CHAPTER 15

Epidemiology of Infectious Bronchitis Virus

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I. INTRODUCTION

Avian infectious bronchitis virus (IBV) is the type species of the genus *Coronavirus* of the family Coronaviridae. It is of considerable economic importance to the poultry industry worldwide, causing a highly contagious disease affecting the respiratory, reproductive, and renal systems.

II. HOST RANGE

The chicken is believed to be the only natural host for IBV; all ages can be infected and both sexes appear equally susceptible. Vindevogel *et al.* (1976) found that pigeons were resistant to IBV infection. However, Biondi and Schiavo (1966) successfully infected quail but not seven other avian species experimentally with IBV, and Cumming (1969) was able to recover the virus from the kidneys of one of six magpies inoculated via the conjunctiva. Barr *et al.* (1988) isolated IBV from a flock of racing pigeons with respiratory signs, but failed to transmit infection to pigeons experimentally. Based solely on serological data, Weisman *et al.* (1987) claim to have demonstrated the presence of IBV in healthy turkeys, although Cumming (1969) was unable to infect turkeys experimentally with IBV. Spackman and Cameron (1983) isolated IBV from pheasants with a history of respiratory signs and aberrant egg production, and

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the disease problem in the pheasants was successfully controlled by the use of an oil-based inactivated IB vaccine. This and a further report (Lister *et al.*, 1985) suggest that the pheasant could be a second natural host for IBV and is the only evidence of the ability of IBV to infect avian species other than the chicken.

III. INCIDENCE

Avian infectious bronchitis is reported from all countries where an intensive poultry industry has developed; in most countries it appears as a serious disease problem shortly after the development of the industry in that area. The relative importance of IBV in a country appears to fluctuate from time to time, being dependent on such factors as the percentage of the poultry population that is vaccinated and on the emergence of virus types against which current vaccines are not wholly effective (see Section VIIA.). Another factor governing the reported incidence of IBV is the efficacy of diagnostic and research facilities in different countries. A detailed review of the incidence of IBV worldwide can be found in the proceedings of the first and second international symposia on infectious bronchitis (Kaleta and Heffels-Redwan, 1988, 1991).

IV. TRANSMISSION

The most important route of spread of IBV is from the respiratory tract, probably by aerosol. During the clinical phase of the disease and for approximately 28 days after infection, virus can be recovered intermittently from the respiratory tract (Cook, 1968). However, IBV replicates in many tissues throughout the chicken (Hofstad and Yoder, 1966; Doherty, 1967), and once chickens have recovered clinically, the feces are possibly the best site from which to recover virus (Cook, 1984), the intestinal tract (El Houadfi *et al.*, 1986; Ambali and Jones, 1990) and specifically the cecal tonsil (Cook, 1968) being suggested as a likely reservoir for IBV. These findings emphasize that the importance of fecal shedding in the spread of the virus should not be overlooked; although inhalation of airborne virus may be the most common method of transmission, ingestion of virus via contaminated food, drinking water, or litter plays an important role in the epidemiology of IBV.

While lateral transmission is undoubtedly the most important method of IBV spread, it has been shown that virus can be recovered from eggs laid by experimentally inoculated hens for up to 6 weeks after inoculation with a high virus dose (Cook, 1971) and also from a small number of day-old chicks hatched from those hens. However, the progeny showed no signs of IB infection. Earlier work (Cook and Garside, 1967) had shown that it was possible to hatch and rear IB-free chicks from dams showing a high incidence of infection; the conclusion was drawn that egg transmission is of negligible importance in the epizootiology of IBV infections. One interesting finding in that work was that IBV could be recovered from semen of infected cockerels for up to 14 days after inoculation, a finding that could have implications for the spread of IBV.

A. Carriers/Persistent Infections

1. In the Chicken

There is no conclusive evidence that a true carrier state exists with IB, but virus clearly persists for a considerable time in experimentally inoculated chicks. Cook (1968) recovered virus for 49 days from experimentally inoculated chicks housed in strict isolation, and could recover it for over 4 months when isolation was less effective. It was suggested in the latter case that continual reinfection from shedders had occurred. Alexander *et al.* (1978) isolated IBV from the feces of chickens up to 227 days after inoculation of two different strains of IBV. These birds were not housed in isolation and again reinfection was a possibility. Therefore, although IBV can be isolated in the presence of high concentrations of humoral antibody, it was concluded that a true carrier state does not occur. However, the demonstration by Jones and Ambali (1987) of reexcretion of an enterotropic IBV strain at point of lay, with virus being recovered from both tracheal and cloacal swabs, suggests that a state of latency may exist.

2. In the Environment

The question of persistence of IBV in the environment is a complex one because of conflicting evidence on the survival of IBV under different environmental conditions. Otsuki *et al.* (1979) demonstrated variation in the resistance of ten IBV strains to chemical and physical treatments. During epidemics the virus can certainly be transmitted between farms. For example, the same IBV serotype has been isolated from four different farms within a 10-mile radius over a period of 13 months (Cook, 1988). Airborne spread is likely to have been involved, but movement of personnel and equipment probably aided transmission. The climatic and other conditions that govern spread are not fully understood, and many aspects of IBV transmission still require elucidation. Moreover, the importance of strain variation in the transmission and persistence of IBV has not received the attention which the subject merits.

V. PATHOGENESIS

A. Clinical Signs

IBV can induce respiratory signs, affect the reproductive tract, and cause nephritis. Young chickens up to 4 weeks of age appear to be most susceptible to respiratory disease. The clinical signs, which appear two to three days after infection, include nasal discharge, sneezing, coughing, and tracheal rales. Caseous or catarrhal plugs may be present in the trachea or bronchi. Swollen sinuses may occur in some chickens. IBV respiratory infection predisposes chickens to infection with secondary pathogens (see Section V.C.), although chickens will usually recover quickly from an uncomplicated viral infection.

IBV infection of susceptible layers can result in a drop in egg production or failure to lay at full potential. The extent of the drop varies, depending on such factors as time of infection in relation to stage of egg production and the general health of the flock. Drops in production of 5 to 20% are not uncommon, together with the production of increased numbers of downgraded eggs with thin, misshapen, rough, and soft shells. Watery whites, in which there is no clear demarcation between the thick and thin albumins, are often produced. Although layers usually recover from the disease, their egg production rarely returns to preinfection levels. IBV infection of chickens less than 2 weeks of age can cause damage to the developing reproductive tract, resulting in "false layers" that do not lay normally at sexual maturity (Broadfoot *et al.*, 1956; Jones and Jordan, 1970).

Nephritis has been reported as a result of IBV infection. Early reports were from Australia and showed that certain strains, such as the T strain, had a predilection for the kidney (Cumming, 1963). However, the incidence of nephritis associated with IBV has increased and has been reported from other countries, including Italy (Zanella *et al.*, 1988), Belgium (Froyman *et al.*, 1985), France (Picault *et al.*, 1987), Japan (Otsuki, 1988), and the United States (Kinde *et al.*, 1991). The reason for this increased incidence of renal involvement remains unclear, and particular IBV serotypes do not necessarily appear to be responsible. Swollen, pale kidneys with the tubules and ureters enlarged, due to the presence of urates, are often observed. Polydipsia may also be present. In some outbreaks in Belgium, mortality rates of up to 20% have been reported (Nauwynck and Pensaert, 1988).

B. Histopathology

This has been covered in previous reviews, for example, King and Cavanagh (1991).

C. Secondary Infections

The severity of the infection caused by IBV can be increased both by environmental factors and by the presence of other microorganisms that may or may not be pathogens in their own right. In such cases, severe air sacculitis, often accompanied by peritonitis and pericarditis, can result and mortality may occur. In adult chickens combined infection with IBV and *Mycoplasma gallisepticum* has been shown to cause a more severe effect on egg production and quality than inoculation with either agent alone (Blake, 1962).

Other viruses, bacteria, and mycoplasmas have all been shown to exacerbate respiratory disease caused by IBV (Jordan, 1972), although most studies have incriminated *Escherichia coli* and *Mycoplasma synoviae*. Fabricant and Levine (1962) and Springer *et al.* (1974) found that while synergism, as based on severity of air sac lesions, could be demonstrated following inoculation with IBV and either *E. coli* or *M. synoviae*, the most severe infection resulted from

inoculation of the three agents together. However, Smith *et al.* (1985) were able to reproduce a highly lethal infection, closely resembling the natural disease, by intranasal inoculation of IBV and invasive serotypes of *E. coli*. Infection of similar severity was caused if the *E. coli* were given at any time between 7 days before and 7 days after the virus. Some insight into the mechanism involved was provided by the observation that, when inoculated intranasally alone, the *E. coli* replicated poorly in the upper respiratory tract and did not invade. However, when IBV was included in the inoculum, *E. coli* multiplied to very high titer in the respiratory tract with subsequent penetration of the invasive *E. coli* strains but not of commensal coliforms. These authors suggested that the *E. coli* were able to invade as a result of the damage that IBV had caused in the respiratory tract, a finding confirmed by Nakamura *et al.* (1992), who showed that histopathological lesions in the respiratory tract were more severe when IB and *E. coli* were inoculated together than when either agent was inoculated alone.

D. Immunity

It is well established that the chicken responds to IBV infection by producing specific antibodies of the immunoglobulin IgM, IgG, and IgA classes, although the main serum antibody is IgG. Chickens with high levels of specific humoral antibodies can still be susceptible to infection, however, suggesting that immune factors other than humoral antibody are involved in protection.

IgM is the first class of antibody to be detected in the serum following IBV infection; peak concentration is reached about 8 days after infection and levels then decline (Mockett and Cook, 1986). As the presence of this antibody is only transitory, it may be useful in the diagnosis of recent infection (Martins *et al.*, 1991). Since plasma cells soon switch from producing specific IgM to IgG, the latter immunoglobulin, although present at relatively low concentrations 7 days after infection, reaches highest concentrations by about 10 to 14 days, and these levels are maintained for a considerable time. The antiviral IgG response, shown by enzyme-linked immunosorbent assay (ELISA), is similar to that shown by the hemagglutination inhibition test, although the former is much more sensitive. Virus-neutralizing (VN) antibodies are detectable from 9 days after infection, but take longer to reach a peak (Mockett and Darbyshire, 1981). Similar antibody profiles to both the spike and membrane protein have been demonstrated using ELISA (Mockett, 1985). It is generally believed that the spike protein is responsible for inducing VN antibodies and protective immunity (Mockett, 1985; Tomley *et al.*, 1987; Cavanagh *et al.*, 1984, 1988), although recent evidence suggests that such immunity may also be induced by the nucleocapsid protein (Boots *et al.*, 1992).

High concentrations of specific VN antibodies are thought to be important in preventing dissemination of the virus from its primary replication site in the respiratory tract (Box, 1988; Otsuki, 1988). Hence, such antibodies may prevent viral replication in and damage to the reproductive tract and the kidneys. It is also known that high concentrations of maternally derived antibodies, which

are transmitted from the hen to the chick via the yolk, can help prevent infection, although for only until 1 to 2 weeks of age (Mockett *et al.*, 1987).

IBV-specific local antibodies have been detected in lachrymal fluid and in nasal and tracheal washings. The antibodies are thought to be produced by plasma cells in lymphoid tissue such as the Harderian gland. The production of both IgG and IgA has been reported (Holmes, 1977; Davelaar *et al.*, 1982; Hawkes *et al.*, 1983; Lütticken *et al.*, 1988). Recent work has suggested that the amount of IgA produced in saliva and lachrymal fluid after infection may be related to the reduced pathogenicity of IBV for certain inbred lines of chickens (Cook *et al.*, 1992). It is interesting to note that aerosol vaccination of day-old chicks results in protective immunity, presumably because local immune mechanisms have been stimulated (Davelaar and Kouwenhoven, 1980).

Although the subject has received little attention, cell-mediated immune responses have been reported following IBV infection. Systemic cell-mediated immunity (CMI) has been detected using a lymphoblast transformation technique (Timms *et al.*, 1980). There was no direct correlation between CMI and humoral antibody production (Timms and Bracewell, 1981). CMI has been demonstrated to the S1, N, and M proteins of IBV (Ignjatovic and Galli, 1993). Although in that work only the S1 glycoprotein protected against challenge, Boots *et al.* (1992) demonstrated that a recombinant product expressing the N protein induced a CMI response and some protective immunity.

E. Host Factors

1. Age Resistance

Although chickens of all ages are susceptible to infection, very young chicks tend to show more severe respiratory signs than older ones and morbidity is generally only observed in very young chicks. Smith *et al.* (1985) found that chicks rapidly became more resistant to a mixed IBV and *E. coli* infection with increasing age. Whereas approximately 90% mortality was recorded following day-old inoculation, this fell to 20% when inoculation was delayed until 6 weeks.

2. Genetic Resistance

The availability of genetically defined, inbred lines of chicken has enabled genetic differences in the response to IBV infection to be studied. Purchase *et al.* (1966) demonstrated some variation in mortality following IBV inoculation of embryos from different inbred lines. Using a mixed IBV and *E. coli* infection, Smith *et al.* (1985) and Bumstead *et al.* (1989) have demonstrated large differences in mortality, ranging from 3 to 87%, in different inbred and partially inbred lines of chicken. However, even in the more resistant lines clinical signs of respiratory infection were recorded and pericarditis and peritonitis observed in some of the chicks, indicating that the severity of the IBV infection was

sufficient to allow *E. coli* to invade, although not to cause a lethal infection. As a result of challenging with IBV or *E. coli* alone, Bumstead *et al.* (1989) concluded that the variation in mortality in different lines reflected differences in resistance to IBV rather than to *E. coli*. Examination of F1 crosses suggested that mortality differences could not be attributed to maternal effects and that inheritance of resistance was fully dominant. The mortality pattern in F2 and backcross generations was compatible with the inheritance of a dominant autosomal resistance gene and showed no evidence of association with the major histocompatibility complex.

During this study, it was found that up to 87% mortality occurred in some inbred lines of chicken following intranasal inoculation of IBV alone. This enabled a comparison to be made of different parameters of an IBV infection in highly resistant (White Leghorn line C) and highly susceptible (White Leghorn line 15I) chickens. It was found (Otsuki *et al.*, 1990; Nakamura *et al.*, 1991) that the respiratory infection and degree of damage to the trachea were more severe and longer lasting in the susceptible line. While similar amounts of virus were recovered initially from tissues of both lines, overall more was recovered and for longer from the susceptible line. However, tissues from each line were equally susceptible to *in vitro* inoculation of IBV, suggesting that resistance to IBV infection is not related to a lack of specific viral receptors by target cells of the resistant line, as is the case with murine hepatitis virus (Boyle *et al.*, 1987). This finding and the observation of some increased susceptibility to IB infection in bursectomized chicks (Cook *et al.*, 1991a) suggests that some other mechanism must play a role in resistance to IBV infections. The nature of this mechanism has yet to be identified.

F. Viral Factors

The ability of IBV to vary antigenically has been known for many years, based mainly on the identification of different serological types using virus neutralizing tests (Hopkins, 1974; Darbyshire *et al.*, 1979). As techniques for performing such tests have improved, it has became increasingly easy to define serotypes clearly. Many new serotypes have now been identified, particularly in Europe where most of such work has been done (Davelaar *et al.*, 1983; Cook, 1984; Cook and Huggins, 1986), but also in other countries, for example, Chile (Cubillos *et al.*, 1991) and the United States (Gelb *et al.*, 1991) (Table I). One reason for the increase in numbers of antigenic types of IBV may be the widespread use of live attenuated IB vaccines, leading to the preferential multiplication of virus populations able to escape neutralization by vaccine-induced antibodies (Kusters *et al.*, 1987). However, nucleotide sequencing has indicated that some IBV isolates are genetic recombinants. The possession by two serologically distinct IBV strains of very similar M but very distinct S1 proteins was suggested as evidence of recombination (Cavanagh and Davis, 1988). Kusters *et al.* (1989) compared S1 sequence data from several IBV isolates with T1 fingerprints of the entire IBV genome and reached the same conclusion. Using nucleotide sequencing, Cavanagh *et al.* (1992a) showed that although the S

TABLE I. Cross-Neutralization between IBV Isolates from Different Countries[a]

Antiserum	UK	Holland	France	Germany	Italy	Portugal	Israel	Morocco	S. Africa	Chile	USA	Australia
UK	*380*[b]	—[c]	—	—	—	—	—	—	—	—	—	—
Holland	—	*870*	—	—	—	—	—	—	—	—	—	—
France	20	20	*3200*	—	26	—	50	—	26	—	—	—
W. Germany	—	—	—	*300*	NE	—	—	—	—	—	—	—
Italy	—	—	—	—	*420*	—	—	—	—	—	—	20
Portugal	—	—	—	—	—	*180*	—	—	—	—	—	—
Israel	—	—	—	—	—	—	*870*	—	—	—	—	—
Morocco	—	—	—	—	—	—	—	*400*	—	—	—	—
S. Africa	—	—	—	—	—	—	—	—	*490*	—	—	—
Chile	—	—	—	NE	—	—	—	—	NE	*1100*	NE	NE
USA	—	—	—	—	—	—	—	—	—	—	*244*	—
Australia	—	—	—	—	20	—	—	—	—	—	—	*1300*

[a]Virus ($\log_{10}$ 2.0 CD_{50}).
[b]Reciprocal neutralization titer; homologous reaction in italics.
[c]Titer <1:10.
NE, Not examined.

sequence of a Massachusetts serotype strain (322/82) was very similar to Massachusetts, the 3C and M gene sequences showed greater homology to those of European non-Massachusetts serotypes, thereby providing evidence of recombination between IBV strains. Similarly, Wang *et al.* (1993) have shown a US field isolate of IBV to be a recombination between the Massachusetts and Arkansas serotypes, the recombination apparently having occurred within S1.

The finding of sharply defined serological differences between IBV strains, however, does not imply lack of *in vivo* cross-protection between them. Using as a challenge system a mixed IBV and *E. coli* infection, it has been shown (Cook *et al.*, 1986) that IBV strains may protect against challenge with antigenically distinct isolates and that protection is much more broadly based than might be inferred from serological data. It may eventually be shown that the grouping of strains into antigenic groups by the use of, for example, monoclonal antibody (MAb)-based ELISAs (Ignjatovic *et al.*, 1991) will correlate better with cross-protection data than do the results of virus neutralizing tests.

It is known that serotype-specific VN antibodies are induced by the highly variable amino-terminal S1 subunit of the spike glycoprotein (Mockett *et al.*, 1984; Cavanagh *et al.*, 1986; Karaca *et al.*, 1992). A surprising finding from sequence analysis of S1 of several British isolates belonging to different serotypes was the similarity of their S1 sequence, the strains differing by only 2% of their S1 residues (Cavanagh *et al.*, 1992b). This provides further evidence that only a few amino acids on S1 form epitopes responsible for inducing VN antibody. Thus, a few key amino acid substitutions can define a new IBV serotype. Analysis of MAb-resistant mutants has shown that an area near the N-terminus of S1 is associated with neutralization epitopes (Cavanagh *et al.*, 1988), and other data (Cavanagh *et al.*, 1992b; Kant *et al.*, 1992) suggest that the region of the first 300 N-terminal residues is an important hypervariable region. As has been shown with transmissible gastroenteritis virus (Jimenez *et al.*, 1986), it is probable that the tertiary structure of the IBV spike protein is important in defining antigenic sites (Koch and Kant, 1990), some of which may be formed by juxtapositioning of linearly well separated amino acids. Recent work (Boots *et al.*, 1991), using a mouse model, has suggested that antigenic variation may also occur in the nucleocapsid of IBV.

The availability of MAbs (Mockett *et al.*, 1984; Koch *et al.*, 1990; Ignjatovic and McWaters, 1991) and the application of molecular techniques to epidemiological studies are permitting the evolution of IBV strains to be studied. It is already clear that the situation is complex; Cavanagh *et al.* (1988) showed that strains of the Massachusetts serotype isolated over many years have retained an S1 protein with over 94% homology, while strains with only 50% homology coexist (Kusters *et al.*, 1989). It has also been shown (Cavanagh *et al.*, 1992b), using neutralizing MAbs, that isolates belonging to different serotypes (defined using pooled, polyclonal chicken antibodies) can share common neutralizing antibody-inducing epitopes. Thus we have examples of S gene homology both within and between serotypes. Much work remains to be done to clarify IBV evolution, a subject further complicated by genetic recombination, but it appears the IBV strains are evolving in different geographical areas by more than one line simultaneously (Cavanagh and Davis, 1992) but at varying rates, de-

pending on many factors such as poultry density, vaccination procedures, and so on.

Progress in studying IBV evolution is now coming both from the application of MAb analysis and the use of the polymerase chain reaction (PCR), which has recently become a useful additional tool to study IBV. Lin *et al.*(1991a) identified a pair of oligonucleotides spaced 400 bases apart in a conserved region of S2. These should identify all IB viruses and indeed they have been used successfully to rapidly confirm a viral isolate as IBV (Parsons *et al.*, 1992). Oligonucleotides have now been identified that enable the DNA of only S1 to be synthesized (Kwon *et al.*, 1993a). PCR products have also been taken, the DNA cut using appropriate restriction enzymes, and IBV strains typed more rapidly than by using conventional virus neutralizing tests (Lin *et al.*, 1991b; Kwon *et al.*, 1993a).

VI. DIAGNOSIS

A. Clinical

Avian infectious bronchitis infection may be suspected in a flock because of the clinical signs, which include respiratory signs, drops in egg production, or nephritis. However, other diseases such as Newcastle disease, infectious laryngotracheitis, and turkey rhinotracheitis can produce similar clinical signs. Hence, in order to diagnose an IBV infection it is important to isolate the virus and to demonstrate a specific antibody response.

B. Virus Isolation

There are a number of ways to propagate IBV, principally tracheal organ cultures, embryonated eggs, and tissue culture. Passage of IBV in embryonated eggs inoculated via the allantoic route may produce no effects on the embryo initially, but after two or three passages dwarfing or death of the embryo is usually observed and high titers of infectious virus can be present in the allantoic fluid. Probably all IBV strains grow in tracheal organ cultures or embryonated eggs but adaption is required for growth in tissue culture. Hence, this substrate is not used for primary virus isolation. As mentioned above, swabs of nasal discharge collected in the early stages of the disease are a rich source of virus as, in the later stages, is fecal material.

Infected allantoic fluid and tracheal organ culture supernatant fluid frequently contain enough virus particles to be visualised by electron microscopy. The typical size (about 80–100 nm) and shape (usually round with projecting 20-nm spikes) of the coronavirus (Berry *et al.*, 1964) enables a presumptive diagnosis to be made. However, the electron microscopy facility is usually found only in research establishments.

The immunofluorescence test is useful in confirming the identity of isolates as IBV, and a technique for classification based on neutralization of immuno-

fluorescent foci (Csermelyi *et al.*, 1988) has been used successfully. Polyclonal antisera, which react with antigenic determinants common to all IBV strains, were used and smears of infected chorioallantoic membranes provided the substrate (Clarke *et al.*, 1972).

Antigen assays, based on MAbs, are now becoming more widely used. A MAb-based immunoperoxidase procedure has been found to be highly sensitive for detecting IBV in infected tissues or chorioallantoic membranes of infected embryos (Naqi, 1990). Yagyu and Ohta (1990) used an indirect immunofluorescence assay with a MAb specific for the IBV nucleocapsid protein and were able to detect IBV in tracheas of infected chickens for longer than was possible by virus isolation. Surprisingly, a streptavidin–biotin immunohistochemical assay was less sensitive than virus isolation (Owen *et al.*, 1991).

By means of the PCR and a biotin-labeled DNA probe it has been possible to amplify and detect IBV in allantoic fluid (Jackwood *et al.*, 1992), in tracheal swabs taken from chickens inoculated with different IBV serotypes, and even in allantoic fluid of embryos inoculated with field samples from IB outbreaks (Kwon *et al.*, 1993b). These authors claim that this technique detects IBV more rapidly and efficiently than more conventional methods.

With the availability of MAbs that are either group-specific or serotype-specific (Koch *et al.*, 1990; Karaca *et al.*, 1992), various ELISAs, such as antigen capture assays, have now been developed (Cavanagh *et al.*, 1992b; Naqi *et al.*, 1993) that allow detection of either all IBVs or particular serotypes. However, the sensitivity of such assays needs improvement (Naqi *et al.*, 1993). The large panel of MAbs now available in The Netherlands has enabled extensive characterization of existing IB strains to be carried out (Cavanagh *et al.*, 1992b).

C. Serological Methods

A range of serological tests is available to detect IBV antibodies, including agar gel precipitation test, immunofluorescence, hemagglutination inhibition, ELISA, and virus neutralization. The agar gel precipitation test requires the presence of high concentrations of specific antibodies (precipitins) in order to produce a precipitin line; thus the test is relatively insensitive. Precipitins are only present for a short period after infection; MacDonald *et al.* (1982) suggested that their presence indicates recent exposure to the virus. The immunofluorescence test is a useful, relatively sensitive assay, but it has not been used extensively as a serological test for IBV.

The hemagglutination inhibition test gained favor when it was found that IBV could be made to hemagglutinate chicken red blood cells following treatment with crude preparations of the enzyme phospholipase C (Bingham *et al.*, 1975), although more recently neuraminidase has been shown to be the active constituent (Schultze *et al.*, 1992). Interestingly, Davelaar *et al.* (1983) have shown that certain IBV strains may spontaneously hemagglutinate chicken red blood cells. The hemagglutination inhibition test detects antibodies as early as 7 days after infection, and these antibodies persist for some time. This test is used routinely for flock monitoring and in most research or diagnostic laborato-

ries. However, considerable care is required in the performance and interpretation of the test (Cook *et al.*, 1987), which should always be carried out under carefully controlled conditions such as those described by Alexander *et al.* (1983).

In some laboratories, the ELISA has gained acceptance. The test is very sensitive (Garcia and Bankowski, 1981) and can detect antibodies as early as 3 days after infection (Mockett and Darbyshire, 1981). ELISAs that use purified virus (from isopycnic sucrose gradients) as antigen produce highly specific results. As with the hemagglutination inhibition test, antibodies reach highest concentrations 14 to 17 days after infection, after which there is a gradual decline. It is probable that the ELISA will become the serological test of choice for monitoring responses to vaccination since it is especially useful for the large-scale screening of poultry flocks. However, neither the ELISA nor the hemagglutination inhibition test identify a particular serotype of IBV. For this, the virus neutralizing test performed in embryonated eggs (King and Cavanagh, 1991), cell culture after adaptation of the virus to that system (Hopkins, 1974), or tracheal organ cultures (Darbyshire *et al.*, 1979) was until recently the only available assay. However, the existence of strain-specific MAbs has led to the recent development of serotype-specific ELISAs (Karaca and Naqi, 1993). It seems likely that the use of such techniques will increase in the future.

Since a humoral antibody response is produced following vaccination, it is frequently necessary to be able to differentiate between the response to vaccination and to field challenge. An infection might be indicated by a rise in antibody titer to well above the anticipated vaccinal response level. In order to demonstrate evidence of a field challenge, it is necessary to take sequential sets of serum samples and demonstrate a rise in antibody titer between them.

VII. CONTROL

A. Vaccines

In most situations the only practical means of preventing IB is to vaccinate against the infection, and both live attenuated and inactivated vaccines are available. Live attenuated vaccines may be given by eyedrop, aerosol, or in the drinking water and are effective in the face of maternally derived immunity (Cook *et al.*, 1991b). With live vaccines it may be possible to prevent disease but not prevent infection. The more highly passaged virus strains, which have reduced pathogenicity, also have reduced immunogenicity. Furthermore, live vaccines may only induce short-lived immunity, and hence repeated vaccinations may be required. It is advisable to monitor regularly the specific antibody status of the flock.

Initially, most of the virulent IBV strains isolated were of the Massachusetts type. Indeed, it was thought for many years that there was only one serotype of IBV. However, other serologically distinct serotypes were subsequently isolated: Connecticut (Jungherr *et al.*, 1956) followed by others such as Arkansas (Johnson *et al.*, 1973) or 06 (Gelb *et al.*, 1991) in the United States;

D212 and D274 in Holland (Davelaar *et al.*, 1981); A to J in England (Cook and Ellis, 1988); PL84084 in France (Picault *et al.*, 1986); Az-23/74 in Italy (Zanella, 1988) and G in Morocco (El Houadfi *et al.*, 1986) (Table I). Different IBV serotypes appear to be present in distinct geographical areas. Thus the Arkansas serotype has not been isolated in Europe, while the D274 serotype has not been isolated in the United States. Even in particular countries distinct serotypes may be confined to local areas although more than one serotype can coexist in the same area (Gelb *et al.*, 1991).

Live vaccines have been developed to combat some of these serotypes, although it is clear that a new IB vaccine is not required for every new serotype since protection is more broadly based than serological tests might suggest (Cook *et al.*, 1986). From time to time, however, new serotypes emerge against which existing vaccines are not fully effective (Picault *et al.*, 1986; Cubillos *et al.*, 1991; Parsons *et al.*, 1992; Lambrechts *et al.*, 1993; Pensaert and Lambrecht, 1994); only in those areas where a known serotype regularly causes disease should a live vaccine containing that serotype be recommended.

It has been assumed that high concentrations of specific IgG antibody can protect the kidney and the reproductive tract from the effects of IBV. The aim therefore is to induce high concentrations of such antibody. This can be achieved by the use of an inactivated IB vaccine given as an oil-in-water emulsion to chickens that have already been "primed" by vaccination with a live-attenuated IB vaccine. The slow release of antigen stimulates and maintains the specific immune response for a considerable time. The timing of administration of inactivated vaccine is important. Chickens that have high concentrations of circulating antibody do not respond well to inactivated vaccine, possibly because the antibody reacts with the antigen and prevents stimulation of the B-cell response. If the correct time interval is allowed, however, a chicken already primed with a live IB vaccine produces a vigorous B-cell response to the inactivated vaccine.

B. Management of Environmental Factors

Avian infectious bronchitis is a very labile but highly infectious virus. Thus, commonly used disinfectants can kill the virus, and material containing IBV is rapidly made noninfectious usually within 24 hr at room temperature. However, because IBV is readily spread by fomites and is airborne, careful procedures must be undertaken to keep premises clean, for example, by providing foot baths and restricted access to the premises.

The large numbers of chickens commonly housed together provide ideal conditions for the spread of IBV, and since the virus is highly infectious it spreads rapidly from chicken to chicken in the aerosols created by the coughing and sneezing of infected birds. Management should avoid any factors that might irritate the chicken's respiratory tract, for example, by preventing the build up of ammonia and ensuring adequate ventilation in poultry houses. It cannot be stressed too strongly that good vaccination programs go hand in hand with good management.

VIII. REFERENCES

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