JARQ 44 (1), 73 - 80 (2010) http://www.jircas.affrc.go.jp

Comparative Pathology of Chickens and Domestic Ducks Experimentally Infected with Highly Pathogenic Avian Influenza Viruses (H5N1) Isolated in Japan in 2007 and 2008

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Abstract

Chickens and domestic ducks were inoculated with highly pathogenic avian influenza viruses (H5N1) isolated in Japan in 2007 and 2008. The virus strain A/chicken/Miyazaki/K11/2007 caused 100% mortality in chickens with characteristic skin lesions on the head that were similar to those found in field chicken outbreaks in 2007. The virus strain A/whooper swan/Akita/1/2008 (Ws/Akita/1/08) isolated from dead wild swans was also highly pathogenic against chickens. When domestic ducks were inoculated with each virus, only Ws/Akita/1/08 caused mortality. Two characteristic clinical signs, a neurologic sign and corneal opacity, were observed in domestic ducks. Histologically, edematous and hemorrhagic skin lesions at the comb and wattle were the most prominent findings in dead chickens. In domestic ducks, non-suppurative meningoencephalitis, myocarditis, pancreatic focal necrosis, keratitis, and epidermal necrosis of the feathers and beak were observed, depending on the course of infection. Immunohistochemical testing revealed that, compared to chickens in which the virus preferably replicated in systemic endothelial cells, antigen distribution in domestic ducks was confined to the parenchymal cells of some organs such as the brain, heart, pancreas, and epidermis of the feathers and beak. Our data suggest that, in addition to the increased mortality rate, skin lesions of the head including the wattle and comb comprise an important clinical sign in chickens for detection of highly pathogenic avian influenza viruses (H5N1) isolated in Japan. On the other hand, the neurologic signs and corneal opacity can be useful indications for detecting infected waterfowl.

Discipline: Animal health
Additional key words: clinical signs, experimental infection, histopathology, immunohistochemistry

Introduction

To date, outbreaks of Asian lineage highly pathogenic avian influenza (HPAI) (H5N1) have occurred in Japan in 2004, 2007 and 2008, with the former two cases causing significant economic losses in the poultry industry[5,13,15]. The outbreaks in 2008 occurred in migrating wild swans which can serve as carriers of the virus[10,22]. HPAI viruses (H5N1) are still circulating especially in Asia[25], indicating that Japan is still at risk of reintroduction of the virus.

Early reporting of suspected cases by farmers and subsequent detection by official veterinarians are key factors for successful control of the disease. However, clinical manifestations and gross lesions of chickens infected with HPAI virus usually vary greatly, depending on many factors involving the birds and the viruses[20,21]. Therefore, it would be a great advantage for early detection to update the kinds of symptoms and lesions that can be readily created in chickens by the currently circulating HPAI virus (H5N1).

The current Asian HPAI virus (H5N1) can cause clinical symptoms including mortality and pathological lesions to wild waterfowl which have been considered natural reservoirs of low pathogenic AI virus circulating in nature[4,11,23]. The susceptibility of waterfowl to the HPAI virus (H5N1) seems lower than that of chickens[8,13,17,18,26]. However, considering the fact that domestic waterfowl can be infected and thus can play a role as carriers of the

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Received 19 January 2009 ; accepted 9 April 2009.

virus[6,8,12,19], understanding the pathogenesis of HPAI (H5N1) in waterfowl is important in detecting infected waterfowl.

The purpose of this study was to examine clinical symptoms and pathological findings useful for detection of infected chickens and domestic ducks after experimental infection with the HPAI viruses (H5N1) isolated in Japan in 2007 and 2008.

Materials and methods

1. Animals and viruses

We used Specific-pathogen-free White Leghorn chickens (*Gallus gallus* var. *domestica*) (Nippon Institute of Biological Science, Tokyo, Japan) and conventional domestic ducks (*Anas platyrhynchos* var. *domestica*; a cross-breed of wild mallard and domestic duck) (Tsumura Inc., Osaka, Japan). The birds were kept in negative-pressure isolators in a bio-safety-level-3 approved laboratory during the experiment. They were provided with food and water *ad libitum*. All experiment procedures were approved by the Ethics Committee of the National Institute of Animal Health, Japan.

We used two HPAI viruses (H5N1) A/chicken/Miyazaki/K11/2007 (Ck/Miya/K11/07) and A/whooper swan/Akita/1/2008 (Ws/Akita/1/08) isolated in Japan. Ck/Miya/K11/07 is genetically classified as clade 2.2 which belongs to the virus lineage spreading from Asia to Europe, the Middle East and Africa[3,5,22]. Ws/Akita/1/08 was isolated from dead wild swans in 2008 and belonged to clade 2.3.2 which is a genetically different lineage from Ck/Miya/K11/07[22]. The stock virus was propagated for two days in the allantoic cavity of 10-day-old embryonated chicken eggs at 37°C. The fresh infectious allantoic fluid was harvested and stored at -80°C until use. The inoculum was made by dilution of the infectious allantoic fluid in phosphate buffered saline on the day of inoculation.

2. Experimental infection

Experiment 1. Eight-week-old chickens (n = 10) were inoculated intravenously (wing vein) with 0.2 ml of a 1:10 dilution of infectious allantoic fluids with a hemagglutination titer >1/16 derived from Ck/Miya/K11/07. Similarly, seven-week-old chickens (n = 10) were inoculated intravenously (wing vein) with Ws/Akita/1/08. The inoculated chickens were examined for clinical signs and mortality for 10 days. All dead birds were examined by pathological analysis. Because eight birds in both groups in this experiment were used for pathogenicity testing under the criteria of the World Organisation for Animal Health[24], the viral titer of the inoculum was not determined.

Experiment 2. Four-week-old domestic ducks (n = 8) were inoculated intravenously (cervical vein) with 10^7 50% egg infectious dose of each virus. The inoculated ducks were examined for clinical signs and mortality. Ducks still surviving on day 10 postinoculation (PI) were euthanized. All birds were examined by pathological analysis.

Experiment 3. Four-week-old ducks (n = 9) were inoculated intranasally with 10^7 50% egg infectious dose of each virus. The intranasal inoculation was chosen as natural-route infection. Three ducks in each inoculation group were euthanized on each of days 3, 6 and 14 PI. One duck inoculated with Ws/Akita/1/08 was examined on day 10 PI because of the unexpected mortality, and therefore two birds in this group were examined on day 14 PI.

3. Pathological analysis

Systemic organs of the chickens and ducks were fixed in 10% neutral-buffered formalin. Samples were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin.

Immunohistochemical testing was performed to detect influenza viral antigens with a Histofine Simple Stain MAX PO (M) kit (Nichirei Inc., Tokyo, Japan). A mouse monoclonal antibody specific for the type A influenza matrix protein (diluted 1:500; clone GA2B, AbD Serotec, Kidlington, UK) was used as the primary antibody. The sections were pretreated with 10 mM citrate buffer (PH 6.0) in a microwave oven at 500 W for 15 minutes for the antigen retrieval. Primary antibodies diluted in phosphate buffered saline containing 1% bovine serum albumin were mounted overnight at 4°C. 3'-3-diaminobenzidine tetrahydrochloride was used as the chromagen. All slides were counterstained with Mayer's hematoxilin. Positive control sections were obtained from the samples in previous experiments[26]. Negative control sections using PBS instead of the primary antibody were included. We found no unexpected reactions of control sections throughout the study.

Results

1. Mortality and gross lesions

Experiment 1. All chickens inoculated with either Ck/Miya/K11/07 or Ws/Akita/1/08 died, with severe depression exhibited shortly before the mortality (Table 1).

In chickens inoculated with Ck/Miya/K11/07, nine chickens including those used for pathogenicity testing died within 26 hours after inoculation. The tenth chicken died on day 3 (53 hours) PI. In this group, most of the gross lesions were hemorrhagic with edematous changes on the head, especially at the wattle (Figs. 1-3). The necrotic changes of the comb (Fig. 2), a swollen head (Fig. 1), and ruffled feathers were observed only in a chicken that died on day 3 PI. Two out of 10 birds did not exhibit any gross lesions.

All chickens inoculated with Ws/Akita/1/08 died

Table 1. Mortality, clinical signs and gross lesions in chickens and domestic ducks inoculated intravenously with HPAI viruses (H5N1) in Experiments 1 and 2

Exp. No.[a)]	Bird	Age	Virus	Mortality rate (dead/total)	Clinical signs and gross lesions (morbidity rate)
1	Chicken	8-week-old	Ck/Miya/K11/07	100% (10/10)	Depression (100%) Dark discoloration and swelling of the wattle and comb (40%; Figs. 1 & 2) Conjunctival hemorrhage (40%; Fig. 3) Petechial hemorrhage of the facial skin (20%) Swollen head (10%; Fig. 1) Ruffled feathers (10%) Increased pericardial fluids (30%) Petechiae of the liver and epicardial adipose tissue (10%)
		7-week-old	Ws/Akita/1/08	100% (10/10)	Depression (100%) Conjunctival hemorrhage (30%) Swollen head (10%) Petechiae of the epicardial adipose tissue (40%; Fig. 4) Pulmonary edema (30%) Increased pericardial fluids (20%)
2	Duck	4-week-old	Ck/Miya/K11/07	0% (0/8)	Corneal opacity (100%) Mild neurologic signs (12.5%)
		4-week-old	Ws/Akita/1/08	100% (8/8)	Depression (100%) Corneal opacity (100%) Severe neurologic signs (87.5%) Pancreatic necrosis (100%)

[a)]: Experiment number.

within 26 hours after inoculation without head lesions except for conjunctival hemorrhage. Following the depression, petechiae of the epicardial adipose tissue was the second most frequent finding (Table 1, Fig. 4).

Experiment 2. All ducks inoculated with Ck/Miya/K11/07 survived for 10 days, although two characteristic clinical signs were observed (Table 1). One clinical sign common to all inoculated ducks was persistent corneal opacity, starting on day 2 or 3 PI. This ocular lesion was observed up to day 10 PI after manifestation. One duck displayed mild neurologic signs of persistent torticollis and circling movement on days 9 and 10 PI. Gross lesions of internal organs were not found in any of the euthanized ducks.

In contrast, Ws/Akita/1/08 caused 100% mortality in ducks on days 4 and 5 PI (Table 1). In addition to the corneal opacity and depression, severe neurologic signs of ataxia and intermittent generalized seizure were observed. All clinical signs started on day 3 PI. Focal to diffuse whitish lesions (necrosis) were observed in the pancreas of all ducks at necropsy. In one case, the pancreatic lesions were recognized as hemorrhagic foci.

Experiment 3. In ducks inoculated intranasally with Ck/Miya/K11/07, except for two birds euthanized on day 3 PI, persistent corneal opacity starting on days 3 to 5 PI was observed in the remaining 7 ducks. One duck showed an intermittent generalized seizure on day 2 PI, but this neurologic sign disappeared on day 3 PI or later. Gross findings of internal organs included small whitish foci (necrosis) of the pancreas on day 3 PI, and scattered depressed lesions corresponding to the former whitish foci on day 6 PI.

Two ducks inoculated with Ws/Akita/1/08 exhibited neurologic signs starting on day 6 or 7 PI, of which one duck unexpectedly died on day 10 PI. Neurologic signs of this dead bird were severe and composed of the circling movement, ataxia and an intermittent generalized seizure. The other duck showed only mild neurologic signs of circling movement and torticollis. Eight out of nine ducks exhibited persistent corneal opacity from day 3 PI.

2. Histopathology

Experiment 1. Histological lesions observed were common to all of the dead chickens and were especially prevalent in the non-feathered skin (the comb and wattle), lungs and brain (Table 2, Figs. 5-10). The slight difference between the two groups is that, as suggested by gross findings, chickens inoculated with Ws/Akita/1/08 had the heterophilic infiltration with abundant viral antigens at the wattle and comb, but lacked the edema, hemorrhage

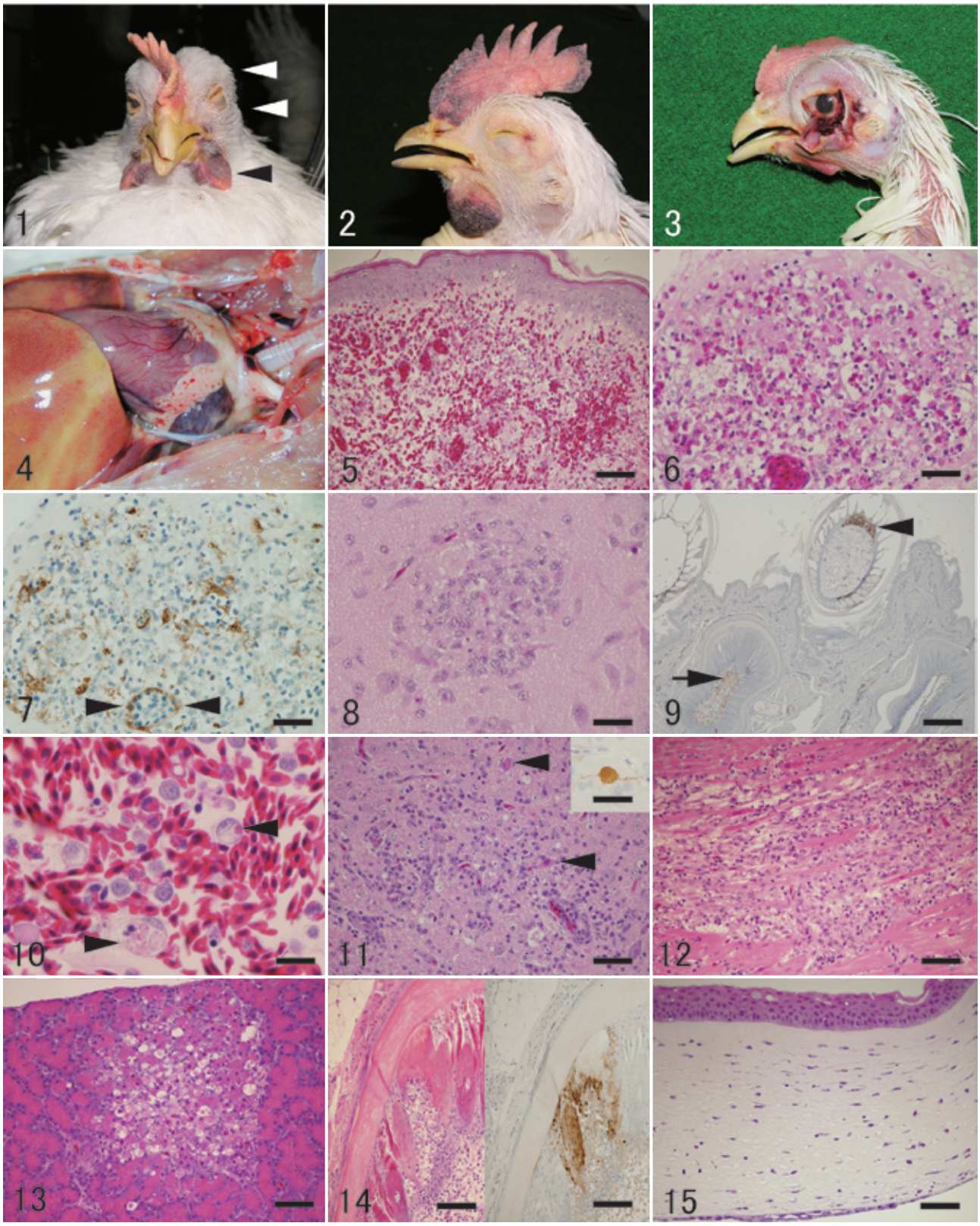

Fig. 1. Chicken inoculated with Ck/Miya/K11/07 on day 3 PI showing unilaterally swollen head (white arrowheads) and dark discoloration of the swollen wattle (black arrowhead)

Fig. 2. Chicken inoculated with Ck/Miya/K11/07 on day 3 PI showing dark discoloration of the comb and wattle with necrotic changes as well as periorbital hemorrhage

Fig. 3. Chicken inoculated with Ck/Miya/K11/07 on day 1 PI showing conjunctival hemorrhage

Fig. 4. Chicken inoculated with Ws/Akita/1/08 on day 1 PI showing petechiae at the pericaridial adipose tissue

and epidermal necrosis. In addition, only Ws/Akita/1/08-inoculated chickens had the necrosis of the periellipsoid lymphocyte sheaths in the spleen.

Rare findings were lymphocytic inflammation with fragmented nuclei of hepatocytes at the portal areas of the liver, hypoplastic bone marrow and small necrotic foci in the pancreas. Some endothelial cells had fragmented nuclei.

Abundant viral antigens were primarily detected in systemic endothelial cells (Table 2, Fig. 7). Antigen-positive cells were also found with various degrees of antigen level in a variety of parenchymal cells of the internal organs such as the brain, heart, kidney, liver, pancreas, skeletal muscle, proventriculus, upper respiratory tract (ciliated cells and gland cells), air sac, salivary gland, and feathers (Table 2, Fig. 9). Some antigen-positive cells lacked any morphological changes under hematoxylin and eosin staining.

Experiment 2. In Ck/Miya/K11/07-inoculatd ducks euthanized on day 10 PI, histological lesions were only mild non-suppurative encephalitis and keratitis without viral antigens. In contrast, all dead ducks inoculated with Ws/Akita/1/08 exhibited severe histological lesions with abundant viral antigens in several organs (Table 3, Figs. 11 & 12). The brain lesions in ducks differed from those in chickens in that an inflammatory reaction was evident in ducks (Figs. 8 & 11).

Unlike chickens in which the virus replicated primarily in endothelial cells, distributions of viral antigens in ducks were strongly associated with necrotic change of parenchymal cells of the affected organs (Table 3). Viral antigens were occasionally detected in the tongue epithelium, skeletal muscle, artery wall, salivary gland, osteoblasts, and osteoclasts.

Experiment 3. Histological findings similar to those in Experiment 2 were observed in both groups, regardless of whether any clinical signs were present (Table 4, Figs. 13-15). However, only limited areas in each organ were affected. On the whole, the lesions were mild to moderate, compared to those of intravenously inoculated ducks in Experiment 2. In ducks inoculated with Ws/Akita/1/08 on day 14 PI, the brain, heart and cornea had histological

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Fig. 5. Chicken inoculated with Ck/Miya/K11/07 on day 1 PI showing severe heterophilic infiltration and hemorrhage at the dermis of the wattle
Hematoxylin and eosin (bar = 60 μm).

Fig. 6. Chicken inoculated with Ck/Miya/K11/07 on day 1 PI showing heterophilic capillaritis with edema at subcutaneous tissue of the wattle
Hematoxylin and eosin (bar = 60 μm).

Fig. 7. Chicken inoculated with Ck/Miya/K11/07 on day 1 PI showing influenza virus antigens detected in endothelial cells (arrowheads) at the same location as Fig. 6
Immunohistochemistry (bar = 60 μm).

Fig. 8. Chicken inoculated with Ck/Miya/K11/07 on day 1 PI showing small focus of gliosis of the brain
Hematoxylin and eosin (bar = 30 μm).

Fig. 9. Chicken inoculated with Ck/Miya/K11/07 on day 1 PI showing viral antigens detected in the feather epidermal cells (arrowhead)
The endothelial cells and stromal cells in the feather pulp were also positive for viral antigens (arrow). Immunohistochemistry (bar = 220 μm).

Fig. 10. Chicken inoculated with Ck/Miya/K11/07 on day 1 PI showing hemophagocytic macrophages (arrowheads) in the vascular space of the pulmonary vein
Hematoxylin and eosin (bar = 15 μm).

Fig. 11. Duck inoculated intravenously with Ws/Akita/1/08 on day 5 PI showing perivascular cuffing and gliosis with a few necrotic neurons (arrowheads) of the brain
Hematoxylin and eosin (bar = 60 μm). The insert shows viral antigens detected in the neuron. Immunohistochemistry (bar = 30 μm).

Fig. 12. Duck inoculated intravenously with Ws/Akita/1/08 on day 5 PI showing lymphohistiocytic myocarditis with fibroblast proliferation of the heart
Hematoxylin and eosin (bar = 60 μm).

Fig. 13. Duck inoculated intranasally with Ck/Miya/K11/07 on day 3 PI showing focal necrosis and vacuolization of acinar cells of the pancreas
Hematoxylin and eosin (bar = 60 μm).

Fig. 14. Duck inoculated intranasally with Ck/Miya/K11/07 on day 6 PI showing epidermal necrosis of the feather on the left and the presence of viral antigens on the right
Left: Hematoxylin and eosin (bar = 120 μm).
Right: Immunohistochemistry (bar = 120 μm).

Fig. 15. Duck inoculated intranasally with Ck/Miya/K11/07 on day 6 PI showing edematous changes with a few heterophils in the stroma of the cornea
Vesicular formations were observed in the corneal epithelium. Hematoxylin and eosin (bar = 50 μm).

Table 2. Major histopathological and immunohistochemical findings in dead chickens inoculated intravenously with HPAI viruses (H5N1) in Experiment 1

Organ	Histological findings	Viral antigen-positive cells
Comb, wattle	Dermatitis (heterophilic infiltration, capillaritis, hemorrhage[a)], edema[a)], epidermal necrosis[a)]; Figs. 5 & 6)	Endothelial cells (Fig.7), epidermal cells
Brain	Focal gliosis (Fig. 8)	Endothelial cells, neurons, glial cells, ependymal cells
Spinal cord	Focal gliosis	Endothelial cells, neurons, glial cells, ependymal cells
Lung	Heterophil and hemophagocytic macrophage infiltration at the blood capillary	Endothelial cells, macrophages
Feathered skin	Heterophilic inflammation at the feather pulp	Endothelial cells, feather stromal cells, feather epidermal cells (Fig. 9)
Conjunctiva	Subepithelial hemorrhage	Endothelial cells
Lymphoid tissue	Lymphocytic depletion	Endothelial cells
Spleen	Necrosis of the periellipsoid lymphocyte sheaths[b)]	Endothelial cells, macrophages
Blood cells, spleen	Hemophagocytic syndrome (Fig. 10)	Endothelial cells, macrophages
Heart, kidney, liver, pancreas, skeletal muscle, proventriculus, upper respiratory tract, air sac, salivary gland	Not significant	Endothelial cells, cardiomyocytes, hepatocytes, pancreatic acinar cells, myocytes, mucosal epithelial cells of the proventriculus, upper respiratory epithelial cells, air sac epithelial cells, salivary gland cells

[a)]: Histological finding observed only in chickens inoculated with Ck/Miya/K11/07.
[b)]: Histological finding observed only in chickens inoculated with Ws/Akita/1/08.

Table 3. Major histopathological and immunohistochemical findings in dead domestic ducks inoculated intravenously with Ws/Akita/1/08 in Experiment 2

Organ	Histological findings	Viral antigen-positive cells
Brain	Non-suppurative meningoencephalitis (neuronal necrosis, gliosis, perivascular cuffing; Fig. 11)	Neurons, glial cells, ependymal cells
Spinal cord	Non-suppurative myelitis	Neurons, glial cells, ependymal cells
Heart	Myocarditis (Fig. 12)	Cardiomyocytes
Pancreas	Focal necrosis	Acinar cells
Feather	Epidermal necrosis	Feather epidermal cells, feather follicle wall epidermal cells
Beak, scaled legs	Epidermal necrosis	Epidermal cells
Ganglion	Neuronal necrosis	Neurons
Cornea	Keratitis	Corneal epithelial cells, corneal endothelial cells
Adrenal gland	Focal necrosis	Adrenal gland cells
Air sac	Airsacculitis	Epithelial cells

lesions without viral antigens. On the contrary, the feather and beak epidermis were rarely positive for viral antigens without any morphological changes.

Discussion

In a series of HPAI (H5N1) field outbreaks in Japan in 2007, clinical signs and gross lesions were found in a limited number of affected chickens[5]. Together with increased mortality rate, clinical findings of drowsiness, depression and ruffled feathers were observed in chickens during the outbreaks[5]. Moreover, swollen heads and cyanosis (dark discoloration) of the wattle, comb and scaled leg were also reported as conspicuous signs[5]. Turning to the experimental infection of the present study, similar lesions were reproduced in chickens inoculated with Ck/Miya/K11/07, suggesting that skin lesions of the head, including the wattle and comb, are important findings in chickens for detection of the HPAI

Table 4. Major histopathological and immunohistochemical findings in domestic ducks infected intranasally with HPAI viruses (H5N1) in Experiment 3

Histological findings	Ck/Miya/K11/07			Ws/Akita/1/08			
	day 3	day 6	day 14	day 3	day 6	day 10	day 14
Non-suppurative meningoencephalitis	2/3[a] (+)[b]	3/3 (+)	3/3 (–)	2/3 (+)	3/3 (+)	1/1 (–)	1/2 (–)
Non-suppurative myelitis	0/3 (–)	1/3 (+)	0/3 (–)	2/3 (+)	2/3 (+)	0/1 (–)	0/2 (–)
Pancreatic focal necrosis (Fig. 13)	2/3 (+)	0/3 (–)	0/3 (–)	3/3 (+)	1/3 (–)	0/1 (–)	0/2 (–)
Myocarditis	0/3 (–)	1/3 (–)	0/3 (–)	0/3 (+)	3/3 (–)	1/1 (–)	1/2 (–)
Feather epidermal necrosis (Fig. 14)	3/3 (+)	3/3 (+)	0/3 (–)	3/3 (+)	3/3 (+)	0/1 (+)	0/2 (+)
Beak epidermal necrosis	3/3 (+)	1/3 (+)	0/3 (–)	3/3 (+)	1/3 (+)	0/1 (–)	0/2 (+)
Keratitis (Fig. 15)	1/3 (+)	3/3 (–)	3/3 (–)	2/3 (+)	3/3 (+)	1/1 (–)	1/2 (–)
Airsucculitis	1/3 (+)	0/3 (–)	0/3 (–)	3/3 (+)	3/3 (–)	0/1 (–)	0/2 (–)

[a]: The number of birds with histological lesions/ The number of total birds.
[b]: (Detected viral antigens); + = positive in one or more birds, – = negative.

virus (H5N1) isolated in 2007 in Japan.

Ws/Akita/1/08 which was isolated from wild swans was a typical highly pathogenic virus against chickens. In the clinical course of chickens infected with Ws/Akita/1/08 in Experiment 1, although only an intravenous inoculation study was performed, inoculated chickens lacked skin lesions on the head that were characteristic for Ck/Miya/K11/07-inoculated chickens. These results were similar to those of chickens after infection with HPAI virus (H5N1) in 2004[14]. The virus strain isolated in Japan in 2004 did not cause apparent clinical signs in chickens despite 100% mortality with a peracute course[14]. The lack of gross lesions in infected chickens would make it more difficult to diagnose the disease in the field outbreaks.

Feathers of infected chickens had minimal morphological changes in the histological section, although viral antigens were occasionally detected in the feather tissue. Viral replication in the feathers of chickens appeared to be secondary lesions following the very active viral replication in systemic endothelial cells. HPAI in chickens are a fatal infection with a large amount of viral load within the body[20]. Therefore, any materials from infected chickens would have the risk of contamination to the environment. Especially, the feathers can easily drop off so that great care must be taken when handling the carcass of infected chickens.

Confirmed clinical signs observed in domestic ducks included corneal opacity and neurologic signs, which were not observed in the chickens. As in a previous report using domestic ducks and Japanese HPAI virus (H5N1) isolated in 2004[26], corneal opacity was the most frequent sign in ducks in the present study, suggesting that ocular lesions can be an indicative sign of ducks infected with H5N1 subtype HPAI viruses isolated in Japan. Moreover, histologic non-suppurative encephalitis was observed in almost all inoculated waterfowl which did not necessarily show perceptible neurologic signs. HPAI (H5N1) should be considered for differential diagnosis when non-suppurative encephalitis is found in waterfowl species by histopathological analysis.

According to the antigen distribution in chickens and domestic ducks, viruses replicated systemically in chicken endothelial cells and a variety of parenchymal cells, whereas in ducks this occurred in the parenchymal cells of some specific organs such as the central nervous system, heart, pancreas, and the epidermis of the feathers and beak. These results indicate that different mechanisms of HPAI (H5N1) pathogenesis between chickens and waterfowl are associated with their clinical signs and pathologic lesions. Although the existence of multiple basic amino acids at the haemagglutinin cleavage site of an AI virus is strongly related to systemic infection leading to mortality in chickens[2,7], this rule does not necessarily apply to infections in waterfowl[1,17,26]. The age of the bird is an important factor related to the severity of the disease in ducks infected with HPAI virus (H5N1)[16,26]. However, 100% mortality caused by Ws/Akita/1/08 in ducks in Experiment 2 indicates that unknown viral factors also would affect the pathogenicity to domestic ducks. Hulse-Post et al. reported a possible link between the PA and PB1 genes of HPAI viruses (H5N1) and lethality in ducks[9]. The epidemiologically important problem is that asymptomatic domestic waterfowl can serve as carriers of the HPAI virus (H5N1)[6,8,12,19]. The clarification of viral factors and host immunity related to active viral replication in waterfowl is of urgent concern for controlling Asian lineage HPAI (H5N1) epidemics.

Acknowledgments

We are grateful to Masaru Kobayashi and Megumi Shimada for their technical assistance. This study was supported by a Grant-in-Aid Project of the Control of Avian

Influenza and other Zoonotic Diseases by the Ministry of Agriculture, Forestry and Fisheries of Japan.

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