# Infectious Bursal Disease of Chickens

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Infectious bursal disease (IBD), formerly termed Gumboro disease had been observed in the U.S.A. towards the end of the 1950 but it was not until 1962 that it was first described by Cosgrove. At first the disease was erroneously called "Avian nephrosis". The disease is a viral disease of young chickens for which the causative virus is tentatively classified as a member of Reoviridae. The virus induces atrophy of the bursa of Fabricius (BF) as a result of necrosis of lymphocytes and also causes a general lymphocidal effect in other lymphoid organs, including the thymus and spleen. The BF has a key role in the development and maturation of the humoral immune response of the chicken. A number of workers have demonstrated that infection with IBD virus at a young age can lead to immunosuppression. Therefore, this disease is of interest immunologically. In the present paper some of our recent studies will be described briefly.

## Morphological characterization of the virion[2,4,7]

The virus was tentatively classified as a member of Reoviridae. The classification was based upon the morphology under ultrathin-section electron microscopy of the BF from infected chickens and upon limited biochemical characterization. Litte imformation is available on the ultrastructure of the IBD virus. In this section, electron micrographs of purified IBD virus, negatively stained with phosphotungstate, are presented.

IBD virus apparently has an outer layer, as indicated by the large size of intact particles with hexagonal outlines (Plate 1 a-f). Moreover, the capsomeric detail on the main capsid surface of IBD virus often appeared partially obscured by such a layer. This outer layer was very thin (7 to 8 nm) and continuous, with T-shaped structures suggestive of the configuration reported for rotavirus but not as clearly defined[12,15,18] Although the outer layers of reovirus and orbivirus are also indistinct, they are featureless[11,18]. The outer layer of IBD virus may exert some stability to the structure of the underlying major capsid layer. By rotational enhancement of image detail (Plate 2 a-f), the results of this study confirmed our previous report that IBD virus has an icosahedral symmetry of T=3, with a probable 32 large capsomeres. This type of symmetry of the unique feature of subunit sharing are morphological characteristics reported for the Reoviridae family[14,15]. The results obtained are summarized as follows. An outer layer surrounding the capsid of IBD virus was evident from electron micrographs of intact virus particles having diameters of 62 to 63 nm. The capsid was found to be composed of large morphological units or capsomeres, measuring about 12 nm in diameter. The architecture of the capsid appears to be that of T=3 symmetry, with probable 32 morphological units by rotational enhancement of image detail.

## Structural proteins of the virion[3,7]

Apart from the initial study by Nick et al.[13], little information is available on the structural proteins of IBD virus, using polypeptide gel electrophoresis. The exact number of polypeptides and their locations within the virion are not yet clearly understood. The results of this study demonstrated that

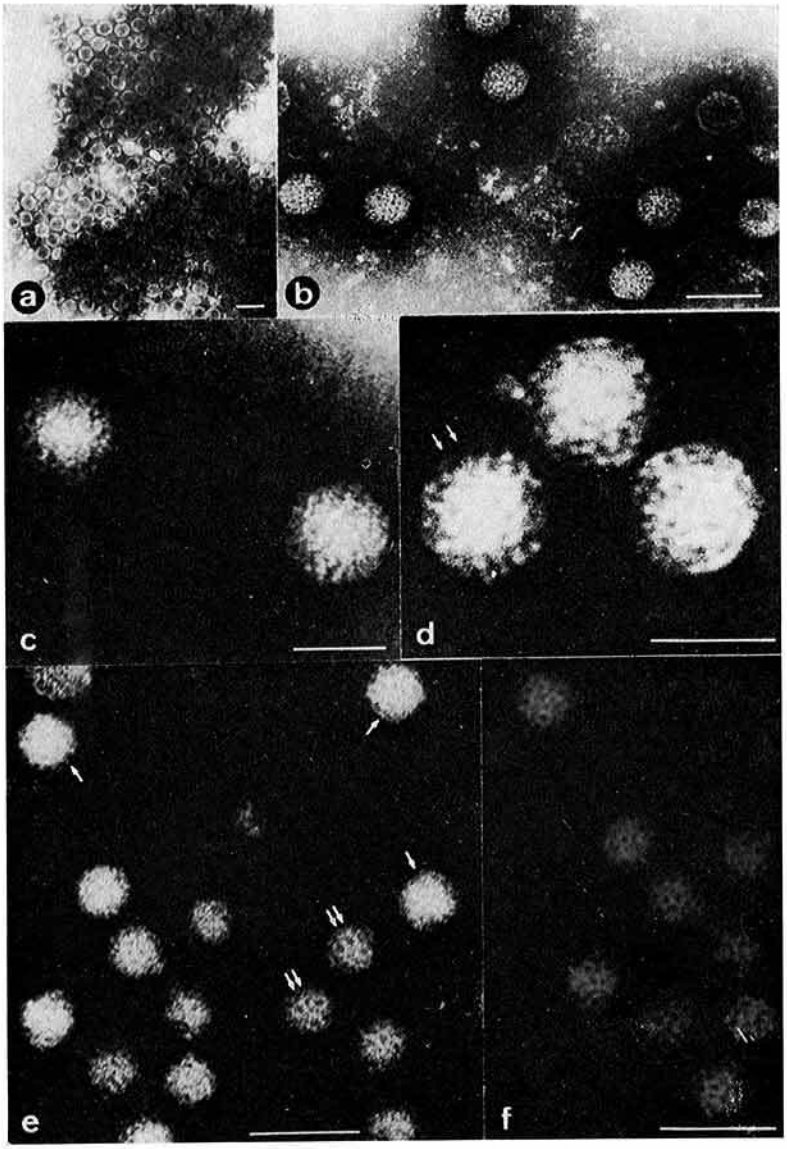


Plate 1. Electron micrographs of negatively stained IBD virus particles.

a. A large number of IBD virus recovered from the 1.34 g/ml CsCl fraction. Bar=100 nm. ×45, 000.
b. Intact virions of IBD virus showing hexagonal outlines. Bar=100 nm. ×130, 000.
c. Intact virions of IBD virus with an outer layer. Bar=50nm. ×330, 000.
d. Higher magnification of an intact virion showing the outer layer. The surface of the particles appear to have T-shaped morphology which is continuous. Bar=50 nm. ×450, 000.
e. IBD virus particles recovered from the 1.35 g/ml CsCl fraction. Intact virions of IBD virus (arrow) and particles with clear capsomeres showing the loss of the outer layer (double arrow). Bar=100 nm. ×190, 000.
f. Particles showing clearly discernible capsomeres. Bar=100 nm. ×200, 000.

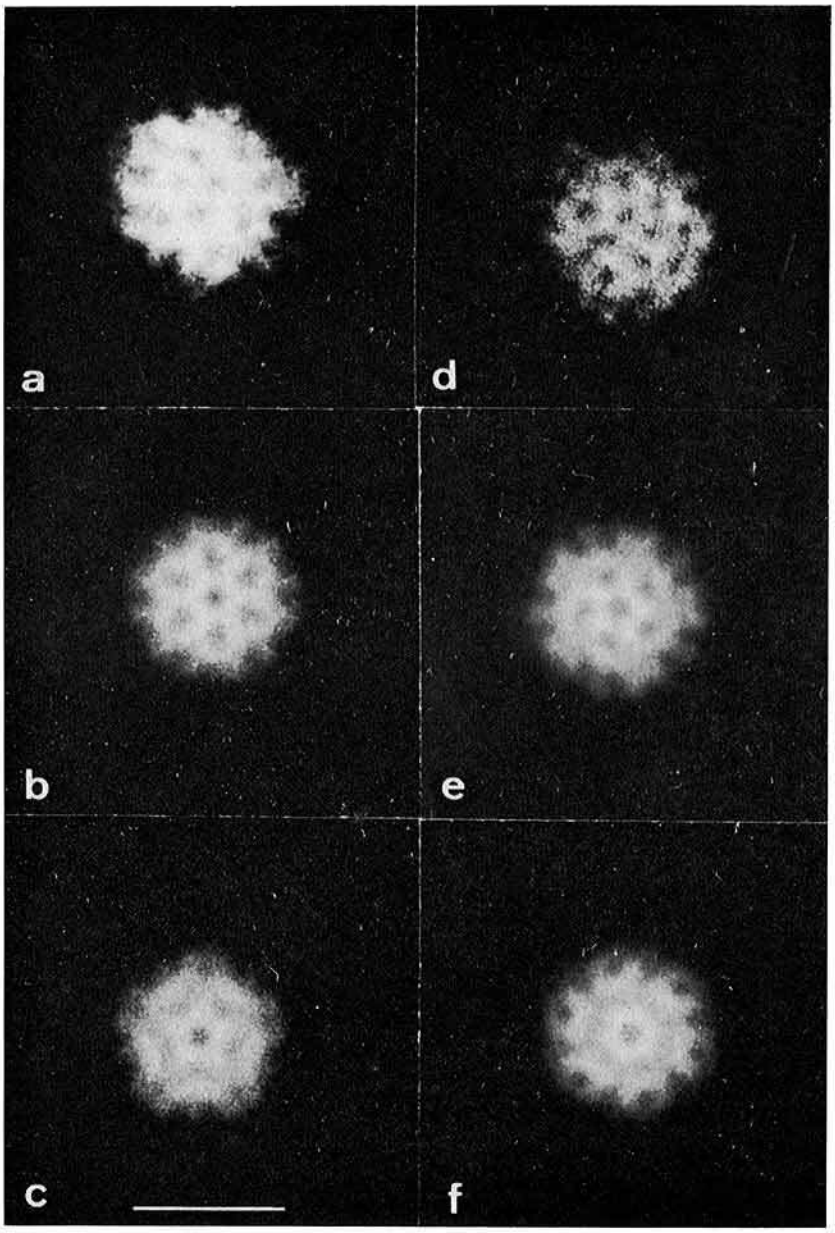


Plate 2. continued

a-c. Single IBD virus particle viewed on a 3-hold axis of symmetry representing n=0, n=6, and n=5 rotations, respectively. Enhancement of capsomeres is evident in n=6 rotation. Bar =50 nm. ×390, 000.

d-f. Single IBD virus particle viewed on a 5-fold axis of symmetry representing n=0, n=5, and n=6 rotations, respectively Enhancement of capsomeres is evident in n=5 rotation. Bar =50 nm. ×390, 000.

**Table 1. A comparison of molecular weights* of IBD virus proteins with those of rotavirus, bluetongue virus and avian reovirus**

| IBDV | Human rotavirus | Bluetongue virus | Avian reovirus |
|---|---|---|---|
| 133 | 127 | 140 | 140 |
| 124 | 103 | 110 | 125 |
| 98 | 97 | 101 | 115 |
| | 88 | 82 | 85 |
| 51 | 58 | 61 | 72 |
| 33 | 32 | 42 | 40 |
| 26.5 | 26 | 29 | 36 |
| 23 | 21 | | 32 |

* Molecular weight $\times 10^3$

structural proteins of IBD virus consist of seven species, two major and five minor polypeptides. These are P1 to P7, with molecular weights of $133\times10^3$, $124\times10^3$, $98\times10^3$, $51\times10^3$, $33\times10^3$, $26\times10^3$, and $23\times10^3$, respectively. The major polypeptides were associated with the smaller subunit particles and string-like structures. Therefore, these polypeptides may be the capsomeral proteins of IBD virus. These results differed from those reported by Nick et al.[13] with regard to the number of polypeptides and their molecular weights. Only four polypeptides were found in the previous study, three of which did compare closely in molecular weight with our P4, P5, and P6 proteins. However, a polypeptide having a molocular weight of $11\times10^3$ was not detected in our preparations. The limited amount of purified IBD virus obtained may not have provided sufficient material or detection of all structural polypeptides. As shown in Table 1, the molecular weights of the IBD virus proteins compared more closely with those reported for the human rotavirus. However, the major polypeptide species (P3 and P4) of IBD virus did not correspond with those major proteins of the human rotavirus, nor did IBD virus have an eighth polypeptide of molecular weight $88\times10^3$. Our results concerning the similarity of its protein composition to the rotavirus group suggest its tentative inclusion into the Reoviridae family.

## Some characterization of immunosuppression in chickens by IBD virus[1,5,8]

This disease is of interest immunologically, since the function of the bursa-dependent lymphoid system is affected in young chickens. The mechanism of the immunosuppression is not fully understood, but presumably it results from the loss of immunocompetent lymphocytes. The present studies were undertaken to compare sequential changes in the proportion of B- and T-lymphocytes and measure serum immunoglobulin concentrations in chickens infected with IBD virus at different ages. The results obtained are summarized as follows. Chickens were infected with IBD virus in ovo or at different times posthatching to 6 weeks of age. The B- and T-cell responses in the lymphoid tissues and blood were examined sequentially to 8 weeks of age by using indirect immunofluorescence. The proportion of B-cells was consistently lower in infected birds than in controls, especially in chicks infected at embryos or at 1 day old. The proportion of T-cells increased following these early infections but was slightly lower in spleen and blood of birds infected at 1, 4, and 6 weeks of age. Serum IgM levels dropped significantly after infection, regardless of the time of infection. IgG levels decreased following early infection but increased after infection at 1 week old or more. The results strongly suggest that B-cells are the target for IBD virus infection.

## Replication of IBD virus in cultured lymphocytes[6]

Hitchner reviewed studies on laboratory host systems for IBD virus and noted that the virus replicates in chicken embryos and that embryos-adapted virus could be cultured in cell cultures of chicken embryo origin, with consequent cytopathic effects. No published

information is available on replication of virulent, nonadapted strains of the virus in cultured lymphocytes. It has been shown in a previous section that a specific lymphocyte type might serve as the target for IBD virus replication *in vivo*. As part of an investigation of the mechanism by which this virus induces immunosuppression, it was of interest to study virus replication *in vitro*. The results obtained are summarized as follows. The *in vitro* susceptibility of chicken lymphocytes to a wild strain of IBD virus was investigated by using immunofluorescence and virus assays as infection criteria. A variety of Marek's disease lymphoblastoid cell lines, all of thymus (T-cell) origin, were refractory to virus exposure. However, a bursa (B-cell)-derived lymphoblastoid cell line from an avian leukosis virus-induced tumor was highly susceptible. Viral antigen appeared in the cytoplasm of 20 to 30% of the cells, and large amounts of cell-free virus were released, with maximum yields occurring by 3 days postinfection. The virus also replicated in a small percentage of normal lymphocytes prepared from lumphoid tissues and peripheral blood of chickens. Pretreatment of the lymphocytes, with heat-inactivated anti-B-cell serum or with antiserum against fowl immunoglobulin M before inoculating them with the virus blocked the virus infection; no blocking occurred with anti-T-cell serum or with specific antiserum against fowl immunoglobulin G or immunoglobulin A. This suggests that surface immunoglobulin M-bearing B-lymphocytes were the target cells for infection.

## Pathogenicity of IBD virus in mouse, rat and hamster[9,10]

Rinaldi et al.[17] and Petek et al.[16] reported that egg-adapted strain of IBD virus was pathogenic for suckling mice. Very little is known of the pathogenicity to laboratory animals. The results obtained are summarized as follows. The susceptibility of mice and rats became lower according with the advance in age regardless of inoculation routes. Mice and rats were the most susceptible to intracerebral inoculation. The infectivity titers in the brains were the highest regardless of the routes of virus inoculation. The titer in the brains increased logarithmically from 12 to 60 hrs postinoculation. A plateau of $10^{6.8}$ to $10^{7.7}$ PFU/0.2 ml was maintained between 60 to 72 hrs. It is also noted that precipitating antigen was produced in the brain of mice and rats inoculated with the virus. The antigen formed 2 or 3 precipitin lines against specific antiserum. The principal changes of the brains were degeneration and destruction of the nerve cells in theramus and cortex. A specific fluorescence was found in the cytoplasm of nerve cells. The virus particles were observed in the cytoplasm of the infected nerve cells. Suckling hamsters inoculated intracerebrally with the virus developed listlessness and marked weight loss begining on postinoculation days 12 to 14. The occipital curvature of the skull became prominent at 17 to 19 days after inoculation. All had mild to severe hydrocephalus. The gross hydrocephalus was first seen at day 14 and reached a maximum on the 21 day.

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# A New *in vitro* Method for Estimating Digestibility of Animal Feeds

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Digestibility experiments have a considerable value in the estimation of nutritive values of animal diets. However, the determination of digestibility is not only tedious and time-consuming but also requires large quantities of diets.

Recently, the author proposed a new *in vitro* method to estimate the digestibility of diets for pigs. The method is based on a simulation of gastric followed by intestinal digestion. Test substances (feed) are first incubated with acid pepsin followed by incubation with intestinal fluid obtained from a pig fitted with a simple cannula in the upper jejunum. This method was also applied to estimate the digestibility of poultry diets, and activity changes of the intestinal fluid when the fluid was lyophilized were examined.

The present paper reports results of study on the *in vitro* method for the estimation of digestibility using the intestinal fluid of the pig.

## Standard procedures in *in vitro* digestion

The method had two stages. In the first stage, 0.5 g each of duplicate samples of each diet was weighed into 100 ml Erlenmeyer flask, to which 20 mg pepsin in 10 ml 0.075 M-hydrochloric acid was added and incubated with shaking at 80 oscillations/min for 4 hrs at 37°C in a water-bath. At the end of the first incubation period, the content was neutralized with 0.2 M-sodium hydroxide. In the second stage, 10 ml of intestinal fluid was added and the digestion mixture was incubated for an additional 4 hrs at 37°C.

A female pig weighing approximately 25 kg was used as a host animal to obtain the intestinal fluid for *in vitro* digestion experiments. The pig was fitted with a simple ('T'-shaped) cannula in the upper jejunum 500 mm beyond the pylorus and distal to the common bile duct. Approximately 500 g of intestinal contents was removed daily between 10.00 and 11.00 a.m. through the cannula and centrifuged for 10 min at 1250 or 1500 g. The supernatant fraction (intestinal fluid) was used immediately or stored at −20°C for *in vitro* digestion experiments.

At the completion of the second incubation the content of the flask was transferred to 120 ml centrifuge-tube and centrifuged immediately for 10 min at 1250 g. The supernatant was resuspended in 50 ml water and recentrifuged for 10 min at 1250 g. The second supernatant fraction was discarded. The insoluble residue in the tube was mixed with a little water and filtered through a weighed filter paper. The paper containing the residue was dried for 5 hrs at 105°C and transferred to a Kjeldahl flask for determination of crude protein (CP). The digestibilities of dry matter (DM) and CP were calculated:

$$1-\frac{R}{S}$$

where R is the weight of the oven-dry sample residue and S is the weight of the sample for each constituent.

For the *in vitro* determinations each diet was ground in a laboratory mill with a 0.5 (poultry diets) or 1 (pig diets) mm screen.