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THE PATHOGENICITY OF VELOGENIC VISCEROTROPIC NEWCASTLE DISEASE VIRUS (VVNDV) IN THE BURSA OF FABRICIUS OF BROILERS

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SUMMARY

The effects of a velogenic viscerotropic Newcastle Disease Virus (VVNDV) on the bursa of Fabricius of Newcastle disease vaccinated chickens were determined by infecting the vaccinated chickens via contact with non-vaccinated chickens challenged intranasally with 0.1 mL of an inoculum containing 10^6 EID_{50} of the virus. All non-vaccinated challenged birds developed clinical signs of Newcastle Disease (ND) and died within 3 to 9 days. However, 90% of the vaccinated challenged birds remained clinically normal. The bursa of Fabricius was examined grossly and histopathologically, while virus was isolated in embryonated chicken eggs and detected by immunoperoxidase staining and transmission electron microscopy examinations. The results showed that VVNDV damaged and replicated in the bursa of Fabricius of vaccinated birds. Grossly, the bursa was swollen, oedematous, haemorrhagic and necrotic. Histopathology sections showed that there were haemorrhages, oedema and cystic cavities in the follicles containing mucus, necrosis of the follicles and presence of a reduced number of lymphocytes but an increased number of heterophils, macrophages and plasma cells. Immunoperoxidase and electron microscopy demonstrated the presence of NDV antigens in both vaccinated and non-vaccinated challenged birds. Viral isolation and electron microscopy showed evidence of viral replication in the bursa of vaccinated and non-vaccinated birds.

Keywords: Velogenic viscerotropic Newcastle disease virus, bursa of Fabricius, pathogenicity, vaccinated birds

INTRODUCTION

Newcastle disease (ND) is one of the important viral diseases of poultry in the world. It occurs in most countries and is the cause of heavy economic losses to poultry farmers particularly in the developing countries. Although the disease can be controlled through vaccination, outbreaks have been reported in vaccinated flocks. The success of any vaccination programme depends on several factors, which include the integrity of immune system (Glick *et al.*, 1956). Thus, the bursa of Fabricius, which is responsible for humoral immunity against ND is an important organ.

Although the NDV vaccinated chickens are protected against clinical manifestation when exposed to virulent strain of NDV, they are not 100% protected against infection by NDV (Cheville and Beard, 1972; Lai, 1985; Parede and Young, 1990). However, humoral antibody is important in protecting chickens against infection with NDV (Levy *et al.*, 1975; Allan *et al.*, 1978; Giambrone, 1979). As the bursa of Fabricius is responsible for humoral antibody production, any damage on the organ will predispose chickens to infections.

The pathogenicity of VVNDV in the trachea has been reported (Lai, 1985), while the pathogenicity of GB strain of NDV in the bursa of NDV-vaccinated chickens has been reported by Cheville and Beard (1972). However, there was no report on the pathogenicity of VVNDV in the bursa. Therefore, this study was carried out to determine the pathogenicity of VVNDV in the bursa of NDV-vaccinated chickens.

MATERIALS AND METHODS

Animals

Two hundred, day-old Lohman broiler chicks were divided into two equal groups and were kept in separate isolation units. Birds of group A were vaccinated intranasally with 0.1 mL vaccine containing 10^6 EID_{50} of live lentogenic F NDV per chicken at day-old, three and six weeks old. Birds of group B were the non-vaccinated control. Serum samples were collected weekly until challenge at 8 weeks of age. At this time, 50 birds from each group were used for challenge while the remaining 50 were kept as non-challenged control.

Serology

Thirty birds were randomly selected from each group and were bled weekly. The antibody titre against NDV was determined by haemagglutination inhibition test according to the method of Allan and Gough (1974). Serum samples collected on the eighth week were used for the agar gel precipitation test (AGPT) to screen for infectious bursal disease (IBD) according to the method of Milford-Ward (1977).

Virus

The NDV for challenge, designated as AF2240, was prepared as an inoculum at the concentration of 10^6 EID_{50} per 0.1 mL. The inoculum was administered intranasally at the dose rate of 0.1 mL per bird into 8 non-vaccinated birds before the challenged birds were used as a source of virus to challenge the rest of the birds through in-contact method.

Challenge procedure

The eight-week-old birds that were to be challenged were tagged, bled and kept in isolation sheds. One hundred birds, 50 from each of groups A and B were challenged (Table 1) while the remaining 100 birds, 50 from each of groups A and B were the non-challenged control birds. Birds in shed 1 were used for sample collection and birds in shed 2 were used for clinical observations.

Clinical and pathological examinations

Three birds per group from the vaccinated challenged and non-vaccinated challenged from shed 1 were collected at 6 hours post-challenge and on the first, second, third, fourth, eighth and fourteenth days post-challenge. Three birds per group from vaccinated and non-vaccinated groups were also collected at the same time intervals to represent the control non-challenged group.

The birds were slaughtered, bled and the bursa of Fabricius was collected. The organ was excised at the neck of the bursa before it was weighed and the size was determined using a pair of calipers while gross lesions were scored according to Chulan (1986). Part of the bursa was used for virus isolation while the remaining was divided further. One portion was fixed in 10% formalin for histopathology and immunoperoxidase staining (Chulan, 1986) while the other portion was fixed in 4% gluteraldehyde for electron microscopy. Birds from challenge shed 2 were observed for two weeks. Post-mortem was carried out on dead birds and ND lesions were recorded.

Virus isolation

The bursa was grounded with fine sand in a mortar before 10% bursal homogenate was prepared by adding Hank's balanced salt solution containing penicillin/streptomycin. The homogenate was centrifuged at 3,000rpm for 10 minutes in a refrigerated centrifuge (MSE Mistral 4L). The supernatant was filtered through 0.45μm millipore filters and was used as the inoculum. Approximately 0.1 mL of the inoculum was inoculated into the allantoic fluid of three 9-day-old embryonated chicken eggs and incubated at 37°C. Embryos that were found dead within 24 hours of incubation were discarded. After the first 24 hours, eggs with dead embryos were collected and chilled before the allantoic fluid was harvested. The presence of NDV was confirmed by demonstrating the presence of haemagglutinin in the allantoic fluid. Surviving eggs were harvested after 5 days of incubation and the allantoic fluid was tested for the presence of NDV.

RESULTS

Serology

During the first three weeks, the titre of the vaccinated birds was low. At week 4, the geometric mean titre (GMT) was 0, after which it increased markedly to reach 32 GMT by 8 weeks of age (Table 2).

The antibody titres of the non-vaccinated birds were low during the first three weeks, after which no antibody was detected. All birds were negative for IBD.

Table 1. Vaccination and challenge programme

Group	No. of birds	Age vacc. (weeks)	Route of vaccination	Age (weeks) challenge	Route of challenge
Shed 1					
A (vaccinated)	21	1	intra-nasal	8	in-contact
		3	intra-nasal	8	
		6	intra-nasal	8	
B (non-vaccinated)	21		not vaccinated	8	in-contact
	8		not vaccinated	8	intra-nasal
Shed 2					
A (vaccinated)	21	1	intra-nasal	8	in-contact
		3	intra-nasal		
		6	intra-nasal		
B (non-vaccinated)	21		not vaccinated	8	in-contact
	8		not vaccinated	8	intra-nasal

Clinical signs

All non-vaccinated challenged birds developed clinical signs of Newcastle disease from day 3 post-challenge. They showed difficulty in breathing, prostration, clonic spasms, paralysis, torticollis and watery-greenish diarrhoea. All birds died within 3 to 9 days post-challenge.

Ninety percent of the vaccinated challenged birds remained clinically normal. Two birds showed difficulty in breathing, watery-greenish diarrhoea and subsequently died on day 9 and 10 post-challenge.

Gross lesions

There was congestion of the trachea and lungs, haemorrhages and necrosis of the proventriculus, intestine, caecum, caecal tonsil and bursa. The lesions were more severe in the non-vaccinated birds.

Gross lesions in the bursa include swelling, oedema, haemorrhage and necrosis. Chickens with gross lesions in the bursa, with or without antibody titre, were positive on viral isolation (Table 3). However, birds with low lesion score were negative.

Table 2. Antibody titre prior to challenge and survivability following challenge with NDV

Group	Haemagglutination inhibition titre* Age (weeks)								Survivability	
	1	2	3	4	5	6	7	8	No. Survived	% Survived
Vaccinated	0.2	0.5	0.1	0.0	1.7	4.6	4.6	4.9	19/21	90%
Non-vaccinated Challenged	0.2	0.5	0.1	0.0	0.0	0.0	0.0	0.0	0/21	0%

*Haemagglutination inhibition (HI) geometric mean titre (GMT) of 30 birds (log_2)

Table 3. Gross lesion score and virus isolation

Days post-challenge	No. of birds	Vaccinated & challenged			Non-vaccinated & challenged		
		HI titre	Lesion score	Virus isolation	HI titre	Lesion score	Virus isolation
0	1	4	1+	-	0	1+	-
(6 hours)	2	4	0	-	0	1+	-
	3	2	1+	-	0	1+	-
1	1	4	1+	+	0	1+	+
	2	4	1+	-	0	2+	+
	3	4	2+	+	0	2+	+
2	1	1	1+	+	0	2+	+
	2	4	1+	+	0	2+	+
	3	2	1+	+	0	1+	+
3	1	1	1+	+	0	1+	+
	2	5	2+	+	0	2+	+
	3	4	3+	+	0	4+	+
4	1	2	4+	+	0	4+	+
	2	5	2+	+	0	4+	+
	3	4	1+	+	0	1+	+
8	1	4	3+	+	0	4+	+
	2	5	2+	+	0	4+	+
	3	6	3+	+	0	4+	+
14	1	5	1+	+	-	-	-
	2	5	2+	+	-	-	-
	3	5	1+	+	-	-	-

0, no lesion; 1+, mild haemorrhage; 2+, mild swelling, oedema and mild haemorrhage; 3+, mild swelling, oedema and severe haemorrhage; 4+, severe necrosis, severe haemorrhage, oedema and swelling

The mean bursal weights at various intervals post-challenge are shown in Table 4. There was no significant ($p>0.05$) difference in the bursal weights between the vaccinated challenge and non-vaccinated challenge throughout the challenge period. Similarly, there were no significant ($p>0.05$) differences in the bursal weight between the vaccinated and non-vaccinated control non-challenged birds as well as between the non-vaccinated challenged and non-vaccinated non-challenged control birds.

The mean bursal size at various intervals post-challenge is shown in Table 5. There was no significant ($p>0.05$) difference in the mean bursal size between the non-vaccinated challenged and vaccinated challenged group. Similarly, there were no significant ($p>0.05$) differences between the bursal size of vaccinated challenged and vaccinated non-challenged control as well as between the non-vaccinated challenged and non-vaccinated non-challenged control birds.

Histopathology

Histopathological findings of the bursa comprised of haemorrhages, oedema and cystic cavities in the follicles containing mucous, necrosis of the follicles and the presence of a reduced number of lymphocytes but an increased number of heterophils, plasma cells and macrophages. There was no obvious follicular and plical atrophy. Lesions in the vaccinated challenged group progressed with time to become most severe on day 8 before started to regress on day 14. In the non-vaccinated challenged group, the severity of the lesions increased with time and was most severe on day 8 post-challenge before death on day 9.

All infected tissues of vaccinated and non-vaccinated birds showed positive immunoperoxidase staining, while all non-infected tissues from the vaccinated and non-vaccinated birds were negative. The positive reactions were located mainly in the cortex, cortico-medullary area and epithelial cell but less frequently in the medulla (Fig. 1). Following the electron microscopic examinations, the ND virus was found to replicate in the cytoplasm of both lymphoid cells and macrophages (Fig. 2).

Table 4. Mean weights of bursa of Fabricius at various intervals post-challenge with NDV

Days post-challenge	Mean weight of the bursa (g)			
	Vaccinated		Non-vaccinated	
	Control	Challenged	Control	Challenged
0	0.88±0.41	0.88±0.41	1.38±0.15	1.38±0.15
1	0.88±0.41	0.77±0.12	1.38±0.15	0.88±0.12
2	1.03±0.02	0.70±0.18	0.80±0.15	0.90±0.06
3	0.62±0.04	0.62±0.10	0.75±0.13	0.75±0.19
4	0.80±0.06	0.72±0.10	0.90±0.12	0.70±0.14
8	1.30±0.40	0.53±0.15	0.70±0.06	0.60±0.00
14	0.88±0.32	1.35±0.05	0.58±0.07	No data

Table 5. Mean size of bursa of Fabricius at various intervals post-challenge with NDV

Days post-challenge	Mean weight of the bursa (g)			
	Vaccinated		Non-vaccinated	
	Control	Challenged	Control	Challenged
0	1.92±0.11	1.92±0.11	2.12±0.25	2.12±0.25
1	1.92±0.11	1.65±0.08	2.12±0.25	1.64±0.29
2	1.70±0.02	1.39±0.02	1.64±0.09	1.14±0.26
3	1.47±0.21	1.14±0.10	1.65±0.17	1.49±0.19
4	0.98±0.38	1.24±0.12	1.34±0.19	1.16±0.18
8	1.29±0.21	0.98±0.18	1.37±0.09	1.34±0.16
14	1.11±0.26	1.62±0.22	0.89±0.05	No data

DISCUSSION

Maternal antibody was present in the non-vaccinated birds at day-old, slowly decreased with time and was not detectable at 4-weeks-old. The antibody titre of the vaccinated birds increased from GMT O to 32 following the second vaccination as expected in ND vaccination programme.

None of the non-vaccinated birds were protected from the NDV challenge whereas only two birds were not protected in the vaccinated group. This is in agreement with Chu and Rizk (1975), who reported that a high level of vaccine induced antibody was important in preventing mortality. The 90% protection level in the vaccinated birds might be due to the presence of residual maternal immunity, which interfered with vaccination.

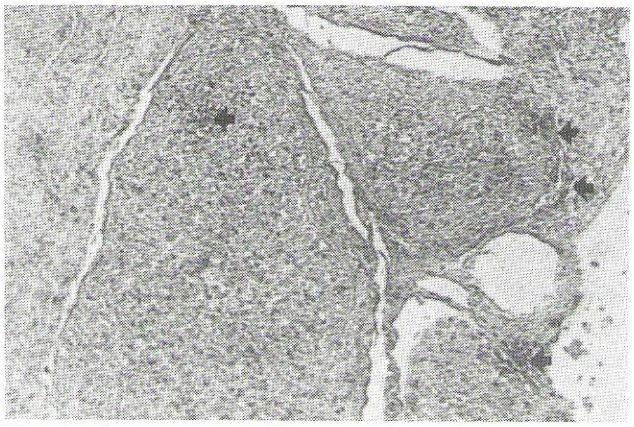

Fig. 1. Immunnoperoxidase staining of the bursa of vaccinated challenge bird 3 days post-challenge. The blue-stained cells (arrows) indicates the presence of VNDV virions. x300.

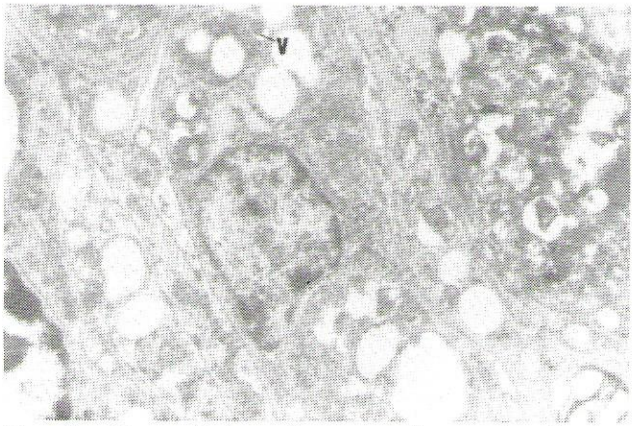

Fig. 2. Transmission electron micrograph showing budding of virus at vacuolar membrane (V) within the cytoplasm of a macrophage of a non-vaccinated bird at day 4 post-challenge. x11,550

The gross lesions observed were consistent for VVNDV infection. In the bursa, the lesions observed were swelling, oedema, haemorrhages and necrosis. There was no significant difference ($p > 0.05$) in the bursal weight and size of both vaccinated challenged and non-vaccinated challenged birds. Different types of changes were expected in infection with IBDV since IBDV causes severe degeneration and necrosis leading to shrinkage and decrease in weight of the bursa (Kaufer and Weiss, 1980; Hafiza, 1993).

NDV was isolated from all infected bursa of the vaccinated and non-vaccinated challenged birds throughout the challenge period except at the first 6 hours post-challenge. This agrees with findings by Parede and Young (1990), who concluded that the gross lesion observations correlated well with the results of viral isolation. The VVNDV was seen replicating in the cytoplasm of both lymphoid cells and macrophages in the bursa of Fabricius of the vaccinated as well as the non-vaccinated bird. Similar viral replications were observed in these cells following infection by IBDV (Hafiza, 1993; Hair-Bejo, 1993).

Histopathology of the bursa showed presence of haemorrhages, oedema, cystic cavities in the follicles containing mucous, necrosis of the follicles, and the presence of reduced number of lymphocytes but an increased number of heterophils, macrophages and plasma cells. These histopathological findings are in agreement with that of Chevile and Beard (1972). Since the VVNDV was found to infect the bursa of both vaccinated and non-vaccinated birds, further study should be carried out to determine whether the infection leads to immunosuppression.

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RINGKASAN

KEPATOGENAN VIRUS PENYAKIT NEWCASTLE VISEROTROPIK VELOGENIC (VVNDV) DALAM BURSA FIBRICIUS AYAM PEDAGING

Kesan virus penyakit Newcastle viserotropik velogenik (VVNDV) terhadap bursa Fabricius ayam tervaksin untuk penyakit Newcastle telah ditentukan dengan menjangkitkan ayam tervaksin tersebut secara sentuhan dengan ayam bukan tervaksin yang tercabar secara intranasum dengan 0.1mL inokulum mengandungi 10^6EID_{50} virus. Kesemua ayam bukan tervaksin tercabar menunjukkan petanda klinikal penyakit Newcastle (ND) dan mati dalam tempoh 3 hingga 9 hari. Bagaimanapun, 90% daripada ayam tercabar tervaksin kekal normal klinikal. Bursa Fibricius diperiksa secara kasar dan secara histologi, sambil virus dipencilkan dalam telur ayam berembrio dan dikesan melalui pewarnaan imunoperoksidase dan pemeriksaan mikroskopi elektron pancaran. Hasilnya menunjukkan VVNDV merosakkan dan mereplikat dalam bursa Fabricius ayam tervaksin. Secara kasar bursa nampak bengkak, beredema, berhemoraj dan bernekrosis. Irisan histopatologi menunjukkan bahawa ada hemoraj, edema dan rongga sista dalam folikel yang mengandungi mukus, nekrosis folikel dan bilangan limfosit dalamnya kurang, sambil bilangan heterofil, makrofaj dan sel plasma pula meningkat. Imunoperoksidase dan mikroskopi elektron menunjukkan wujudnya antigen NDV dalam kedua-duanya, ayam tervaksin dan bukan tervaksin. Pemencilan virus dan mikroskopi elektron juga menunjukkan bukti berlakunya pereplikatan virus dalam bursa ayam tervaksin dan bukan tervaksin.