofstad, M.S. (1971a) Pathogenesis of oviduct lesions in immature chickens following exposure to infectious bronchitis virus at one day-old. *Avian Diseases, 15*, 32–41.

Crinion, R.A.P., Ball, R.A. & Hofstad, M.S. (1971b) Abnormalities in laying chickens following exposure to infectious bronchitis virus at one day-old. *Avian Diseases, 15*, 42–48.

Cubillos, A., Ulloa, J., Cubillos, V. & Cook, J.K.A. (1991) Characterisation of strains of infectious bronchitis virus isolated in Chile. *Avian Pathology, 20*, 85–99.

Cumming, R.B. (1962) The etiology of uraemia of chickens. *Australian Veterinary Journal, 38*, 554.

Cumming, R.B. (1963) Infectious avian nephrosis (uraemia) in Australia. *Australian Veterinary Journal, 39*, 145–147.

Cumming, R.B. (1969) The control of avian infectious bronchitis/nephrosis in Australia. *Australian Veterinary Journal, 45*, 200–203.

Cumming, R.B. & Chubb, R.C. (1988) The pathogenesis of nephritis evoked by Australian IB viruses. *Proceedings of the Ist International Symposium on Infectious Bronchitis*, Rauischholzhausen, Germany, pp. 129–132.

Darbyshire, J.H. (1980) Assessment of cross-immunity in chickens to strains of avian infectious bronchitis virus using tracheal organ cultures. *Avian Pathology, 9*, 179–184.

Darbyshire, J.H. (1985) A clearance test to assess protection in chickens vaccinated against infectious bronchitis virus. *Avian Pathology, 14*, 497–508.

Darbyshire, J.H. & Peters, R.W. (1984) Sequential development of humoral immunity and assessment of protection in chickens following vaccination and challenge with avian infectious bronchitis virus. *Research in Veterinary Science, 37*, 77–86.

Darbyshire, J.H. & Peters, R.W. (1985) Humoral antibody response and assessment of protection following primary vaccination of chicks with maternally-derived antibody against avian infectious bronchitis virus. *Research in Veterinary Science, 38*, 14–21.

Darbyshire, J.H., Cook, J.K.A. & Peters, R.W. (1976) Organ culture studies on the efficiency of infection of chicken tissues with avian infectious bronchitis virus. *British Journal of Experimental Pathology, 57*, 443–454.

Darbyshire, J.H., Cook, J.K.A. & Peters, R.W. (1978) Growth comparisons of avian infectious bronchitis virus strains in organ cultures of chicken tissues. *Archives of Virology, 56*, 317–325.

Darbyshire, J.H., Rowell, R.G., Cook, J.K.A. & Peters, R.W. (1979) Taxonomic studies on strains of avian infectious bronchitis virus using neutralisation tests in tracheal organ cultures. *Archives of Virology, 61*, 227–238

Davelaar, F.G. & Kouwenhoven, B. (1976) Changes in the Harderian gland of the chicken following conjunctival and intranasal infection with infectious bronchitis virus in one and 20 day-old chickens. *Avian Pathology, 5*, 39–50.

Davelaar, F.G. & Kouwenhoven, B. (1977) Influence of maternal antibodies on vaccination of chicks of different ages against infectious bronchitis virus. *Avian Pathology, 6*, 41–50.

Davelaar, F.G. & Kouwenhoven, B. (1980) Effect of removal of the Harderian gland in one day-old chicks on immunity following infectious bronchitis vaccination. *Avian Pathology, 9*, 489–497.

Davelaar, F.G. & Kouwenhoven, B. (1981) Study on the local effect of eye-drop vaccination against infectious bronchitis in one day-old chicks with maternal antibodies. *Avian Pathology, 10*, 83–90.

Davelaar, F.G., Noordzij, A. & Vander Donk, J.A. (1982) A study on the synthesis and secretion of immunoglobulins by the Harderian gland of fowl after eye-drop vaccination against infectious bronchitis at 1 day-old. *Avian Pathology, 11*, 63–79.

De Wit, J.J., Davelaar, F.G. & Braunius, W.W. (1992) Comparison of the enzyme linked immunosorbent assay, the haemagglutination-inhibition test and agar gel precipitation test for the detection of antibody against infectious bronchitis and Newcastle disease in commercial broilers. *Avian Pathology, 21*, 651–658.

Dhinakar Raj, G. & Jones, R.C. (1996a) Immunopathogenesis of infection in SPF chicks and commercial broilers of a variant infectious bronchitis virus of economic importance. *Avian Pathology, 25*, 481–501.

Dhinakar Raj, G. & Jones, R.C. (1996b) Local antibody production in the oviduct and gut of hens infected with an enterotropic strain of infectious bronchitis virus. *Veterinary Immunology and Immunopathology, 53*, 147–161.

Dhinakar Raj, G. & Jones, R.C. (1996c) An *in-vitro* comparison of the virulence of seven strains of infectious bronchitis virus using tracheal and oviduct organ cultures. *Avian Pathology, 25*, 649–662.

Dhinakar Raj, G. & Jones, R.C. (1996d) Protectotypic differentiation of avian infectious bronchitis viruses using an *in-vitro* challenge model. *Veterinary Microbiology, 53*, 239–252.

Dhinakar Raj, G. & Jones, R.C. (1997a) Effect of T-cell suppression by cyclosporin on primary and persistent infections of infectious bronchitis virus in chickens. *Avian Pathology, 26*, 257–276.

Dhinakar Raj, G. & Jones, R.C. (1997b) Cross-reactive cellular immune responses in chickens vaccinated with live infectious bronchitis vaccine. *Avian Pathology, 26*, 641–649.

Dhinakar Raj, G., Savage, C.E. & Jones, R.C. (1997) Effect of heterophil depletion by 5-fluorouracil on infectious bronchitis virus infection in chickens. *Avian Pathology, 26*, 427–432.

Droual, R. & Woolcock, P.R. (1994) Swollen head syndrome associated with *Escherichia coli* and infectious bronchitis virus in the Central Valley of California. *Avian Pathology, 23*, 733–742.

El Houadfi, M.D., Jones, R.C., Cook, J.K.A. & Ambali, A.G. (1986) The isolation and characterisation of six avian infectious bronchitis virus isolated in Morocco. *Avian Pathology, 15*, 93–105.

Fabricant, J. & Levine, P.P. (1962) Experimental production of complicated chronic respiratory disease (air-sac disease). *Avian Diseases, 6*, 13–23.

Fulton, R.M., Reed, M. & Thacker, H.L. (1993) Cellular response of the respiratory tract of chickens to infection with Massachusetts 41 and Australian T infectious bronchitis viruses. *Avian Diseases, 37,* 951–960.

Gelb, J.J., Wolff, J.B. & Moran, C.A. (1991a) Variant serotypes of infectious bronchitis virus isolated from commercial layer and broiler chickens. *Avian Diseases, 35,* 82–87.

Gelb, J., Lunt, R.L., Metz, A.L. & Fries, P.A. (1991b) Attenuation of avian infectious bronchitis virus by cold adaptation. *Avian Diseases, 35,* 847–853.

Giambrone, J.J., Eidson, C.S. & Kleven, S.H. (1977) Effects of infectious bursal disease on the responsiveness of the chickens to *Mycoplasma synoviae,* Newcastle disease and infectious bronchitis. *American Journal of Veterinary Research, 38,* 252–253.

Gillette, K.G. (1974) Avian infectious bronchitis: demonstration of serum IgG and IgM neutralising antibody by sucrose density gradient centrifugation and mercaptoethanol reduction. *Avian Diseases, 18,* 515–525.

Gillette, K.G. (1981) Local antibody responses to avian infectious bronchitis: virus neutralising antibody in tracheobronchial secretions. *Avian Diseases, 25,* 431–443.

Gomez, L. & Raggi, L.G. (1974) Local immunity to avian infectious bronchitis virus in tracheal organ cultures. *Avian Diseases, 18,* 346–368.

Goodwin, M.A., Brown, J., Smeltzer, M.A., Girshick, T., Miller, S.L. & Dickson, T.G. (1992) Relationship of common avian pathogen antibody-titres in so-called chicken anaemia agent (CAA)-antibody-positive chicks to titers in CAA-antibody-negative chicks. *Avian Diseases, 36,* 356–358.

Gough, R.E. & Alexander, D.J. (1977) Comparison of serological tests for the measurement of primary immune response to avian infectious bronchitis virus vaccines. *Veterinary Microbiology, 2,* 289–301.

Gough, R.E., Randall, C.J., Dagless, M., Alexander, D.J., Cox, W.J. & Pearson, D. (1992) A new strain of infectious bronchitis virus infecting domestic fowl in Great Britain. *Veterinary Record, 130,* 493–494.

Gross, W.B. (1958) The role of *Escherichia coli* in the cause of chronic respiratory disease and certain other poultry diseases. *American Journal of Veterinary Research, 19,* 448–452.

Guittet, M., Picault, J.P., Le Coq, H., Lamande, J. & Bennejean, G. (1988) Strategy of protection of the layer against infectious bronchitis virus. *Proceedings of the 1st International Symposium on Infectious Bronchitis,* Rauischholzhausen, Germany, pp. 298–307.

Hawkes, R.A., Darbyshire, J.H., Peters, R.W., Mockett, A.P.A. & Cavanagh, D. (1983) Presence of viral antigens and antibody in the trachea of chickens infected with avian infectious bronchitis virus. *Avian Pathology, 12,* 331–340.

Heath, B.C. (1970) Chemical pathology of nephrosis induced by infectious bronchitis virus. *Avian Diseases, 14,* 95–106.

Hinze, V., Lohr, J.E. & Kaleta, E.F. (1991) Infectious bronchitis virus strain differentiation attempts by cross-immunity studies in tracheal organ cultures derived from immunised chickens. *Proceedings of the IInd International Symposium on Infectious Bronchitis,* Rauischholzhausen, Germany, pp. 73–86.

Hitchner, S.B., Appleton, G.S. & Winterfield, R.W. (1964) Evaluation of the immune response to infectious bronchitis virus. *Avian Diseases, 8,* 153–162.

Holmes, H.C. & Darbyshire, J.H. (1978) Induction of chicken interferon by avian infectious bronchitis virus. *Research in Veterinary Science, 25,* 178–181.

Hopkins, S.R. & Yoder, H.W. (1982) Influence of infectious bronchitis strains and vaccines on the incidence of *Mycoplasma synoviae* airsacculitis. *Avian Diseases, 26,* 741–752.

Ignjatovic, J. (1988) Epidemiology of infectious bronchitis in Australia. *Proceedings of the Ist International Symposium on Infectious Bronchitis, Rauischholzhausen,* Germany, pp. 84–88.

Ignjatovic, J. & Galli. L. (1994) The S1 glycoprotein but not the N or M proteins of avian infectious bronchitis virus induces protection in vaccinated chickens. *Archives of Virology, 138,* 117–134.

Ignjatovic, J. & Galli. L. (1995) Immune responses to structural proteins of avian infectious bronchitis virus. *Avian Pathology, 24,* 313–332.

Janse, E.M., Van Roozelaar, D. & Koch, G. (1994) Leukocyte subpopulations in kidney and trachea of chickens infected with infectious bronchitis virus. *Avian Pathology, 23,* 513–523.

Jia, W., Karaca, K., Parrish, C.R. & Naqi, S.A. (1995) A novel variant of avian infectious bronchitis virus resulting from recombination from three different strains. *Archives of Virology, 140,* 259–271.

Jones, R.C. (1974) Nephrosis in laying chickens caused by Massachusetts-type of infectious bronchitis virus. *Veterinary Record, 95,* 319.

Jones, R.C. & Ambali, A.G. (1987) Re-excretion of an enterotropic infectious bronchitis virus by hens at point of lay after experimental infection at day-old. *Veterinary Record, 120,* 617–619.

Jones, R.C. & Jordan, F.T.W. (1970) The exposure of day-old chicks to infectious bronchitis and subsequent development of the oviduct. *Veterinary Record, 87,* 504–505.

Jones, R.C. & Jordan, F.T.W. (1971) The site of replication of infectious bronchitis virus in the oviducts of experimentally infected hens. *Veterinary Record, 89*, 317–318.

Jones, R.C. & Jordan, F.T.W. (1972) Persistence of the virus in the tissues and development of the oviduct in the fowl following infection at day-old with infectious bronchitis virus. *Research in Veterinary Science, 13*, 52–60.

Jungherr, E.L., Chomiak, T.W. & Luginbuhl, R.E. (1956) Immunological difference in strains of infectious bronchitis virus. *60th meeting of the U.S. Livestock Sanitary Association*, pp. 203–209.

Kant, A., Koch, G., Van Roozelaar, D.J., Kusters, J.G., Poelwijk, F.A.J. & Van der Zeilst, B.A.M. (1992) Location of antigenic sites defined by neutralising monoclonal antibodies on the S1 avian infectious bronchitis virus glycopolypeptide. *Journal of General Virology, 73*, 591–596.

Kinde, H., Daft, B.M., Castro, A.E., Bickford, A.A., Gelb, J. & Reynolds, B. (1991) Viral pathogenesis of a nephrotropic infectious bronchitis virus isolated from commercial pullets. *Avian Diseases, 35*, 415–421.

King, D.J. & Cavanagh, D. (1991) Infectious Bronchitis. In B. W. Calnek, J. H. Barnes, C. W. Beard, W. M. Reid and H. W. Yoder, Jr (Eds) *Diseases of Poultry*. Ames, Iowa: *Iowa State University Press*.

Kleven, S.H., King, D.D. & Anderson, D.P. (1972) Airsacculitis in broilers from *Mycoplasma synoviae*: effects on air sac lesions of vaccinating with infectious bronchitis or Newcastle disease vaccines. *Avian Diseases, 16*, 915–924.

Klieve, A.V. & Cumming, R.B. (1990) Respiratory disease and immunity to challenge produced by Australian strains of infectious bronchitis virus. *Avian Pathology, 19*, 305–312.

Koch, G., Hartog, L., Kant, A. & Van Roozelaar, D.J. (1990) Antigenic domains on the peplomer proteins of avian infectious bronchitis virus: correlation with biological functions. *Journal of General Virology, 71*, 1929–1935.

Kogut, M.H., Tellez, G., Hargis, B.M., Corrier, D.E. & DeLoach, J.R. (1993) The effect of 5-fluorouracil treatment of chicks: a cell depletion model for the study of avian polymorphonuclear leukocytes and natural host defences. *Poultry Science, 72*, 1873–1880.

Kottier, S.A., Cavanagh, D. & Britton, P. (1995) Experimental evidence of recombination in coronavirus infectious bronchitis virus. *Virology, 213*, 569–580.

Kusters, J.G., Niesters, H.G.M., Lenstra, J.A., Horzinek, M.C. & Van der Zeijst, B.A.M. (1989) Phylogeny of antigenic variants of avian coronavirus, infectious bronchitis virus. *Virology, 169*, 217–221.

Kusters, J.G., Jager, E.J., Neisters, H.G.M. & Van der Zeijst, B.A.M. (1990) Sequence evidence for RNA recombination in field isolates of avian corona virus, infectious bronchitis virus. *Vaccine, 8*, 605–608.

Kwon, H.M. & Jackwood, M.W. (1995) Molecular cloning and sequence comparison of the S1 glycoprotein of the Gray and JMK strains of avian infectious bronchitis virus. *Virus Genes, 9*, 219–229.

Lambrechts, C., Pensaert, M. & Ducatelle, R. (1991) Comparative study of the infectivity of Belgian NIBV isolates for the kidney after intravenous inoculation in chickens. *Proceedings of the IInd International Symposium on Infectious bronchitis*, Rauischholzhausen, Germany, pp. 113–119.

Lambrechts, C., Pensaert, M. & Ducatelle, R. (1993) Challenge experiments to evaluate cross-protection induced at the trachea and kidney level by vaccine strains and Belgian nephropathogenic isolates of avian infectious bronchitis virus. *Avian Pathology, 22*, 577–590.

Lillehoj, H.S., Lillehoj, E.P., Weinstock, D. & Schat, K. (1988) Functional and biochemical characterisation of chicken T-lymphocyte antigens. *European Journal of Immunology, 18*, 2059–2065.

Lohr, J.E. (1988) Differentiation of IBV strains. *Proceedings of the Ist International Symposium on Infectious Bronchitis*, Rauischholzhausen, Germany, pp. 199–207.

Lohr, J.E., Hinze, V. & Kaleta, E.F. (1991) Immunological relationship between the New Zealand A and the Australian T strains of infectious bronchitis virus as measured by cross-immunisation tests in tracheal organ cultures from immunised birds. *New Zealand Veterinary Journal, 39*, 113–114.

Lomniczi, B. (1974) Interferon induction by different strains of infectious bronchitis virus. *Acta Veterinarica Hungarica, 24*, 199–204.

Lu, Y.S., Shien, Y.S., Tsai, H.J., Tseng, C.S., Lee, S.H. & Lin, D.F. (1994) Swollen head syndrome in Taiwan—isolation of an avian pneumovirus and serological survey. *Avian Pathology, 23*, 169–174.

Lucio, B. & Fabricant, J. (1990) Tissue tropism of three cloacal isolates and Massachusetts strain of infectious bronchitis virus. *Avian Diseases, 26*, 508–519

Lutticken, D., Rijke, E.O., Loeffen, T. & Hesselink, W.G. (1988) Aspects of local immune response to IBV. *Proceedings of the Ist International Symposium on Infectious Bronchitis*, Rauischholzhausen, Germany, pp. 173–181.

MacDonald, J.W. & McMartin, D.A. (1976) Observations on the effects of the H52 and H120 vaccine strains of infectious bronchitis virus in the domestic fowl. *Avian Pathology, 5*, 157–173.

MacDonald, J.W., Randall, C.J. & McMartin, D.A. (1980) An inverse age resistance of chicken kidneys to infectious bronchitis virus. *Avian Pathology, 9,* 245–259.

MacDonald, J.W., Randall, C.J., McMartin, D.A., Dagless, M.D. & Gazdzinski, P. (1981) Active and passive immunisation against nephritis induced by avian infectious bronchitis virus. *Avian Pathology, 10,* 121–129.

MacDonald, J.W., Randall, C.J., McMartin, D.A. & Dagless, M.D. (1983) Immunity following inoculation of H120 and H52 vaccine strains of infectious bronchitis virus into the crop of the domestic fowl. *Avian Pathology, 12,* 379–383.

Martins, N.R. da Silva, Mockett, A.P.A., Barrett, A.D.T. & Cook, J.K.A. (1991) IgM responses in chicken serum to live and inactivated infectious bronchitis virus vaccines. *Avian Diseases, 35,* 470–475.

McDougall, J.S. (1968) Infectious bronchitis in laying fowls: its effect upon egg production and subsequent egg quality. *Veterinary Record, 83,* 84–86.

McMartin, D.A. (1968) The pathogenicity of an infectious bronchitis virus for laying hens, with observations on pathogenesis. *British Veterinary Journal, 124,* 576–581.

McMartin, D.A. (1993) Infectious bronchitis. In J. B. McFerran. & M. S. McNulty (Eds) *Virus Infections of Vertebrates. Virus Infections of Birds,* Vol. *4,* (pp. 249–275). Amsterdam: Elsevier Science Publishers.

Mimms, C.A. (1964) Aspects of pathogenesis of virus diseases. *Bacteriological Reviews, 28,* 30–45.

Mimms, C.A. (1988) Immunobiology and pathogenesis of persistent virus infections. In: C. Lopez (Ed.) *Immunobiology and Pathogenesis of Persistent Virus Infections* (pp. 3–17). Washington DC: American Society for Microbiology.

Mockett, A.P.A. & Cook, J.K.A. (1986) The detection of specific IgM to infectious bronchitis virus in chicken serum using an ELISA. *Avian Pathology, 15,* 437–446.

Mockett, A.P.A. & Darbyshire, J.H. (1981) Comparative studies with an enzyme linked immunosorbent assay for antibodies to avian infectious bronchitis virus. *Avian Pathology, 10,* 1–10.

Mockett, A.P.A., Cavanagh, D. & Brown, T.D.K. (1984) Monoclonal antibodies to the S1 spike and membrane proteins of avian infectious bronchitis corona virus strain Massachusetts M41. *Journal of General Virology, 65,* 2281–2286.

Mockett, A.P.A., Cook, J.K.A. & Huggins, M.B. (1987) Maternally-derived antibody to infectious bronchitis virus: its detection in chick trachea and serum and its role in protection. *Avian Pathology, 16,* 407–416.

Monreal, G., Bauer, H.J. & Wiegmann, J. (1985) Comparison of enzyme linked immunosorbent assay (ELISA), haemagglutination-inhibition test and agar-gel precipitation test for detection of antibodies to avian infectious bronchitis virus. *Avian Pathology, 14,* 421–434.

Montgomery, R.D., Maslin, W.R., Boyle, C.R., Pledser, T. AND Wu, Ching-Ching (1991) Effects of an Arkansas strain of infectious bronchitis vaccine on the head-associated lymphoid tissue (HALT). *Avian Diseases, 35,* 302–307.

Montgomery, R.D., Maslin, W.R., Magee, D.L. & Boyle, C.R. (1994) Effects of modified live infectious bronchitis virus vaccines on the head-associated lymphoid tissue. *Avian Diseases, 38,* 847–856.

Morley, A.J. & Thompson, D.K. (1984) Swollen head syndrome in broiler chickens. *Avian Diseases, 28,* 238–243

Muneer, M.A., Halvorson, D.A., Sivanandan, V., Newman, J.A. & Coon, C.N. (1986) Effects of infectious bronchitis (Arkansas strain) on laying chickens. *Avian Diseases, 30,* 644–647.

Muneer, M.A., Newman, J.A., Halvorson, D.A., Sivanandan, V. & Coon, C.N. (1987) Effects of avian infectious bronchitis virus (Arkansas strain) on vaccinated laying chickens. *Avian Diseases, 31,* 820–828.

Muneer, M.A., Newman, J.A., Halvorson, D.A., Sivanandan, V., Nagaraja, K.V. & Coon, C.N. (1988) Efficacy of infectious bronchitis virus vaccines against heterologous challenge. *Research in Veterinary Science, 45,* 22–27.

Nakamura, K., Cook, J.K.A., Otsuki, K., Huggins, M.B. & Frazier, J.A. (1991) Comparative study of respiratory lesions in two chicken lines of different susceptibility , infected with infectious bronchitis virus: histology, ultrastructure and immunochemistry. *Avian Pathology, 20,* 245–261.

Nakamura, K., Cook, J.K.A., Frazier, J.A. & Narita, M. (1992) *Escherichia coli* multiplication and lesions in the respiratory tract of chickens inoculated with infectious bronchitis virus and/or *Escherichia coli. Avian Diseases, 36,* 881–890.

Nakamura, K., Ueda, H., Tanimura, T. & Noguchi, K. (1994) Effect of mixed live vaccine (ND and IB) and *Mycoplasma gallisepticum* on the chicken respiratory tract and on *Escherichia coli* infection. *Journal of Comparative Pathology, 111,* 33–42.

Nakamura, K., Imai, K. & Tanimura, N. (1996) Comparison of the effects of infectious bronchitis and infectious laryngotracheitis on the chicken respiratory tract. *Journal of Comparative Pathology, 114,* 11–21.

Nauwynck, H. & Pensaert, M. (1988) Studies on the pathogenesis of infections with a nephropathogenic variant of infectious bronchitis virus in chickens. In *Proceedings of the Ist International Symposium on Infectious Bronchitis*, Rauischholzhausen, Germany, pp. 113–119.

Niesters, H.G.M., Bleuminkpluym, N.M.C., Osterhaus, A.D.M.E., Horzinek, M.C. & Van der Zeijst, B.A.M. (1987) Epitopes on the peplomer protein of infectious bronchitis virus strain M41 as defined by monoclonal antibodies. *Virology, 161*, 511–519.

Olson, N.O., Adler, H.E., Da Massa, A.J. & Corstvet, R.E. (1964) The effect of intranasal exposure to *Mycoplasma synoviae* and infectious bronchitis virus on the development of lesions and agglutinins. *Avian Diseases, 8*, 623–631.

Otsuki, K., Yamamoto, H. & Tsubokura, M. (1979a) Studies on avian infectious bronchitis virus (IBV). 1. Resistance of IBV to chemical and physical treatments. *Archives of Virology, 60*, 25–32.

Otsuki, K., Maeda, J., Yamamoto, H. & Tsubokura, M. (1979b) Studies on avian infectious bronchitis virus (IBV). III. Interferon induction by and sensitivity to interferon of IBV. *Archives of Virology, 60*, 249–255.

Otsuki, K., Nakamura, T., Kawaoka, Y. & Tsubokura, M. (1988) Interferon induction by several strains of avian infectious bronchitis virus, a corona virus, in chickens. *Acta Virologica, 32*, 55–59.

Otsuki, K., Huggins, M.B. & Cook, J.K.A. (1990) Comparison of the susceptibility to avian infectious bronchitis virus infection of two inbred lines of white leghorn chickens. *Avian Pathology, 19*, 467–475.

Otsuki, K., Davison, T.F. & Cook, J.K.A. (1991) Interferon induction by avian infectious bronchitis virus. *Proceedings of the IInd International Symposium on Infectious Bronchitis*, Rauischholzhausen, Germany, pp. 137–142.

Owen, R.L., Cowen, B.S., Hattel, A.L., Naqi, S.A. & Wilson, R.A. (1991) Detection of viral antigen following exposure of one-day old chickens to the Holland-52 strain of infectious bronchitis virus. *Avian Pathology, 20*, 663–673.

Parsons, D., Ellis, M.M., Cavanagh, D. & Cook, J.K.A. (1992) Characterisation of an infectious bronchitis virus isolated from vaccinated broiler breeder flocks. *Veterinary Record, 131*, 408–411.

Patterson, S. & Bingham, R.W. (1976) Electron microscope observations on the entry of avian infectious bronchitis virus into susceptible cells. *Archives of Virology, 52*, 191–200.

Pattison, M., Gough, R.E. & Dawson, P.S. (1971) The effect of live infectious bronchitis vaccine on the course of infectious laryngotracheitis. *Veterinary Record, 75*, 643–644.

Peters, R.W., Darbyshire, J.H. & Cook, J.K.A. (1979) The susceptibility of chicken kidney and oviduct organ cultures to a vaccine strain of infectious bronchitis virus. *Research in Veterinary Science, 26*, 38–40.

Picault, J.P., Drouin, P., Guittet, M., Bennejean, G., Protuis, J., L'Hospitalien, R., Gillet, J.P., Lamande, J. & Buchelien, A. (1986) Isolation, characterisation and preliminary cross-protection studies with a new pathogenic avian infectious bronchitis virus (strain PL-84084). *Avian Pathology, 15*, 367–383.

Picault, J.P., Giraud, P., Drouin, P., Guittet, M., Bennejean, G., Lamande, J., Toquin, D. & Gueguen, C. (1987) Isolation of a turkey rhinotracheitis-like virus from chickens with swollen head syndrome. *Veterinary Record, 121*, 135.

Picault, J.P., Drouin, P., Toux, J.Y., Guittet, M. & Bennejean, G. (1991) Infectious bronchitis in France: epidemiological situation and prophylactic practices. *Proceedings of the IInd International Symposium on Infectious Bronchitis*, Rauischholzhausen, Germany, pp. 3–22.

Pohl, R. (1974) The histopathogenesis of the nephrosis-nephritis syndrome. *Avian Pathology, 3*, 1–13.

Pradhan, H.K., Mohanty, G.C. & Rajya, B.S. (1984) Comparative sensitivities of oviduct and tracheal organ cultures and chicken embryo kidney cultures to infectious bronchitis virus. *Avian Diseases, 27*, 594–601.

Purcell, D.A. & Clarke, J.D. (1972) The replication of infectious bronchitis virus in fowl trachea. *Archiv fur die Geasmte Virusforschung, 39*, 248–256.

Purcell, D.A. & McFerran, J.B. (1972) The histopathology of infectious bronchitis in the domestic fowl. *Research in Veterinary Science, 13*, 116–122.

Purcell, D.A., Tham, V.L. & Surman, P.G. (1976) The histopathology of infectious bronchitis in fowls infected with a nephrotropic T strain of virus. *Australian Veterinary Journal, 52*, 85–91.

Purchase, H.G., Cunningham, C.H. & Burmester, B.R. (1966) Genetic differences among chicken embryos in response to inoculation with an isolate of infectious bronchitis virus. *Avian Diseases, 10*, 162–Raggi, L.G. & Lee, G.G. (1964) Infectious bronchitis virus interference with Newcastle disease virus; II interference in chickens. *Avian Diseases, 8*, 471–480.

Raggi, L.G. & Lee, G.G. (1965) Lack of correlation between infectivity, serologic response and challenge results in immunisation with an avian infectious bronchitis vaccine. *Journal of Immunology, 94*, 538–543.

Raggi, L.G., Asmar, J. & Lee, G.G. (1967a) Co-existence of infectious bronchitis and infectious laryngotracheitis. *Avian Diseases, 11*, 419–426.

Raggi, L.G., Young, D.C. & Sharma, J.D. (1967b) Synergism between avian infectious bronchitis and *Haemophilus gallinarum*. *Avian Diseases, 11*, 308–321.

Ratanasethakul, C. & Cumming, R.B. (1983) Effect of environmental temperature on the mortality in vaccinated chickens after challenge with Australian infectious bronchitis virus. *Australian Veterinary Journal, 60*, 254–256.

Rosenberger, J.K. & Gelb, J. (1978) Response to several avian respiratory viruses as affected by infectious bursal disease virus. *Avian Diseases, 22*, 95–105.

Sapats, S.I., Ashton, F., Wright, P.J. & Ignjatovic, J. (1996) Sequence analysis of the S1 glycoprotein of infectious bronchitis viruses—identification of a novel genotypic group in Australia. *Journal of General Virology, 77*, 413–418.

Schalk, A.E. & Hawn, M.C. (1931) An apparently new respiratory disease of baby chicks. *Journal of American Veterinary Medical Association, 78*, 413–422.

Sevoian, M. & Levine, P.P. (1957) Effects of infectious bronchitis on the reproductive tract, egg production and egg quality of laying chickens. *Avian Diseases, 1*, 136–164.

Shirai, J., Maeda, M., Fujii, M. & Kuniyoshi, S. (1993) Swollen head syndrome is not associated with turkey rhinotracheitis virus. *Veterinary Record, 132*, 41–42.

Siller, W.G. & Cumming, R.B. (1974) The histopathology of an interstitial nephritis in the fowl produced experimentally with infectious bronchitis virus. *Journal of Pathology, 114*, 163–173.

Smith, H.W., Cook, J.K.A. & Parcell, Z.E. (1985) The experimental infection of chickens with mixtures of infectious bronchitis virus and *Escherichia coli*. *Journal of General Virology, 66*, 777–786.

Survashe, B.D., Aitken, I.D. & Powell, J.R. (1979) The response of the Harderian gland of the fowl to antigen given by the ocular route. I. Histologic changes. *Avian Pathology, 8*, 77–93.

Timms, L.M. & Bracewell, C.D. (1981) Cell mediated and humoral immune response of chickens to live infectious bronchitis vaccines. *Research in Veterinary Science, 31*, 182–189.

Timms, L.M. & Bracewell, C.D. (1983) Cell mediated and humoral immune response of chickens to inactivated oil-emulsion infectious bronchitis vaccines. *Research in Veterinary Science, 34*, 224–230.

Timms, L.M. & Bracewell, C.D. & Alexander, D.J. (1980) Cell mediated and humoral immune response in chickens infected with avian infectious bronchitis. *British Veterinary Journal, 136*, 349–354.

Toro, H. & Fernandez, I. (1994) Avian infectious bronchitis—specific lachrymal IgA level and resistance to challenge. *Journal of Veterinary Medicine B Series 41*, 467–472.

Toro, H., Lavand, P., Vallejos, P. & Ferreira, A. (1993) Transfer of IgG from serum to lachrymal fluid in chickens. *Avian Diseases, 37*, 60–66.

Toro, H., Hidalgo, H. & Collingwoodselby, A.N. (1994) Lachrymal antibody response of specific-pathogen-free chickens and chickens with maternal immunity after infectious bronchitis virus vaccination. *Preventive Veterinary Medicine, 18*, 267–274.

Toro, H., Godoy, V., Larenas, J., Reyes, E. & Kaleta, E.F. (1996a) Avian infectious bronchitis—viral persistence in the Harderian gland and histological changes after eye-drop vaccination. *Avian Diseases, 40*, 114–120.

Toro, H., Reyes, E., Redmann, T. & Kaleta, E.F. (1996b) Local and systemic antibody response of different chicken lines after ocular vaccination against infectious bronchitis. *Journal of Veterinary Medicine B, 43*, 449–454.

Van Roekel, H., Clarke, M.K., Bullis, K.L., Olesiuk, O.M. & Sperling, F.G. (1951) Infectious bronchitis. *American Journal of Veterinary Research, 12*, 140–146.

von Bulow, V. & Klasen, A. (1983) Effects of avian viruses on cultured chicken bone marrow-derived macrophages. *Avian Pathology, 12*, 179–198.

Wadey, C.N. & Westaway, E.G. (1981) Structural proteins and glycoproteins of infectious bronchitis virus particles labelled during growth in chick embryo cells. *Intervirology, 15*, 19–27.

Wakenell, P.S., Sharma, J.M. & Slocombe, R.F. (1995) Embryo vaccination of chickens with infectious bronchitis virus—histologic and ultrastructural lesion response and immunological response to vaccination. *Avian Diseases, 39*, 752–765.

Wang, L., Junker, D., Hock, L., Ebiary, E. & Collisson, E.W. (1994) Evolutionary implications of genetic variations in the S1 gene of infectious bronchitis virus. *Virus Research, 34*, 327–338.

Wilcox, G.E., Nandapalan, N., Flower, R.L.P. & Frysmith, D. (1983) Comparison of a microneutralisation test with ELISA and precipitin tests for detection of antibodies to infectious bronchitis virus in chickens. *Australian Veterinary Journal, 60*, 119–122.

Winterfield, R.W. & Albassam, M.A. (1984) Nephropathogenicity of infectious bronchitis virus. *Poultry Science, 63*, 2358–2363.

Winterfield, R.W. & Fadly, A.M. (1972) Some characteristics of isolates of infectious bronchitis virus from commercial vaccines. *Avian Diseases, 16,* 746–755.

Winterfield, R.W. & Hitchner, S.B. (1962) Etiology of an infectious nephritis-nephrosis syndrome of chickens. *American Journal of Veterinary Research, 23,* 1273–1279.

Winterfield, R.W., Fadly, A.M. & Hoerr, F.J. (1976) Immunity to infectious bronchitis virus from spray vaccination with derivatives of Holland strain. *Avian Diseases, 20,* 42–48.

Winterfield, R.W., Hoerr, F.J. & Fadly, A.M. (1978) Vaccination against infectious bronchitis virus and immunosuppressive effects of infectious bursal disease. *Poultry Science, 57,* 386–391.

Yachida, S., Aoyama, S., Sawaguchi, K., Takahashi, N., Iritani, Y. & Hayashi, Y. (1985) Relationship between several criteria of challenge-immunity and humoral immunity in chickens vaccinated with avian infectious bronchitis vaccines. *Avian Pathology, 14,* 199–211.

Yagyu, K. & Ohta, S. (1990) Detection of infectious bronchitis virus antigen from experimentally infected chickens by indirect immunofluorescent assay with monoclonal antibodies. *Avian Diseases, 34,* 246–252.

Yoder, H.W., Jr, Drury, L.N. & Hopkins, S.R. (1977) Influence of environment on airsacculitis: effects of relative humidity and air temperature on broilers infected with *Mycoplasma synoviae* and infectious bronchitis. *Avian Diseases, 21,* 195–208.

Zanella, A. (1988) Avian infectious bronchitis: properties and application of attenuated vaccine prepared with nephropathogenic strain AZ-23/74. *Proceedings of the Ist International Symposium on Infectious Bronchitis,* Rauischholzhausen, Germany, pp. 335–342.

RÉSUMÉ

Virus de la bronchite infectieuse: immunopathogènie de l'infection chez le poulet

L'immunopathogénie du virus de la bronchite infectieuse (IBV) chez le poulet fait l'objet d'un bilan. Bien que la bronchite infectieuse (IB) soit d'abord considérée comme une maladie du système respiratoire, différentes souches d'IBV peuvent montrer des tropismes tissulaires variables et affectent aussi l'oviducte et les reins avec des conséquences sérieuses. Quelques souches se multiplient dans l'intestin mais apparemment sans troubles pathologiques. Une myopathie pectorale a été associée à l'apparition récente d'un virus variant important. Différents facteurs peuvent influencer le cours de l'infection par l'IBV, incluant l'âge, la variété commerciale et l'alimentation des poulets, l'environnement et les infections intercurrentes dues à d'autres agents infectieux. Les composants immunogéniques du virus incluent les protéines S (de spicule) et la nucléoprotéine N. Les réponses humorales, locale et cellulaire du poulet à l'IBV sont étudiées, ainsi que la résistance génétique. En ce qui concerne la persistance à long terme de l'IBV, les amygdales caecales ou les reins ont été proposés pour être des sites de persistance. La variation antigénique parmi les souches d'IBV est reliée à des différences relativement faibles au niveau de la séquence en acides aminés de la protéine de la spicule S1. Néanmoins, les études antigéniques seules ne définissent pas de façon adéquat les relations immunologiques existant entre les souches, et des essais d'immunisation croisées ont été utilisés pour classer les isolats d'IBV en 'protectotypes'. Il a été spéculé que les changements au niveau de la protéine S1 peuvent être reliés à des différences de tropismes tissulaires mises en évidence pour certaines souches. Dans le futur, de nouvelles souches pourraient apparaître et affecter des organes ou systèmes non-habituellement associés à l'IB.

ZUSAMMENFASSUNG

Virus der infektösen Bronchitis: Immunpathogenese der Infektion beim Huhn

Es wird eine Übersicht über die Immunpathogenese der Bronchitisvirus (IBV)-Infektion beim Huhn gegeben. Obwohl die infektiöse Bronchitis (IB) primär als eine Erkrankung des Respira-

tionstraktes angesehen wird, können verschiedene IBV-Stämme unterschiedliche Gewebstropismen aufweisen und, mit ernsten Folgen, auch den Eileiter und die Nieren befallen. Manche Stämme vermehren sich im Darm, anscheinend aber ohne pathologische Veränderungen. Pektorale Myopathie ist mit einer wichtigen neuen Variante in Verbindung gebracht worden. Mehrere Faktoren können den Verlauf der Infektion mit IBV beeinflussen und umfassen das Alter, die Rasse und die Fütterung des Huhnes, die Umgebung und die interkurrente Infektion mit anderen Infektionserregern. Die immunogenen Bestandteile des Virus sind die S (spike)-Proteine und das N-Nukleoprotein. Die humoralen, lokalen und zellulären Reaktionen des Huhnes gegen IBV sowie die genetische Resistenz des Huhnes werden besprochen. Bei der langfristigen IBV-Persistenz sind die Blinddarmtonsillen oder die Nieren als Orte der Persistenz vermutet worden. Die Antigenvariation bei IBV-Stämmen hängt mit relativ kleinen Unterschieden der Aminosäuresequenzen im Spike-Protein S1 zusammen. Antigenuntersuchungen alleine reichen jedoch nicht aus, um immunologische Beziehungen zwischen Virusstämmen ausreichend zu definieren, und Kreuzimmunisierungsversuche sind benutzt worden, um IBV-Isolate in 'Protektotypen' zu klassifizieren. Es sind Vermutungen darüber angestellt worden, daß Veränderungen im Spike-Protein S1 eine Beziehung zu Unterschieden der Gewebstropismen, die verschiedene Stämme aufweisen, haben könnten. Vielleicht könnten in der Zukunft neue IBV-Stämme auftauchen, die Organe oder Systeme befallen, welche normalerweise keinen Zusammenhang mit der IB haben.

RESUMEN

Virus de la bronquitis infecciosa: Inmunopatogenia de la infeccion en la gallina

Se revisa la inmunopatogenia de la infección por el virus de la bronquitis infecciosa en la gallina. Mientras que la bronquitis infecciosa (IB) es considerada primariamente una enfermedad del aparato respiratorio, distintas cepas de IBV pueden mostrar tropismos diversos y afectar también al oviducto y el riñón con consecuencias serias. Algunas cepas se replican en el intestino pero aparentemente sin producir lesiones. Una reciente variante de IBV ha sido relacionada con la miopatía pectoral. Diversos factores pueden influir el curos de la infección por IBV incluyendo la edad, estirpe y estado nutritivo del ave, el medio ambiente e infecciones intercurrentes con otros agentes infecciosos. Los componentes inmunogénicos del virus incluyen las proteínas S y la nucleoproteína N. Se revisan las respuestas inmunes humorales y celulares de las gallinas frente a IBV así como la resistencia genética de la gallina. En situaciones de persistencia de IBV se considera que las tonsilas cecales o el riñón son los lugares probables de acantonamiento. Las variaciones antigénicas entre las distintas cepas de IBV están en relación con diferencias aminoacídicas pequeñas en la proteína S1. No obstante, los estudios antigénicos por si solos no son capaces de definir adecuadamente las relaciones entre las cepas, y se han utilizado estudios de inmunización cruzada para clasificar los aislamientos de IBV en "protectotipos". Se especula que los cambios en la proteína S1 puedan estar relacionados con las diferencias en el tropismo tisular que presentan ciertas cepas. Probablemente en el futuro se aislarán nuevas cepas de IBV que afecten a órganos o sistemas no asociados normalmente con IB.