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Comparative Pathology of Chickens Experimentally Inoculated with Virulent Viscerotropic Newcastle Disease Viruses isolated in Korea

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강병원성 뉴캣슬병 바이러스 한국분리주의 SPF 닭 접종에 따른 병리학적 변화 비교

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ABSTRACT: Pathologic changes and distribution of viral antigen as determined by immunohistochemistry were compared among 4-wk-old specific-pathogen free (SPF) chickens inoculated intratracheally with velogenic viscerotropic Newcastle disease virus isolated in Korea. Although the pattern of organ involvement and severity of lesion was different among chickens infected with different velogenic viscerotropic Newcastle disease (VVND) viruses, the pathological types of lesion was similar among the chickens. Severe lymphocytic necrosis and depletion were main histologic lesions in the immune related organs such as thymus, Fabricius bursa and spleen. The frequency of IP positive staining was variable depends on the types of tissues but not types of the kinds of VVND viruses infected. Brain, Fabricius bursa, thymus, cecal tonsil and trachea were IP positive with fairly high frequency and spleen, lung, proventriculus, intestine, pancreas, liver, kidney, heart and Harderian gland were with relatively low frequency. These results suggest that histologic evaluation and viral antigen specific immunohistochemical staining methods to determine virus distribution will be useful for pathogenic study of velogenic viscerotropic Newcastle disease virus infection in chicken.

(Key words: newcastle disease virus, velogenic, pathology)

INTRODUCTION

Newcastle disease (ND) is a highly contagious disease of chickens caused by a Newcastle disease virus (NDV), a member of the genus Rubulavirus, family Paramyxoviridae. Highly virulent ND is a Office of International Epizootics (OIE) list A disease and requires reporting to the OIE (Alexander, 1997).

ND can be classified as velogenic, mesogenic, and lentogenic (Alexander, 1997; Hanson et al, 1973) pathotype based on clinical signs and pathological changes. Velogenic isolates are further divided into a velogenic viscerotropic pathotype and a velogenic neurotropic pathotype. Velogenic viscerotropic Newcastle disease (VVND) virus causes visceral hemorrhage and intestinal necrosis.

Since the first outbreaks of Newcastle Disease in Korea, which was reported in 1929, the disease has been continuously found at some restricted areas throughout the country (Konno et al, 1929). Although most of the poultry farms used live and killed oil-emulsion vaccine commercially available, sometimes, the respiratory distresses with high mortality and the egg drop problems caused by NDV infection were observed in young broiler flocks and laying flocks.

Pathogenesis study of VVND has been performed to

reduce the incidence of field ND outbreak and to find interaction between VVND virus and host using immunohistochemistry technique, which may find relation of viral antigen distribution to lesions (Brown et al., 1999a; Brown et al., 1999b; Lockaby et al., 1993; Ojok and Brown, 1996).

The purpose of this study is to evaluate the relation between histopathology and immunohistochemistry and to compare the pathologic profile using the highly pathogenic Newcastle disease viruses isolated in Korea.

MATERIALS AND METHODS

1. Viruses

For this study, six different VVND viruses were used such as KJW, 97147, 90163 and 8248, which were obtained from National Veterinary Research and Quarantine Service (NVRQS, Anyang, Korea), 9358GS and 95132 isolates, which were obtained from Seoul National University (Suwon, Korea). The KJW strain is isolated from chickens in 1950 and official standard challenge virus for evaluation of ND vaccines in Korea. The history of these 6 VVND viruses used in this study is summarized in Table 1.

2. Chickens

Four-week-old SPF chickens were housed in negative pressure isolators in a high containment facility at the NVRQS. Feed and water were provided *ad libitum*.

3. Experimental Design

Fifteen chickens were intratracheally inoculated with each VVND virus and maintained in separate isolation units within the same room. The experimental groups and inoculums are shown in Table 1. Fifteen uninoculated control chickens were housed in isolation unit of different room. Blood samples were obtained from 5 uninoculated chickens at 0 DPI (preinoculation) and tested for the presence of Newcastle disease virus specific antibody by hemagglutination inhibition (HI) test

4. Pathology

Tissue samples were collected daily from any chickens that died during the experiment. Tissue samples were also collected each of uninoculated chickens on 10 DPI. The following tissues were collected and fixed for at least 2 weeks in buffered formalin: brain, heart, pancreas, intestine, trachea, lung, Harderian gland, Fabricius bursa, thymus, proventriculus, liver, kidney, spleen and spinal cord. Tissue samples for light microscopic examination were stained with hematoxylin and eosin (H&E).

5. Lesion Scoring

A previously described numeric scoring system was used to record data on histological observations (Mo et al., 1997). Histologic lesions of all tissues collected from each chicken were scored numerically as follows: 1 = absence, 2 = focal, 3 = multifocal and 4 = diffuse

Table 1. Summary of Korean ND isolates used in this study

Strains	Year isolated	Animal isolated	History of passage
KJW	1950	Chicken	CEF[a]1, CE[b]10
9358GS	1993	Chicken	CEL[c]4, CEF3[d], CE5
97147	1997	Chicken	CE2
95132	1995	Chicken	CEL1, CE3
90163	1990	Chicken	CE3
8248	1982	Chicken	CEF1, CE5

[a] CEF: chicken embryo fibroblast.

[b] CE: chicken embryo.

[c] CEL: chicken embryo liver.

[d] Number of passages.

lesion. The mean histologic lesion score was calculated by averaging individual scores for all sampling days within each group.

6. Immunoperoxidase (IP) Staining

Immunoperoxidase (IP) staining of formalin fixed tissues was performed using a commercially available avidin-biotin-peroxidase complex (ABC) staining kit (Vector, Laboratory, Inc. Burlingame CA). Monoclonal chicken antibody, Q24, against Newcastle disease virus was obtained from Australia (provided by Dr. Hooper, CSIRO, Queensland, Australia) and used as the primary antibody. Each time unknown tissue sections were stained with monoclonal antibody, tissue samples from positive and negative controls were used and processed identically to the unknown tissue samples. To determine whether a given tissue was IP positive, a minimum of one section of the tissue was immunostained and the entire section was examined for IP staining.

RESULTS

1. Clinical Responses

The clinical signs were similar in the chickens inoculated with VVND viruses and were characteristics of highly pathogenic Newcastle disease. The first clinical signs were observed after 3 DPI in most chickens with gasping and ruffled feather and were progress to severe and ending with prostration, moribund and death.

The first mortality was observed at 4 DPI in chickens inoculated with 9358GS virus and majority of other chickens inoculated with different viruses were died between 5 and 6 DPI except the chickens inoculated with 8248 virus, which cause first mortality at 7 DPI and last mortality at 9 DPI. In the group of chickens inoculated with 8248 virus, first mortality was observed at 7DPI and last mortality at 9DPI.

2. Pathology

Mean histologic lesion scores for chickens infected with different VVND viruses are summarized in Table 2. These scores reflect the severity of lesion and the different patterns of organ involvement among groups.

The histologic lesion score of Fabricius bursa, thymus and spleen is higher than those of other organs in most chickens infected with different VVND viruses. Lung, heart and Harderian gland were less affected organs and brain was the least affected organ of the chickens.

The pattern of organ involvement among these chickens is similar and indicative of systemic infection. However, some of the groups showed different pattern of lesion severity in certain organs such as Harderian gland and brain. In the Harderian gland, the lesion score of the chickens infected with KJW was higher than those of the chickens infected with other VVND viruses, which produce only mild lesion or absence of lesion. There was difference of lesion severity among chickens infected with VVND viruses in the brain. The

Table 2. Mortality data for chickens inoculated intratracheally with intratracheally with VVND viruses

Group	No. of chickens	Dose [a]	Days post inoculation										Mortality (%)
			1	2	3	4	5	6	7	8	9	10	
CONT	15	No											0
KJW	15	10^{5a}					10	5					100
9358GS	15	10^5				3	11	1					100
97147	15	10^5					4	10	1				100
95132	15	10^5					5	8	2				100
90163	15	10^5					8	7					100
8248	15	10^5							7	6	2		100

[a] Each chick of groups inoculated with different viruses was inoculated with 10^5ELD50 per chick.

chickens infected with 8248, 95132 or 97147 VVND viruses has more pathological lesion than chickens infected with other viruses. In contrast to 8248 group, which more than 90% chickens has pathological lesions, the chickens infected with 9358GS, only 5 chickens of total 15 chickens has only mild histological changes in the brain.

With tubular necrosis, pancreas with vacuolation and atrophy of acinar gland. There was no specific histologic lesion in the heart.

3. Immunohistochemistry

Marked differences were observed (Table 4) when IP staining methods were used to compare viral antigen distribution among tissues of chickens infected with VVND viruses. Viral antigen was detected more frequently in the chickens infected by KJW, 97147 and 8248 than in the chickens infected with 9358GS, 90163 and 95132. The 95132 group have only 3 chickens with IP positive staining from total 14 chickens and the lowest frequency of viral antigen distribution compare to other groups (Table 4).

The viral antigen was first found in the chicken of 9358GS group, which was dead at 4DPI. IP positive staining was observed at the Fabricius bursa, thymus and trachea. IP positive staining was not always present in the all chickens but in the chickens, which were dead at certain time. For example, 9 chickens of total 10 chickens, which were dead at 5DPI in KJW group had IP positive. In contrast, there was no IP positive in any 5 chickens, which were dead at 6DPI in same group.

The frequency of IP positive staining was variable depends on the types of tissues but not types of the kinds of VVND viruses infected. Brain, Fabricius bursa, thymus, cecal tonsil and trachea were IP positive with fairly high frequency and spleen, lung, proventriculus, intestine, pancreas, liver, kidney, heart and Harderian gland were with relatively low frequency.

Immune related tissues such as Fabricius bursa and thymus were mostly IP positive except spleen, which was IP positive only in the chickens infected with 97147 strain(Fig. 1). The IP positive was usually present in the lymphocytes in the Fabricius bursa, macrophages and monocytes in the thymus. No IP positive was present in the spleen of any groups. .

IP staining positive were easily found in the mucosa of respiratory and digestive system, such as lung, trachea, proventriculus and intestine. Among groups, the chickens infected with 97147 strain had high frequen-

Table 3. Frequency of lesions and mean histologic lesion scores among chickens inoculated intratracheally with VVND viruses

Tissues	NDV isolates					
	KJW	9358GS	97147	95132	90163	8248
Harderian gland	14/14(3.2)	3/12(1.3)	0/ 8(1.0)	0/ 9(1.0)	1/ 8(1.3)	0/10(1.0)
Trachea	10/12(2.7)	14/15(2.5)	12/15(2.0)	13/15(1.9)	11/12(2.3)	10/14(2.1)
Lung	2/14(1.1)	5/14(1.4)	5/14(1.4)	8/15(1.6)	8/14(1.6)	5/15(1.3)
Proventriculus	14/15(2.8)	13/15(2.2)	15/15(2.6)	13/15(2.6)	14/14(2.9)	12/14(2.0)
Intestine	13/15(2.7)	10/15(2.3)	14/15(2.9)	13/14(2.9)	14/14(3.4)	13/15(2.3)
F. bursa	14/14(3.2)	13/14(2.5)	14/15(3.0)	14/14(3.2)	14/14(3.4)	14/15(3.1)
Thymus	14/14(3.2)	13/14(2.5)	12/12(3.1)	14/14(3.6)	10/10(3.3)	12/13(3.5)
Spleen	15/15(3.1)	13/15(2.7)	15/15(3.5)	15/15(3.5)	14/14(3.9)	15/15(2.7)
Pancreas	9/15(1.8)	12/15(2.0)	11/15(2.0)	10/14(2.0)	7/14(1.9)	14/15(2.1)
Liver	14/15(2.0)	11/15(1.9)	14/15(2.1)	13/15(2.3)	10/13(2.0)	15/15(2.1)
Kidney	14/14(3.1)	10/14(2.7)	13/14(2.6)	13/15(2.7)	11/14(2.7)	15/15(3.0)
Heart	2/13(1.2)	3/15(1.3)	10/15(1.7)	10/15(1.9)	7/14(1.7)	10/15(1.9)
Brain	7/15(1.6)	5/15(1.3)	11/15(1.9)	13/15(2.2)	7/13(1.5)	14/15(2.6)

Table 4. Comparison of immunohistochemical distribution of viral antigen in various tissues among chickens inoculated with VVND viruses

Tissues	NDV isolates					
	KJW	9358GS	97147	95132	90163	8248
Harderian gland	1/15	0/14	0/15	0/14	0/15	0/15
Trachea	7/15	6/14	6/15	1/14	2/15	3/15
Lung	2/15	2/14	4/15	2/14	0/15	4/15
Proventriculus	5/15	2/14	3/15	1/14	2/15	1/15
Intestine	1/15	0/14	1/15	1/14	3/15	0/15
F. bursa	2/15	3/14	7/15	3/14	1/15	5/15
Cecal Tonsil	5/14	1/14	3/15	2/14	4/15	7/15
Thymus	5/15	5/14	7/15	2/14	3/15	3/15
Spleen	0/15	0/14	1/15	0/14	0/15	0/15
Pancreas	0/15	1/14	3/15	0/14	0/15	2/15
Liver	0/15	0/14	0/15	0/14	0/15	0/15
Kidney	2/15	0/14	1/15	0/14	0/15	2/15
Heart	0/15	0/14	1/15	0/14	1/15	0/15
Brain	3/14	1/14	5/14	1/14	1/15	7/15

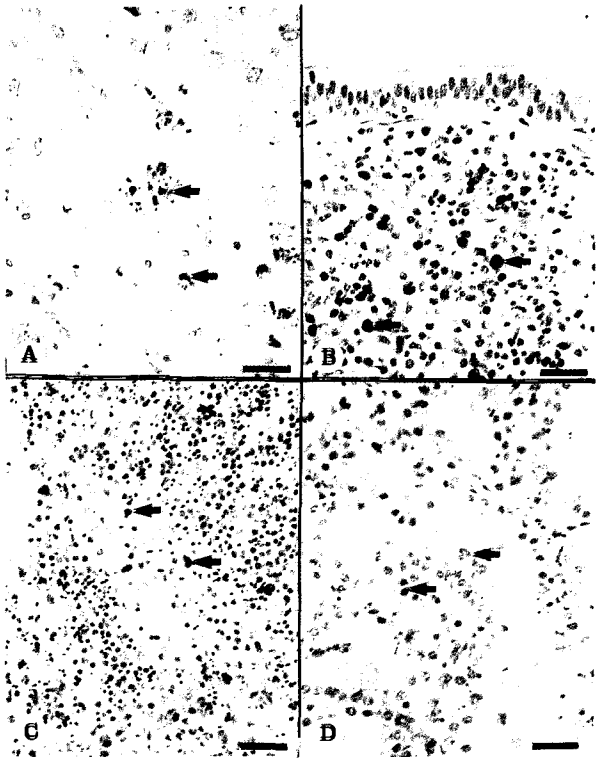

Fig. 1. Anti-Newcastle virus IP monoclonal antibody staining of tissues collected day 8 PI from chickens inoculated intratracheally with 8248 VVND virus. Note IP staining(arrows) of (A) Brain, (B) F. bursa, (C) Thymus and (D) Harderian gland. Hematoxylin counter stain. Bar=25㎛.

cy of IP positive staining in trachea and lung. The IP positive was usually present in the epithelium and macrophages of the trachea. Macrophage and lymphocytes usually were IP positive in lung, proventriculus and intestine.

IP staining was present with low frequency in the pancreas, heart and kidney and was not detected in the liver. The IP positive cells were only found in the epithelial cells of the kidney. No IP positive staining was present in the glomerulus of kidney. Only a chicken, which was inoculated with KJW has IP positive in the Harderian gland. The IP positive was present in the epithelium.

One of the interested observation was the different frequency of IP positive staining in the brain. The chickens infected with either 97147 or 8248 strain has higher IP positive frequency than the chickens infected with 9358GS, 95132 and 90163, which has only a chicken with IP positive staining each (Fig. 1).

DISCUSSION

Highly pathogenic Newcastle disease can be classi-

fied into two different forms based on clinical signs and pathological lesions in chickens and summarized (Alexander, 1997): 1) Doyle's form, Viscerotropic velogenic Newcastle disease(VVND). 2) Beach's form, Neurotropic velogenic Newcastle disease (NVND). Most of the chickens infected with different isolates in this experiments produced severe hemorrhagic lesion in the proventriculus and intestine and were died within 6 DPI except group of 8248, which were died between 7 and 9 DPI. The result were similar to those of previous study which described the systemic infection and acute death of chickens infected with VVND virus(Brown et al., 1999a). Although there is some differences of time to dead, the isolates of this study could be classified as VVND virus based on the pathological changes and mortality.

The histologic lesion have been reported in the various organs of chickens infected with different pathotype of VVND viruses by several researchers (Cheville et al., 1972; Hamid et al., 1991; Hanson et al., 1973; Jungherr et al., 1946; Katoh, 1977). Histological lesions which has been previously described in the chickens infected with VVND virus were almost similar to those of chickens infected with VVND virus in this study. Most chickens infected with VVND virus in this experiment had lymphoid cellular necrosis of spleen, thymus and F.bursa which have been described in the previous literature (Brown et al., 1999a; Ojok and Brown, 1996). Therefore, lymphoid cellular necrosis of immune organ may be common histological lesions of chickens infected with VVND virus(Ojok and Brown, 1996; Cheville et al., 1972; Hamid et al., 1991). The histological lesion of brain was also described in the both previous studies (Brown et al., 1999a; Cheville and Beard, 1972; Parede and Young, 1990) and this experiment. However, the 8248, 97147 and 95132 group showed more frequent and severe histological lesion in the brain than other group. This observation is considered that the lesion of the brain is closely related to the tissue tropism of the virus inoculated. In the heart, severe necrosis which was described(Ojok and Brown, 1996) was not present in this experiment. The differences of this results may be related with various reasons such as the virulence of the viruses infected, host age and dose of inoculum etc. Another explanation for this may be the difference of duration taken to death after challenge. If a chicken have enough time until death, the chicken may develop histological lesions in the several tissues including heart.

There was a difference of observation on histological lesion, but also many unmatching results on IP staining in the chickens infected with virulent Newcastle disease viruses (Brown et al., 1999a; Brown et al., 1999b; Ojok and Brown, 1996; Lockaby et al., 1993). Immune organs such as F. bursa, thymus showed positive IP staining in the most previous studies, but another immune organ spleen showed different results between researchers. IP positive reactions in the spleen was not described(Brown et al., 1999a) but described by this study and others (Ojok and Brown 1996). However, all these studies agree with the histological lesions in the spleen of chickens infected with VVND virus.

In this experiment, though not much as immune organs, entrances where most pathological agents invade, such as lung, proventriculus and intestines, also had positive reactions. These results, which turn out different IP staining reaction in immune organs between researchers, also had the same consequences(Brown et al., 1999a; Brown et al., 1999b; Ojok and Brown, 1996). IP positive staining was detected in the lung of chickens either infected with VVND virus or mesogenic ND virus by previous studies (Lockaby et al., 1993) and this study but not described by other researchers (Brown et al., 1999b). However, the intestine or cecal tonsil has been described as IP positive tissues in most studies (Brown et al., 1999a; Brown et al., 1999b; Ojok and Brown, 1996).

Visceral organs, such as heart, liver, kidney and pancreas, showed very few or no positive reactions in this experiment, and these results matched with the results of the other researcher.(Brown et al., 1999a; Ojok and Brown, 1996). The process of the infections with virulent Newcastle viruses is per-acute in the

visceral organs, and the reasons of these consequences turned out to be the insufficient quantity and/or replication of viruses(Ojok and Brown, 1996).

On the whole it was considered generally that there are no positive reactions in the brain, but from this experiment and previous study(Brown et al., 1999a) it was discovered that the brain of the inoculation group has positive reactions. Practically during this IP staining study, the part that was the main problem was the IP staining reaction of the brain. The reactions are different according by the kinds of the MCA.

In conclusion, although it is considered that the main reason why the staining reaction is different from each tissue is related to the difference of the virus, following the results of the brain experiment, it can also be predicted that this difference can be occurred by the difference of each antibody which was used in staining, so it is estimated that there should be more staining samples by using more different kinds of antibodies.

적 요

본 시험의 목적은 국내에서 분리된 강병원성 뉴캣슬병 바이러스를 SPF(specific pathogen free)닭에 접종을 하여 병리학적 변화와 각 조직내의 뉴캣슬병 바이러스 항원의 분포를 조사하여 국내분리 바이러스들간의 조직내 친화성을 비교함으로써 분리 바이러스간 병리학적 차이점을 규명하는 것이다. 병리학적 검사를 위하여 가능한 대부분의 조직을 채취하였으며 항원검사는 특이성을 높이기 위하여 단클론성 항체를 이용하였다. 검사결과를 요약하면 뉴캣슬병 바이러스를 접종한 모든 닭들은 접종 후 3일부터 폐사가 시작되어 접종 후 9일에 100% 폐사하였으며 대부분의 접종군은 접종후 5일에서 6일 사이에 폐사가 있었으나 8248 계군은 접종후 7일에서 9일 사이에 모두 폐사를 하여 다른 접종군과 확연히 다른 폐사양상을 보였다. 그러나, 폐사한 닭들은 접종 바이러스에 관계없이 임상증상은 비슷하였다. 일반적으로 접종 후 3일이 경과하면 급격한 활동저하와 함께 빈사상태를 보였고 일단 빈사상태까지 진행된 접종 닭은 다시 회복하지 못하였다. 병리조직학적 소견은 과거 연구자들이 강병원성 뉴캣슬병에서 흔히 관찰하였던 장, 기낭, 폐장에서의 출혈소견 들을 발견할 수 있었으며 접종한 바이러스에 따른 병리학적 변화의 차이는 뚜렷하지 않았다. 공통적인 병변으로서 대표적인 것은 면역기관인 F. bursa, 흉선, 비장에서의 탐식세포 및 단핵세포의 괴사로서 과거의 문헌들에서 기술된 병변과 비교해 볼 때 이 면역기관에서의 괴사소견은 강병원성 뉴캣슬병의 가장 특징적인 조직학적 병변인 것으로 판단된다. 조직병변과 바이러스간의 유기적 관계를 알아보기 위해 면역화학조직검사법(Immuno histo chemistry)이 많이 이용되는데 본 실험에서도 이 방법을 사용하여 국내분리주들 간의 조직친화성을 비교하여 보았다. 대부분의 면역조직화학 양성은 병리조직병변이 있는 곳에서 발견되었으나 병리조직소견이 심하였다는 사실과 비교하여 볼 때 면역조직화학의 양성 발현 빈도수는 낮았다. 그러나, 본 실험에서 특이한 점은 일반적으로 다른 논문에서는 검출이 비교적 어려웠던 뇌에서 면역조직화학 양성율이 높게 검출 되었다는 것으로 이러한 사실은 아마도 이번에 사용한 단크론성 항체의 특이성이 매우 좋았기 때문인 것으로 판단되었다. 이상의 결과를 종합하여 볼 때 강독형 뉴캣슬병의 병성기전을 연구하는데 있어서 병리조직소견과 면역조직화학법을 이용한 조직친화성 평가는 매우 유용할 것으로 판단되며 8248접종군은 다른 접종군과 병리학적으로 비교할 때 병성기전이 다른 것으로 추정되었다.

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