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PATHOGENICITY AND IMMUNOHISTOCHEMICAL DETECTION OF INFECTIOUS BURSAL DISEASE VIRUS IN SPECIFIC PATHOGEN-FREE CHICKENS

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SUMMARY

Only two out of eight infectious bursal disease (IBD) virus isolated from the field were capable of causing 100% mortality of 4 week-old specific pathogen free chickens within 5 days post-inoculation (pi). Typical gross and histological IBD lesions were seen in all lymphoid organs of chickens that died within 5 days pi. Chickens that were culled at 6 and 10 days pi showed only atrophy in the bursa of Fabricius (BF). Immunoperoxidase staining revealed positive reactions in the tissues of the BF, spleen, thymus and caecal tonsil of chickens that died within 5 days pi and in the BF of chickens culled within 10 days pi.

Keywords: Infectious bursal disease, pathogenicity, specific pathogen free chickens, immunoperoxidase technique

INTRODUCTION

Acute infectious bursal disease (IBD) outbreaks with high mortality were first reported in Malaysia in mid 1991 (Loganathan et *al.*, 1992; Hair Bejo, 1993). With concurrent infections involving IBD in both vaccinated and non-vaccinated chickens, detection of gross and histological lesions on the lymphoid organs alone could not confirm the disease. The fluorescence antibody (FA) technique is useful for *in situ* detection of IBD viral antigens but the usage is limited on frozen sections.

Recently, the immunoperoxidase (IP) staining has emerged as a valuable tool for localisation of antigens in both routine histopathology and research materials (Jonsson and Engstrom, 1986; Nunoya *et al.*, 1992; Mahani and Narita, 1993). This technique is sensitive and specific in detection of various virus antigens in the formalin fixed, paraffin embedded tissues from both experimental and field samples. This paper describes the pathogenicity of eight field isolates of IBD virus and *in situ* detection of the viral antigens using the IP technique.

MATERIALS AND METHODS

Animals

A total of 162 four-week-old specific pathogen free (SPF) chickens were divided into 9 groups consisting of 18 chicks per group. Eight groups were infected with different IBD virus isolates while one group was the non-infected control. Each group was kept in separate negative pressure isolators.

Virus isolates

Eight viral isolates from acute IBD outbreaks with 5-20% field mortality were chosen for inoculum. The viruses were detected and confirmed by the antigen-capture ELISA kit (Trop-Bio, USA) and by the direct FAT (Cheah *et al.*, 1992).

Experimental design

The bursa of Fabricius homogenate from each isolate was diluted at 1:20 with tryptose phosphate buffer, filtered through a 0.22 filter and inoculated into the chickens. The chickens were sacrificed at 5 days pi and the BF were harvested. The BF of each isolate were pooled, homogenised and stored at -70°C for use as inoculum. For the pathogenicity test, chickens were inoculated orally with 0.1 mL of IBD virus in the form of BF suspension diluted 1/20. Chickens were observed daily for clinical signs for up to 10 days pi and culling was carried on surviving chickens at 6 and 10 days pi. At autopsy, the lymphoid and major visceral organs were collected and fixed in 10% buffered formalin.

Tissue processing

Tissues were processed, sectioned at 5μm and stained with haematoxylin and eosin (H&E). The IP staining was conducted on paraffin-embedded sections of the BF, spleen, thymus and caecal tonsils of chickens using the method described previously by Nunoya *et al.* (1992) with slight modifications. The anti-IBDV rabbit serum was used at a dilution of 1:1500 while the biotilylated lgG and the avidin-biotin-complex reagents were purchased commercially. Tissue sections from non-inoculated chickens and serum from a non-immunised rabbit were used as controls.

RESULTS

Pathogenicity study

Table 1 shows the summary of the clinical signs, mortality, gross and histological lesions in the SPF chickens experimentally infected with IBD virus. Six of the isolates caused mortality as early as the third day and continued until the fifth day pi. Depression, ruffling of feathers and whitish or watery diarrhoea were noticed at 1 and 2 days pi. There was no apparent clinical signs or mortality seen in chickens infected with other isolates.

At necropsy, the carcasses of chickens that died within 5 days pi were dehydrated with severe haemorrhages in the thigh muscles. The BF were swollen and surrounded by gelatinous serosal surface while some cases were accompanied by mucosal haemorrhages. The spleens were mottled and friable, the livers were congested while the kidneys were enlarged. In this case, the thymuses were opaque and enclosed by gelatinous substance. No significant lesions were observed in the other organs of these chickens. Chickens that were sacrificed at days 6 and 10 pi only showed yellowish and atrophied BF respectively. No lesion was observed in the other organs.

Histologically, the BF of chickens that died within 5 days pi showed generalised necrosis of the lymphocytes in the cortical and medullar areas. There were also infiltration of heterophils, oedema and haemorrhages of the inter-follicular regions. In culled chicken, the BF follicles consisted of reticular cells, macrophages and cellular debris at day 6 pi while all follicles were found to be severely atrophied and contained cystic structures at day 10. The spleen and thymus of chickens that died within 5 days pi showed focal areas of necrosis. In the spleen, lymphocyte necrosis was more prominent in the white pulp while thymus had lesions in the cortex. Lymphocyte necrosis was also observed in the lymphoid nodule of the caecal tonsil. Reticular hyperplasia in the spleen and lymphocyte depiction of the thymus and caecal tonsil could be observed in the culled chickens infected by the more virulent IBD virus isolates for up to 6 days pi. At 10 days pi, however, all spleen, thymus and caecal tonsils of the infected chickens appeared normal. No histological lesions were observed in the organs of control chickens.

Immunoperoxidase staining

Table 2 summarises the IP staining of the BF, spleen, thymus and caecal tonsil of chickens that died within 5 days pi and those that were sacrificed at days 6 and 10 pi. Positive reactions, indicated by brownish granules, were observed in the necrotic areas of the medulla and cortex of the BF and in the macrophages that infiltrated the inter-follicular areas. Positive reactions were also observed in the follicles, inter-follicular areas and cystic structures of the BF until day 10 pi. In the lamina propria of the caecal tonsil and secondary lymphoid nodules of the spleen, fine brownish granular aggregates were observed for up to day 6 pi. A few foci of positive granules were also present in the necrotic areas of the thymic medulla of the dead chickens.

Table 1. Pathogenicity and histopathological observations of SPF chickens inoculated with field isolates of IBD virus

Isolate	Clinical signs	No. (%) of chicks died[a]	Presence of gross and histological lesions		
			dead chicks[a]	culled at day 6	culled at day 10
1495/93	-	0/18 (0%)	-	BF	BF
391/93	-	0/18 (0%)	-	BF	BF
3426/93	-	0/18 (0%)	-	BF	BF
3647/92	++	5/18 (27%)	BF,sp,th,ct[b]	BF	BF
921/92	++	6/18 (33%)	BF,sp,th,ct	BF	BF
6511/91	++	8/18 (44%)	BF,sp,th,ct	BF	BF
4709/91	++	18/18 (100%)	BF,sp,th,ct	-	-
3529/91	++	18/18 (100%)	BF,sp,th,ct	-	-

[a]chickens died within 3-5 days p.i

[b]bursa of Fabricius, spleen, thymus, caecal tonsil

Table 2. Summary of the immunoperoxidase staining on formalin-fixed tissue of SPF chickens infected with IBD virus

Isolate	Lesion scoring following IP staining in tissues											
	Dead chicks[a]				Culled at day 6 pi				Culled at day 10 pi			
	BF	sp	th	ct[b]	BF	sp	th	ct	BF	sp	th	ct
1495/93	no sample				3+	-	-	1+	2+	-	-	-
391/93	no sample				3+	-	-	1+	1+	-	-	-
3426/93	no sample				2+	-	-	1+	1+	-	-	-
3647/92	4+	1+	1+	1+	3+	1+	-	1+	2+	-	-	-
921/92	4+	1+	1+	1+	3+	1+	-	1+	2+	-	-	-
6511/91	4+	1+	1+	1+	2+	1+	-	1+	1+	-	-	-
4709/91	4+	1+	1+	1+	no sample				no sample			
3529/91	4+	1+	1+	1+	no sample				no sample			

[a]died within 3-5 days pi
[b]bursa of Fabricius, spleen, thymus, caecal tonsil
1+; less than ten positively-stained cells per high-power field
2+; more than ten positively-stained cells per high-power field
3+; more than twenty positively-stained cells per high-power field
4+; numerous positively-stained cells per high-power field

DISCUSSION

Only two of the IBD virus described in the present study could be considered virulent based on the pathogenicity test. Kaufer and Weiss (1980) regarded that 100% mortality in four-week-old SPF chickens were caused by highly virulent IBD virus. Similar cases of IBD caused by highly virulent virus strains have been reported in Japan (Nunoya *et al.*, 1992) and Europe (Stuart, 1989; Van den Berg *et al.*, 1991).

The IBD viral antigens were successfully demonstrated using the IP technique on formalin-fixed, paraffin embedded BF, spleen, thymus and caecal tonsil of chickens infected with IBD virus. The pattern and distribution of positive reactions on these tissues were consistent with those of the histological findings using H&E staining. The strong positive reactions detected in the BF by the IP staining were due to a high concentration of IBD virus at early infection (Winterfield *et al.*, 1972; Kaufer and Weiss, 1980). The sensitivity and specificity of our immunoperoxidase technique using polyclonal antibody were comparable to the method that employed monoclonal antibody (Cruz-Coy *et al.*, 1993) and chicken anti-sera against IBD (Jonsson and Engstorm, 1986). Our results showed that IBD viral antigens were detected from spleen, caecal tonsil and BF at days 6 and 10 pi respectively. Winterfield *et al.* (1972) concluded that IBD virus could not be isolated from tissues except the BF beyond day 10 pi. The IP technique was more sensitive in detecting IBD viral antigen in the thymus compared to the fluorescent antibody technique (Nunoya *et al.*, 1992).

The IP technique, however, is time consuming with the possibility of antigenic site degradation by formalin fixation and tissue processing. However, this technique is very sensitive and recommended in the diagnosis of IBD virus infection.

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RINGKASAN

KEPATOGENAN DAN PENGESANAN IMUNOHISTOKIMIA VIRUS PENYAKIT BURSA BERJANGKIT DALAM AYAM BEBAS PATOGEN KHUSUS

Hanya dua daripada lapan virus penyakit bursa berjangkit (IBD) dipencil daripada luar berupaya menyebabkan 100% kematian ayam bebas patogen khusus berumur 4 minggu dalam tempoh 5 hari pasca-penginokulatan (pi). Lesi kasar dan histologi tipikal dilihat dalam kesemua organ limfoid ayam yang mati dalam tempoh 5 hari pi. Ayam yang ditakai pada 6 dan 10 hari pi menunjukkan atrofi sahaja pada bursa Fabricius (BF). Pewarnaan imunoperoksidase menunjukkan tindak balas positif pada tisu BF, limpa, timus dan tonsil sekum ayam yang mati dalam tempoh 5 hari pi dan pada BF dalam ayam yang ditakai dalam tempoh 10 hari pi.