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PATHOGENESIS OF FIELD ISOLATES OF ACUTE INFECTIOUS BURSAL DISEASE WITH HIGH MORTALITY IN INDONESIA

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ABSTRAK

Studi patogenesis virus infectious bursal disease (IBD) dilakukan pada ayam SPF. dengan menggunakan tiga isolat virus infectious bursal disease berasal dari kasus Gumboro lapang yang akut dengan kematian yang tinggi. Deteksi antigen virus Gumboro tertinggi terdapat pada bursa Fabricius dan terjadi pada hari keempat dan kelima sesudah infeksi. Index bursa menunjukan sifat keganasan virus IBD isolat lapang.

ABSTRACT

A pathogenesis study of infectious bursal disease virus (IBDV) was carried out in SPF flocks. Three isolates of IBDV originated from the outbreaks of an acute IBD with high mortality were used in this study. An antigen detection of IBDV was recorded to be high in bursa Fabricius and occurred at the fourth and fifth days post infection. A Bursal index showed the virulence of field isolates of IBDV.

INTRODUCTION

Gumboro disease caused by Infectious bursal disease virus (IBDV) in Indonesia has been reported serologically by Akiba *et al* in 1976. Pathological finding of IBD was reported by Partadiredja *et al.*, 1984 and viral isolation was reported by Darminto *et al., 1985*). However, Gumboro disease seem not important viral disease until the outbreaks of IBD at the end of the 1991 had caused greater mortality in chicken flocks. The outbreaks were reported in Java island, Bali and North Sumatra and spreading to other provinces in Indonesia. Recent similar problem with severe IBD outbreak occurred in other countries of South-East Asia (Teng-Huat Khoo, 1992). The recent IBD outbreaks cause greater economic losses due to high mortality in commercial layer, body weight losses in broiler and secondary bacterial infection. Cases have also reported in intensive village chicken farms in North Sumatra and West Java.

The more serious form of IBD is called virulent IBD to differentiate it from the originally described disease, which is called classic IBD (Lukert and Saif, 1991). The incubation period and the clinical signs are very short. Striking features of the recent outbreaks were a sudden, sharp rise and fall in the number of deaths, with a peak on the third or fourth day, and rapid flock recovery.

The mortality rate of infected flock can be as high as 60% in young chickens. The available vaccination program was not able to control the disease. The present assessment was to study pathogenesis of the field IBDV isolates selected from the outbreaks. Three organs were selected for antigen detection by capture Elisa.

MATERIALS AND METHODS

Viral stock

A reference IBDV, Lukert strain used as standard IBD virus and four of field isolates (Medan, Cileungsi, Tasik and #9) (Parede *et al.*, 1994) were chosen for this study. The sample was repassage several times into chicken embryo fibroblast (CEF) cells and then reinfected in SPF chicken of 3 weeks of age. The 20% suspension was prepared from each organ sample with

PBS-A sterile and then centrifuged at 100 *g* for 10 minutes. The 20% supernatant suspension was used for infected the experimental chicken.

Experimental infection of IBDV in SPF chickens

The chickens were divided into five groups of forty chickens and each group were infected with IBD from different field isolates (Medan, Cileungsi, #9-5-1) and also with isolate #5 and Lukert. Chickens were infected by oculo-orally with 20% bursal suspension and observation was carried out within 18 days. Organ sampling for bursal index, histopathology examination, antigen detection and serology were taken from 4 birds daily in first week after infection and then twice a week in the second and the third week. Bursal index is determined by calculation of bursa to body weight ratio as described previously (Musket *et al.*, 1979).

Antigen detection

A portion of bursa Fabricius, spleen and thymus were processed for antigen detection. The 20% suspension was prepared from each organ sample with PBS-A sterile and then centrifuged at 100 *g* for 10 minutes. The supernatant was used as source of IBD antigen.

Antibody detection

Sera of 10 µL were collected from individual chicken for measuring the IBD antibody titre by capture ELISA (TropBio. Australia, LTD) as described previously (Parede, 1992).

Histopathological changes

A portion of each organ was fixed in 10% NBF and stained with Haematoxylin and Eosin (H&E) for histopathology examination to assess organ damage.

RESULTS

The observation was done daily based on the clinical signs, gross pathological finding, histopathology, antibody titre and antigen detection by Elisa method.

Clinical signs

The first symptoms appeared on the two day postinfection until day four and five. The classic clinical signs for Gumboro infection such as watery diarrhoea, ruffled feathers, pallor of the comb and wattles and sudden death. The effected mortality was not as high as field cases, although the morbidity was 100%. The birds were recovered by the six days postinfection.

Serological test

Antibody titre against Gumboro was raising from the 7 days after infection and going steady until the end of experiment (Fig. 1).

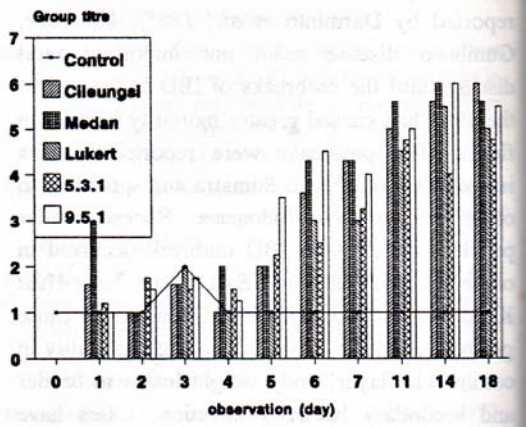

Figure 1. Antibody titre against IBVD in experimental chicken infection

Antigen detection of IBD

The present of IBD antigen in the organ examined was detected from the first day of infection, increased and optimised by the fourth and the fifth day after infection. The antigen was detected nearly from all samples of bursa Fabricius but not from all spleen and thymus (Fig. 2, Fig. 3 and Fig. 4).

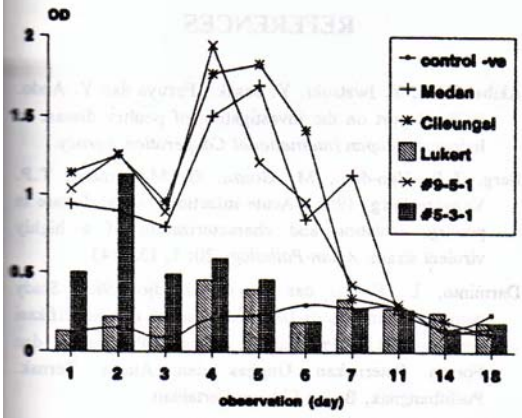

Figure 2. Antigen detection of IBD isolates from bursa Fabricius

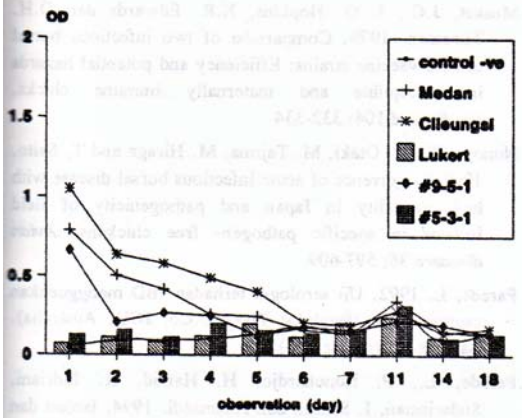

Figure 3. Antigen detection of IBD isolates from spleen

Bursal index

All three field isolate of IBD showed low bursal index (1-2) compare to the Lukert strain and IBDV isolate #5 after the 7th day postinfection. The virulent virus of IBD cause permanent shrinkage compare to the normal bursa due to atrophy and reduced number of lymphoid follicle. The existing lymphoid follicle have scarce amount of lymphoid cells and forming of cystic structure (Fig. 5).

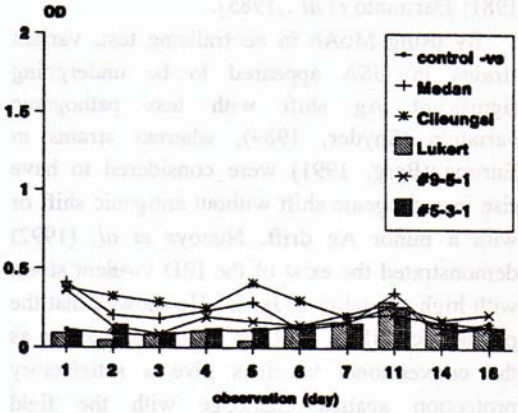

Figure 4. Antigen detection of IBD isolates from thymus

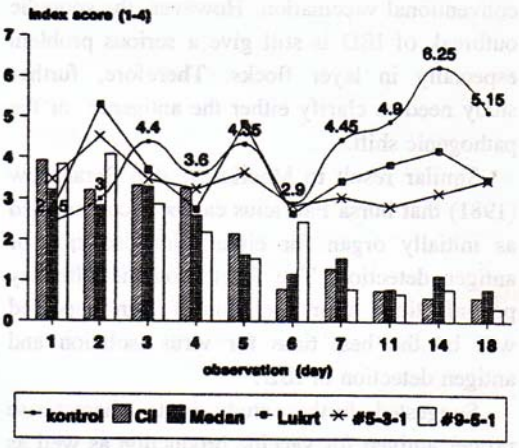

Figure 5. Bursal index of IBVD isolates

DISCUSSION

The origin of our virulent field isolates is unknown, either has already present or viral mutation from the classical IBDV. All isolates reproduced similar IBD with common clinical and pathological findings but in more serious forms. The resulted of bursal index has supported the occurrence of virulence field isolates of IBDV. Our finding indicate that these isolates were more virulent than the originally described Gumboro disease (Partadiredja *et al.*, 1981; Darminto *et al* .,1985).

By using MoAb in neutralising test, variant strains in USA appeared to be undergoing significant Ag shift with less pathogenic variation (Snyder, 1989), whereas strains in Europe (Berg, 1991) were considered to have rise in pathogenic shift without antigenic shift or with a minor Ag drift. Nunoya *et al.* (1992) demonstrated the exist of the IBD virulent strain with high mortality in Japan. He showed that the pathotypic shift of IBD has occurred in Japan as the conventional vaccines give a satisfactory protection against challenge with the field isolates.

In Indonesia situation, the outbreak of IBD in 1991 was reported can be controlled by conventional vaccination. However, the sporadic outbreak of IBD is still give a serious problem especially in layer flocks. Therefore, further study need to clarify either the antigenic or the pathogenic shift.

Similar result to MacKenzie dan Spradbrow (1981) that bursa Fabricius can be recommended as initially organ for either viral isolation or antigen detection. The fourth and the fifth day postinfection, after the clinical sign appeared will be the best time for virus isolation and antigen detection of IBD.

Suggested further study is to characterise proper antigen for vaccine production as well as to design the vaccination program of IBD for Indonesia.

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