Isolation of Virulent Infectious Bursal Disease Virus from Field Outbreaks with High Mortality in Japan

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Since the first report of Cosgrove in 1962 [3], infectious bursal disease (IBD) has been recognized as an economically important viral disease of young chickens in most poultry-producing areas [8]. The IBD virus (IBDV) causes severe immunosuppression in chickens younger than 3 weeks of age and induces bursal lesions in chickens between 3 weeks and 3 months old [8]. IBDV exhibits different degrees of pathogenicity in chickens. Some virus strains induced only subclinical infection, whereas others lead to typical bursal lesions and high mortality [4, 7]. Since the first outbreak of IBD in 1967 in Japan [13], most of the infections except for the outbreaks in the first few years have been subclinical.

However, recently, outbreaks of IBD have caused greater than usual mortality in flocks in Holland [1], England [2] and Belgium [11], and highly virulent strains of type 1 IBDV were isolated. In 1990, IBD with high mortality occurred suddenly in many flocks in western parts of Japan. It is suspected that the highly virulent strain of IBDV invaded Japan.

The present study was conducted to determine the pathogenicity for specific pathogen-free (SPF) chickens of the field isolates of IBDV from the outbreaks with high mortality during 1990 to 1991 in Japan.

The 6 outbreaks of IBD studied are summarized in Table 1. They occurred in 5 layer flocks and a broiler flock, and 3 of the outbreaks had a mortality of more than 10%. Most outbreaks showed a sharp death curve and rapid recovery. On postmortem examination, typical IBD lesions were seen including turgid bursa of Fabricius (BF), hemorrhage in skeletal muscles and renal lesions with swollen lobules. In all cases, the parent flocks were inoculated with IBD vaccine but their progeny were not.

Homogenates (50%) of the BFs which were taken from the affected chickens were prepared with phosphate-buffered saline (PBS) and after centrifugation (3,000 rpm for 10 min) the supernatants were tested by the agar gel immunodiffusion test at each of the prefectural hygiene service centers or in this laboratory, and IBDV antigens were detected in all the samples. The bursal homogenates filtered through a membrane (220 nm) were inoculated into 10-day-old embryonated eggs by the chorioallantoic membrane (CAM) route. All the embryos inoculated showed lesions or died 3 to 8 days after the inoculation. Liver homogenates of the dead embryos passaged twice in embryonated eggs by the CAM route were inoculated onto LSCC-BK3 cells [6]. After 4 days cultivation, the cells were tested for the presence of IBDV antigens with anti-IBDV chicken IgG conjugated with FITC [12], and IBDV was isolated from all the bursal samples tested. Virus neutralization tests with the embryo liver homogenates of the field isolates and antiserum to the J1 strain (serotype 1) of IBDV showed that all the isolates were serotype 1. These embyro liver homogenates were also inoculated onto primary chicken kidney cells. No cytopathic effects were observed after 7 days of cultivation of blind-passaged samples. Therefore, each bursal homogenate selected randomly in each outbreak was inoculated orally into 5 SPF chickens of line PDL-1 [5] to prepare the inoculum. Four days later, all the chickens were killed and the BF was collected. Ten percent homogenates of the BF were prepared with PBS and after centrifugation (3,000 rpm for 10 min) in supernatants, which contained $10^{3.5}$ to $10^{6.0}$ 50% egg infective dose $(EID)_{50}$/0.1 m*l*, were used as the inoculum. Since all the chickens inoculated with the field isolates except for the Osaka strain showed clinical signs and severe edema of the BFs, the pathogenicity of the 5 isolates of IBDV was studied further. The J1 strain of IBDV [12], which was isolated in 1972 in Japan and

Table 1. Six outbreaks of IBD in Japan from 1990 to 1991

Flocks	Chickens	Age of chickens	Date of outbreak	Mortality in the field (%)
Fukuoka-1	Layer	65[a)]	'90, 9, 24–30	14.3
Okayama-1	Layer	62	'90, 12, 8–15	5.3
Yamaguchi-1	Broiler	35	'90, 12, 15–28	6.5
Osaka-1	Layer	47	'90, 12, 23–27	4.0
Oita-1	Layer	35	'90, 12, 23–'91, 1, 4	19.9
Ehime-1	Layer	70	'90, 3, 3–10	10.2

a) Days.

Table 2. Mortality in the chickens inoculated with field isolates and J1 strain of IBDV

Strains of IBDV	No. of chickens inoculated	Days after inoculation					Mortality (%)
		3	4	5	6	7	
Yamaguchi	10	1[a)]	3	0	3	0	70
Ehime	10	2	3	1	0	0	60
Oita	10	0	2	2	1	0	50
Okayama	10	0	4	0	0	0	40
Fukuoka	10	0	3	0	0	0	30
J1	10	0	0	0	0	0	0
Control	10	0	0	0	0	0	0

a) No. of dead chickens.

Table 3. Effect of IBDV infection on bursa of Fabricius, thymus, spleen and whole body 7 days after inoculation

Strains of IBDV	No. of chickens	Bursa of Fabricius	Thymus	Spleen	Whole body (%)
Yamaguchi	3	2.3[a)]	2.0[a)]	1.9[a)]	98.2[b)]
Ehime	4	1.7	2.5	3.0	99.3
Oita	5	2.3	2.9	2.9	100.5
Okayama	6	2.1	2.5	2.5	107.7
Fukuoka	7	1.7	3.0	2.1	95.3
J1	10	1.7	4.2	2.8	118.4
Control	10	3.4	5.9	1.6	122.9

a) Organ weight (g)×1,000 / body weight (g)
Averages of the rate of each chicken were shown.
b) Body weight (BW) PID 7/BW before inoculation (%)
Averages of the rate of each chicken were shown.

passaged 5 times in SPF chickens, was used as a reference strain of IBDV.

A total of 70 4-week-old SPF chickens of line PDL-1 were weighed and divided into 7 groups to give approximately the same average body weight in each group. Each of 10 chickens reared in the same room were inoculated orally with 0.1 m*l* of a field isolate or the J1 strain of IBDV. Observation was done twice a day at fixed times for 7 days.

Watery or whitish diarrhea was observed within 24 hrs in chickens inoculated with the field isolates of IBDV and continued for 5 days. These affected birds showed depression, ruffled feathers, trembling and severe prostration for 2 to 6 days after inoculation. Some chickens inoculated with the field isolates died 3 to 6 days after inoculation (Table 2), although mortality differed among the isolates from 30% to 70%. Chickens inoculated with strain J1 of IBDV and uninoculated control chickens showed no clinical signs and no mortality. The BFs from the dead chickens were edematous and some of them were hemorrhagic. However, BFs of the dead chickens were not enlarged since the BF/body weight ratio (2.7) of the 18 dead chickens 3 to 4 days after inoculation was smaller than that (3.4) of the control chickens. Renal swelling with prominent lobules was found in all the dead birds. Hemorrhage was also observed in about two-thirds of the dead chickens in the mucosa of the proventriculus and in the leg muscles. Atrophy of BFs and the thymus was observed in all the surviving chickens inoculated with IBDV (Tabel 3). Thymic atrophy was pronounced in the surviving chickens inoculated with field isolates of IBDV. The spleen was slightly enlarged in chickens inoculated with all IBDV strains (Table 3).

The chickens surviving after inoculation with the field isolates either did not gain weight or lost weight, except for those inoculated with the Okayama strain, whereas control chickens and J1 strain-inoculated chickens showed a weight gain of about 20% in 7 days (Table 3).

This study shows that virulent strains of IBDV with high mortality were isolated from the field outbreaks. Such virulent strains had not been isolated during the past decade in Japan. Recently, highly virulent strains of IBDV were isolated in Europe [1, 2, 11]. Although mortality of the Japanese isolates was lower than that of the European isolates, it is suspected that the highly virulent strain might have invaded Japan from Europe, since mortality may be influenced by experimental conditions such as virus titers of inoculum, chicken strains and experimental temperature. It is expected that antigenic or genetic analyses of these strains will clarify the relationship between these outbreaks.

The virulent strains of IBDV isolated in this laboratory induced atrophy of the BF even 3 to 4 days after inoculation and atrophy of the thymus was more severe in

chickens inoculated with the field isolates than in chickens inoculated with J1 strain. Since atrophy of the BF caused by the field isolates was accompanied by an inflammatory response, pathogenicity of these Japanese isolates differed from that of a variant strain in the U.S.A., which produces rapid bursal atrophy associated with minimal inflammatory response [10]. Sharma *et al.* suggested that the thymic pathology induced by the conventional serotype 1 of IBDV might be indicative of the highly virulent nature of the virus, but that the lesion might not be associated with extensive viral replication in thymic cells [9]. Atrophy of the thymus caused by these isolates may be a response to an acute virus infection, as they speculated.

Since a difference in antigenicity between the vaccine and field strains decreases the efficacy of the vaccine [8, 10], the antigenicity of the Japanese isolates of IBDV should be compared with that of commercial live vaccines. Also, high titers of maternal antibodies may prevent the replication of a mild live vaccine virus in the chickens, whereas a virulent virus can infect these chickens [8]. Therefore, development of an effective method to control the virulent IBDV infection may be required.

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