STUDIES ON THE EPIDEMIOLOGY OF NEWCASTLE DISEASE IN EASTERN INDONESIA BY SEROLOGY AND CHARACTERISATION OF THE VIRAL ISOLATES USING PANELS OF MONOCLONAL ANTIBODIES

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ABSTRACT

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Epidemiological studies on Newcastle disease (ND) was performed in Eastern Indonesia, which covered Denpasar (Bali), Kupang (Nusa Tenggara Timur, NTT) and Jayapura and Merauke districts (Irian Jaya), by serology and viral isolation. The viral isolates then were characterised using panels of monoclonal antibodies (mAbs). The results indicated that for Denpasar and Kupang, the virulent strains of Newcastle disease virus (NDV) seemed to be more frequently circulated in the environment than the less virulent strains. The serological incidence of ND in these two areas were similar as indicated by the high prevalence of ND during September and October 1989 and remarkable drop in the prevalence in April and July 1990. For two areas at Irian Jaya, the less virulent strains may be more frequently circulating in the environment, except for two occasions in September, 1989 (Merauke) and in February 1990 (Jayapura). Antibodies to NDV were detected both in village chickens and ducks. However, on most occasion, reactors to NDV were more frequently detected in chickens than in ducks. None-the-less the proportions of duck reactors with high titres of HI antibodies in September 1989 were higher in Denpasar and Kupang than that in Irian Jaya. Based on the binding reaction between NDV isolates and mAbs, it could be suggested that most NDV isolates which were tested in this study may share biological properties. Since no NDV isolate demonstrated the similar MAb binding pattern as seen with the V4, suggested no isolates which were tested in this study has a close antigenic relationship to the V4 strain. This study also noted that both village chickens and ducks appeared to play a significant role in the epidemiology of the disease.

Key words: Epidemiology, Newcastle disease, serology and monoclonal antibody, village chickens and ducks.

ABSTRAK

Darminto, P.W. Daniels dan P. Ronohardjo. 1993. Studi epidemiologi penyakit tetelo di daerah Indonesia bagian Timur dengan serologi dan isolasi virus serta karakterisasi isolat-isolat dengan panel antibodi monoklonal. *Penyakit Hewan* 25(46): 67-75.

Studi epidemiologi penyakit tetelo telah dilakukan di wilayah Indonesia bagian Timur yang meliputi Denpasar (Bali), Kupang (Nusa Tenggara Timur, NTT) dan Kabupaten Jayapura serta Kabupaten Merauke (Irian Jaya). Penelitian dilaksanakan dengan survei untuk mengumpulkan bahan-bahan pemeriksaan, baik untuk serologi maupun isolasi virus. Isolat virus penyebab penyakit tetelo yang diperoleh kemudian dikarakterisasi dengan panel antibodi monoklonal. Hasil penelitian menunjukkan bahwa untuk daerah Denpasar dan Kupang tampaknya virus penyebab penyakit tetelo yang virulen lebih banyak bersirkulasi di lingkungan dari pada virus yang tidak virulen. Prevalensi penyakit tetelo dikedua daerah tersebut bervariasi menurut waktu, dimana prevalensi tertinggi pada bulan September dan Oktober 1989 dan prevalensi terendah pada bulan April dan Juli 1990. Untuk daerah di Irian Jaya, virus penyakit tetelo avirulen lebih banyak bersirkulasi di lingkungan dari pada virus yang virulen, kecuali pada dua kasus pada bulan September 1989 (Merauke) dan bulan Februari 1990 (Jayapura). Titer antibodi terhadap penyakit tetelo dapat dideteksi baik pada ayam buras maupun pada itik. Meskipun prevalensi pada itik umumnya lebih rendah pada ayam, namun itik reaktor dengan titer tinggi pada bulan September 1989 tampak lebih tinggi di Denpasar dan Kupang dari pada di Irian Jaya. Dari hasil kharakterisasi isolat virus dengan antibodi monoklonal terbukti bahwa tidak ada isolat yang memiliki persamaan antigenik dengan galur RIVS.V4. Namun umumnya isolat-isolat tersebut memiliki persamaan sifat-sifat biologik. Lebih dari itu, penelitian ini juga menunjukkan bahwa ayam buras dan itik memiliki peranan penting dalam epidemiologi penyakit tetelo.

Kata kunci: Epidemiologi, Newcastle disease, serologi, antibodi monoklonal, ayam buras dan itik.

INTRODUCTION

The first description of Newcastle Disease (ND) in Indonesia was published by Kraneveld (1926) when he reported the disease on the island of Java near Jakarta. ND is now considered to be endemic throughout Indonesia (Ronohardjo *et al.*, 1985; Moerad, 1987). The Research Institute for Veterinary Science (RIVS) at Bogor has Newcastle disease virus (NDV) isolates from West and East Java, Irian Jaya, and West Timor (Parede,

1987). The United States Naval Medical Research Unit (NAMRU II) within the Department of Health in Jakarta has NDV isolates from locations in Sumatra, West Java and Lombok, and from the bird market in Jakarta (Daniels *et al.*, 1987). The regional diagnostic laboratories in other parts of Indonesia have also reported isolations (Sobari, 1980).

There has been extensive growth in the commercial poultry industry in the last five years, particularly in the West Java region (Jackson, 1989). However, at the present time the Government of Indonesia has allocated a high priority to development programs in eastern Indonesia. The programs include the development of animal production including the poultry industry. Since ND is a potentially devastating disease of poultry and the outbreaks of ND have frequently been reported in all areas of Indonesia (Moerad, 1987; Anon., 1990), an intensive study of ND in eastern Indonesia is required to provide basic information which can be used to plan strategies.

In Indonesia, NDV has been reported to infect a wide variety of avian species (Picard, 1928; Ronohardjo and Jan Nari, 1977; Kingston *et al.*, 1977; Ronohardjo, 1980; Parede, 1987; Malole, 1988). However, the relative role of wild birds and village poultry in the transmission of NDV is not known. Domestic poultry such as village chickens and ducks may each have epidemiological significance in the spread of NDV, since these birds are widely reared in rural areas. The birds live under extensive conditions, scavenge for their food and water. Spradbrow (1987) noted that village chickens are apparently a source of infection for commercial poultry. Free-range chickens gain contact with birds in commercial farms that are established in rural areas. Ducks are often infected with NDV, but reported rarely to show clinical signs (McFerran and McCracken, 1988), although Kingston *et al.* (1977) reported severe disease with high mortality in ducks infected with mesogenic strains of NDV in Indonesia. Shortridge and Alexander (1978), during surveillance in Hong Kong, reported the isolation of NDV from apparently healthy ducks. Of five NDV isolates from ducks which were included in their pathogenicity tests, two isolates behaved as velogenic strains, and the others as lentogenic strains.

This chapter describes a study on the epidemiology of ND in eastern Indonesia: Denpasar (Bali), Kupang (Nusa Tenggara Timur, NTT) and Jayapura and Merauke (Irian Jaya). The study comprised a serological survey to define the distribution and prevalence of NDV infection in village chickens and ducks, as well as attempted isolation of the virus from village chickens and ducks. The viral isolates were partially characterised based on the patterns of the binding to a panel of monoclonal antibodies.

MATERIALS AND METHODS

Location and time

This study was conducted in eastern Indonesia, at Denpasar in the province of Bali, at Kupang on the island of Timor in NTT province, and at two districts in Irian Jaya province, Jayapura and Merauke.

At Denpasar, surveys were conducted four times, twice in 1989 (September and October) and twice in 1990 (April and July), At Kupang, surveys were conducted in September and October 1989 and July 1990. In Irian Jaya, at both Jayapura and Merauke, surveys were conducted seven times, twice in 1989 (September and November), four times in 1990 (February, May, August and November) and once in 1991 (May).

Collection of samples

Serum samples for examination of antibodies to NDV and cloacal swabs for viral isolation were collected from either village chickens or ducks.

Blood samples were collected in syringes, then transferred to sterile glass tubes for clotting and centrifugation. Sera were removed from the clots aseptically, transported to RIVS on ice, and stored in serum storage bottles at -20°C until haemagglutination inhibition (HI) tests were performed.

Swabs were taken from the cloaca of birds and placed into 1.8 mL ampoules. The ampoules were transported to the local laboratory on ice and stored in liquid nitrogen before being transported to RIVS.

From each area on each occasion, 30 samples were collected from each of two species tested. This sample size was considered sufficient to detect reactors or viral infections with a greater than 10% prevalence with 95% confidence (Cannon and Roe, 1986).

Serology

The haemagglutination inhibition (HI) test was used to measure antibodies against NDV. The tests were performed based on the method described by Allan and

Gough (1974) with modifications according to Shortridge *et al.*, (1982) and Alexander (1988). All serum samples were heated at 56°C for 30 minutes prior to testing. Serial two-fold dilutions of serum samples were made in PBS pH 7.2 in 25 µL volumes. To each dilution was then added an aliquot of 25 µL of antigen containing 4 HA units. Plates were incubated 20 minutes at room temperature. Aliquots of 50 µL of 0.5% red blood cell suspension were added to each well. Reactor and non-reactor sera as well as red blood cell suspensions were used as controls. The tests were read when the red blood cell control had completely settled. The haemagglutination inhibition titres were expressed as the reciprocal of the highest serum dilution which completely inhibited haemagglutination activity and were expressed as $\log_2$.

Birds were considered as non-reactors if antibody titres were not detected (titre = 0 $\log_2$), as reactors with low antibody titres if the HI titres in the serum samples were in the range 1 to 3 ($\log_2$) and as reactors with high antibody titres if the serum sample had HI titres of ≥4 ($\log_2$).

Viral isolation and identification

Swabs were thawed, placed in 1 mL PBS pH 7.2 containing 5,000 IU penicillin and 5,000 µg streptomycin and allowed to stand at room temperature for one hour before being centrifuged at 2,000 rpm. The supernatant from the immersed swabs were then used as inocula for viral isolation (Shortridge *et al.*, 1982; Alexander, 1988).

Each inoculum was injected into two nine-day old embryonated chicken eggs via the allantoic cavity with a dose of 200 µL per egg. After inoculation, all eggs were incubated at 37°C for 4 days with daily candling. Embryos which died within 24 hours after inoculation were considered to be non-specific deaths. Surviving embryos were chilled at 4°C overnight. Allantoic fluids were harvested from embryos which died after 24 hours of inoculation and from chilled eggs.

The presence of haemagglutinating virus in allantoic fluids was tested using a spot test as described by Shortridge *et al.* (1982). One drop of allantoic fluid was mixed with one drop of a 10% suspension of chicken red blood cells on the microscope slide. The presence of the virus was indicated by the agglutination of red blood cells which occurred within 15 seconds after mixing.

The viral isolates were identified as NDV based on HI tests using standard serum specific for NDV.

Monoclonal antibodies and reagents for capture enzyme-linked immunosorbent assay antigen

A panel of mAbs was used to characterise NDV isolates based on the binding pattern of the mAbs to the isolates in a capture ELISA antigen.

Monoclonal antibodies Q135 and Q93 were provided by Dr T. Della-Porta, Australian Animal Health Laboratory, CSIRO, Geelong, Victoria. These mAbs were prepared against the V4 strain (Della-Porta *et al.*, 1988).

Monoclonal antibodies 5C7, 4A4, AB5 prepared against the V4 strain (Malcolm, 1990) and 4A7, 2G11, 4A10, 1C2, 4E12 prepared against a duck isolate (DI3245) (Parede, 1991) were produced at the Graduate School of Tropical Veterinary Science and Agriculture, James Cook University of North Queensland. These MAbs were made available by Dr. J. Smith.

Affi-gel Blue purified rabbit anti-DI3245 IgG (TropBio) was also provided by Dr. J. Smith.

The V4 strain which had been selected as a heat resistant variant at the RIVS (RIVS.V4) (Ronohardjo *et al.*, 1988) was used for antigen control in the capture ELISA antigen.

Capture enzyme-linked immunosorbent assay antigen

Polystyrene u-bottom 96-well microtitre plates (Disposable Products) were coated with 100 µL per well of Affi-gel Blue purified rabbit anti-DI3245 IgG (TropBio) at a dilution of 1:1,000 in carbonate/bicarbonate buffer, pH 9.6. The coated plates were incubated overnight at room temperature in a humidified container.

The plates were then washed three time manually with PBS-Tween to remove unbound antibody. The NDV isolates and control virus (RIVS.V4) as well as uninfected allantoic fluid were diluted 1:5 in TEN-Tween-Casein (TEN-TC). Aliquots of 50 µL of diluted virus or uninfected allantoic fluid were added to duplicate wells for each mAb being used in the assay. The plates were incubated for one hour at room temperature.

After washing three time as above, aliquots of 50 µL of MAb diluted 1:100 in TEN-TC were added to duplicate wells containing test or control antigens. The plates were incubated at room temperature for one hour.

After washing three times with PBS-T, aliquots of 50 µL of the goat anti-mouse HRPO conjugate (Silenus, Batch No. MI 11 B) diluted 1:1,000 in TEN-TC were added to all wells of the plate. Plates were incubated for

one hour at room temperature before being washed with PBS-Tween.

Subsequently, aliquots of 100 µL of ABTS substrate were added to each well of the plates. The plates were incubated at room temperature for one hour in the dark.

Finally, the plates were read at the dual wavelengths of 414 and 492 nm using an ELISA plate reader (Titertek Multiskan automatic plate reader, Flow Labs.).

Reactions were designated as non-binding (-) if the optical density was <0.100, weak binding reaction (+) if the optical density was in the range of 0.100 to 0.399, moderate binding reaction (++) if the optical density was in the range of 0.400 to 0.699 and strong binding reaction (+++) if the optical density was greater than 0.700.

RESULTS

Serology

Results of the serological examinations are presented in Table 1 for Denpasar, Table 2 for Kupang and Table 3 for two sites (Jayapura and Merauke) at Irian Jaya.

At Denpasar, the proportion of reactors with high antibody titres was higher than those with low titres, except in the survey which was conducted in July 1990 where the chicken reactors with high and low antibody titres were detected in the same proportion (Table 1). Antibodies to NDV were not detected in duck in April 1990, but in the previous survey, in September 1989, reactors in this species were detected with prevalence of 37% with high antibody titres and 18.5% with low antibody titres (Table 1).

Table 1. Percentage distribution of the HI titres of serum samples from Denpasar, Bali

Months	Poultry	HI titres (log2):		
		0	1-3	≥4
Sep 1989	Chickens	19.4	9.7	70.9
	Ducks	44.4	18.5	37.0
Oct 1989	Chickens	9.5	4.8	85.7
	Ducks	-	-	-
Apr 1990	Chickens	42.2	25.0	32.8
	Ducks	100.0	0.0	0.0
Jul 1990	Chickens	60.0	20.0	20.0
	Ducks	-	-	-

At Kupang, on all occasions, chicken and duck reactors with high antibody titres were more frequently detected than those with low antibody titres. The proportion of chicken reactors with high antibody titres in July 1990 was lower than those detected in previous surveys (Table 2).

Table 2. Percentage distribution of the HI titres of serum samples from Kupang, NTT

Months	Poultry	HI titres (Log2):		
		0	1-3	≥4
Sep 1989	Chickens	14.3	0.0	85.7
	Ducks	15.4	7.7	76.9
Oct 1989	Chickens	9.5	4.8	85.7
	Ducks	-	-	-
Jul 1990	Chickens	55.6	7.9	35.5
	Ducks	-	-	-

In Irian Jaya, at both Jayapura and Merauke, percentage of non-reactors either chickens or ducks, was usually higher than for all reactors (Table 3). A high prevalence of high titre reactors were only detected in chickens at Merauke in September 1989 and at Jayapura in February 1990. On the other occasions, the proportions of reactors with high titres did not exceed 18%. Generally, the proportion of reactors with low antibody titres was greater than those with high antibody titres.

Viral isolations

The results of viral isolations are presented in Table 4. Ten NDVs were isolated in swabs from Denpasar, two from chickens and the others from ducks.

At Kupang, six NDV isolates were obtained from chickens. Isolations from duck were not attempted.

In Irian Jaya, nine NDV isolates were obtained at Jayapura, six from chickens and four from ducks. At Merauke, nine NDV isolates were obtained from chickens and eight from ducks.

Reaction of monoclonal antibodies to Newcastle disease viral isolates in a capture ELISA antigen

Of 42 NDVs which were isolated during this study, 25 representative isolated were examined for binding to a panel of mAbs in the antigen capture ELISA.

The binding reactions of mAbs to the viral isolates are summarised in Table 5. Binding of mAbs to the uninfected allantoic fluid which was used as a control antigen was not observed. All mAbs prepared against the V4 strain (Q135, Q93, 5C7, 4A4, AB5) reacted strongly to the RIVS.V4, while mAbs prepared against the duck isolate also demonstrated reactions to the

RIVS.V4, but the degree of reaction varied from weak (4E12), to moderate (1C2 and 2G11) and strong (4A7).

Table 3. Percentage distribution of the HI titres of serum samples from Irian Jaya

Months	Districts	Poultry	HI titres (log2):		
			0	1-3	≥4
Sep 1989	Jaya Pura	Chickens	80.0	6.7	13.3
		Ducks	73.3	20.0	6.7
	Merauke	Chickens	34.6	30.8	34.6
		Ducks	92.0	4.0	4.0
Nov 1989	Jaya Pura	Chickens	77.9	13.2	8.8
		Ducks	89.3	10.7	0.0
	Merauke	Chickens	92.0	8.0	0.0
		Ducks	90.0	0.0	10.0
Feb 1990	Jaya Pura	Chickens	45.4	11.7	42.9
		Ducks	95.3	1.6	3.1
	Merauke	Chickens	81.7	18.3	0.0
		Ducks	95.8	2.1	2.1
May 1990	Jaya Pura	Chickens	60.7	21.4	17.9
		Ducks	83.3	0.0	16.7
	Merauke	Chickens	56.7	43.3	0.0
		Ducks	100.0	0.0	0.0
Aug 1990	Jaya Pura	Chickens	91.3	8.7	0.0
		Ducks	100.0	0.0	0.0
	Merauke	Chickens	100.0	0.0	0.0
		Ducks	96.8	0.0	3.2
Nov 1990	Jaya Pura	Chickens	100.0	0.0	0.0
		Ducks	100.0	0.0	0.0
	Merauke	Chickens	96.8	3.2	0.0
		Ducks	-	-	-
May 1991	Jaya Pura	Chickens	69.6	17.4	13.0
		Ducks	-	-	-
	Merauke	Chickens	90.0	3.3	6.7
		Ducks	93.3	3.3	3.3

Monoclonal antibody Q93 reacted strongly to most NDV isolates, except isolates MCS4, MCS25, KCS50, KCS79, BDS121 and BDS122 which demonstrated a moderate binding reaction. Other mAbs also demonstrated binding reactions to all tested viruses, but the degree of reaction varied from weak to strong.

DISCUSSION

Newcastle disease is considered endemic in Indonesia (Ronohardjo *et al.*, 1985; Moerad, 1987; Anon. 1990) and the disease has also been reported in ducks (Kingston *et al.*, 1977). The present study demonstrated that NDV infections are widespread in eastern Indonesia and the virus could be successfuly isolated from cloacal swabs of apparently healthy chickens and ducks.

It has been recognised that all NDV strains are capable of eliciting antibody responses in chickens, rabbits and other species into which the viruses are introduced (Beard and Hanson, 1984). The level of antibody response is generally considered to be associated with the virulence of the virus. In an experiment in chickens with NDV vaccine strains, Westbury *et al.* (1984b) demonstrated that the La Sota strain was more virulent than either B1 or V4. Furthermore, the La Sota strain was demonstrated to be more effective in stimulating antibody responses than B1 or V4, since, using similar doses and routes of administration, La Sota gave a higher proportion of birds with HI titres of >3 (log_2) than did B1 or V4 at 21 days after vaccination (Westbury, 1984). Furthermore, in experimental infections using velogenic strains of NDV in challenge tests, birds surviving exposure consistently had hig titres of HI antibodies (>3 log_2) (Spradbrow *et al.*, 1980; Bell *et al.*, 1991; Darminto *et al.*, 1992). The association between the viral virulence and the ability to induce HI antibody responses was applied in this study in interpreting the serological data.

In Irian Jaya and Kupang, serum samples were taken from birds which did not have a history of ND vaccination. In Bali, the Veterinary Services reported regular vaccinations of village chickens. In the serological examination, antibodies to NDV were detected in the majority of serum samples with titres varying from 1 to 8 (log_2). By applying the association between viral virulence and the ability to provoke antibody responses, it may be suggested that reactors with low HI titres (1-3 log_2) were possibly exposed to the avirulent or less virulent strains of NDV, while reactors with high titre of HI antibodies (≥4 log_2) were more likely to be infected by the virulent strains. It is recognised that this interpretation does not allow for the possibility of low titres being declining titres, but is none-the-less in helping to interpret high titres.

At Denpasar and Kupang, the virulent strains of NDV seemed to be more frequently circulated in the environment than the less virulent strains as indicated by the demonstration of reactors with high HI titres which were more frequently detected than those with low titres. The serological incidence of ND in these two areas were similar as indicated by the high prevalence of ND during September and October 1989 and remarkable drop in the prevalence in April and July 1990 (Tables 1 and 2).

The epidemiology of ND in Irian Jaya seemed to show a different pattern. Since reators with low HI titres were more frequently detected than those with high titres, it may be suggested that less virulent strains may be more frequently circulating in the environment. Except for two occasions at Merauke in September, 1989 and Jayapura in February 1990, where the proportion of reactors with high HI titres reached 34.6% and 42.9% respectively, the serological prevalence of ND in Irian Jaya was lower than that in Denpasar and Kupang.

Data of this study showed that antibodies to NDV were detected both in chickens and duck. However, on most occasion, reactors to NDV were more frequently detected in chickens than in ducks. None-the-less the proportion of duck reactors with high titres of HI antibodies in September 1989 were higher in Denpasar and Kupang than in Irian Jaya.

The detection of NDV antibodies in ducks is of particular interest. Since no ND vaccination was applied to this species, the antibodies were more likely to be induced by infection with field strains of NDV. This study was in agreement with Asplin (1947) and McFerran and McCracken (1988) who reported that ducks infected with NDV, rarely show clinical signs, but they produced antibodies to the virus.

In Indonesia, ducks are widely reared by small holders under extensive condition, scavenging for their food and water. Free-range ducks may gain contact with village chickens in rural areas and may transmit NDV to the chickens. In this situation, ducks could be play as a source of NDV infection for chickens. Hence, this species was suggested to have a significant role in the epidemiology of ND.

Further evidence of circulation of NDV in the study areas was obtained from viral isolation. Forty two NDVs were successfully isolated from cloacal swabs of apparently healthy chickens and ducks (Table 4). The pathogenicity of the isolates was not determined in this study. However, an other researcher interested in obtaining lentogenic or avirulent strains of NDV for vaccine development has evaluated several NDV isolated from ducks from Bali and Irian Jaya in this study (JDS1, JDS10, JDS14, BDS121 and BDS122) and found that these duck isolates killed chicken embryos whithin 48 hours after infection (Ronohardjo, unpublished data). Therefore, these duck isolates appeared to be velogenic strains.

The isolation of velogenic NDV from eastern Indonesia such as Irian Jaya, Kupang and Bali from organs of sick birds have been previously reported by Parede (1987). The isolation of mesogenic NDV from acute disease of Indonesian ducks was reported by Kingston *et al.* (1977). This study demonstrated the isolation of NDV from the cloacae of apparently healthy ducks. Similar findings have been reported previously in Hong Kong by Shortridge and Alexander (1978). The potential role of ducks in spreading NDV has been demonstrated by Asplin (1947) who described that ducks which were exposed to infection with NDV remained apparently healthy, but that their antibody titres increased, showing an active infection. The infected ducks became infective for chickens. Hence ducks could also be a potential source of NDV infection.

Table 4. Number of sample tested and Newcastle disease virus isolated from eastern Indonesia: Bali, NTT and Irian Jaya

Areas	Poultry	No. sample tested	No. viral isaolated
Denpasar, Bali	Chickens	78	2
	Ducks	61	8
Kupang, NTT	Chickens	14	6
	Ducks	-	-
Jaya Pura, Irja	Chickens	381	6
	Ducks	261	3
Merauke, Irja	Chickens	294	9
	Ducks	268	8
Total		1,357	42

Velogenic NDV has been demonstrated to infect chickens even with hig titres of HI antibodies (Parede, 1987; Parede and Young, 1990) and without inducing clinical disease in such birds. The virus multiplies in these infected chickens and then excreted through the cloaca or respiratory tract (Westbury *et al.*, 1984a; Darminto and Daniels, 1992). If these observations from the commercial birds can be extrapolated to village chickens, the village chicken environment would be expected to remain contaminated with NDV, even with vaccination programs. Hence such chickens could be the potential source of NDV in the environment.

In the capture ELISA antigen, all 25 isolates which were tested showed binding reaction to the panel of mAbs with degrees of reaction varying from non-binding (-) to strong reaction (+++) (Table 5). The mAb Q135 was the only mAb which demonstrated lack of reaction with several isolates (1W-Otak, 1A, JCS32, KCS50, BDS121 and BDS122). The Q93 demonstrated strong binding reaction to most isolates, except to isolates MCS4, MCS25, KCS50, KCS79, BDS121 and BDS122 which showed moderate binding. Three mAbs

against duck isolates (4A10, 1C2 and 4E12) demonstrated weak binding reaction to the majority of the isolates, while 4A7 which was also prepared against duck isolate demonstrated moderate to strong reaction to most isolates, except to isolates 1W-Otak, 2W-Otak, MCS4, MCS12 and BP17/0 which only resulted in weak reaction. The 2G11 gave strong binding reaction to isolates JDS10 and JDS14, moderate binding reaction to isolates 3W-Otak, JDS1 and JDS11 and demonstrated weak binding reaction to other isolates. Overall reaction between isolates and mAbs demonstrated that most isolates which were tested in this study, except isolates 1A, JDS1, JDS10 and JDS11, had similar pattern of binding reaction to a panel of mAbs suggested that these isolated may share biological properties. No NDV isolate demonstrated the similar mAb binding pattern as seen with the V4 suggested no isolates which were tested in this study has a close antigenic relationship to the V4 strain.

This study provided further information on the epizootiology of ND in eastern Indonesia. The virus appeared to be widespread in the environment and has been successfully detected either by serology or vival isolation. Both village chickens and ducks appeared to play an important role in the epidemiology of the disease.

Table 5. Monoclonal antibody binding to Newcastle disease viral isolates present in allantoic fluid

Viruses	Monoclonal antibodies									
	Q135	Q93	5C7	4A4	2G11	4A7	AB5	4A10	1C2	4E12
RIVS.V4	+++	+++	+++	+++	++	+++	+++	nd	++	+
Allant.	-	-	-	-	-	-	-	-	-	-
1W Otak	-	+++	+	++	+	+	+	++	+	+
3W Otak	++	+++	+	+	++	+	++	+	nd	+
1A	-	++	+	+++	+	++	++	+	+	+
JDS1	+	+++	+	+	++	+++	+	++	+	+
JDS10	+	+++	++	+	+++	++	++	++	+	++
JDS11	+	+++	++	++	++	+++	+++	+	+	+
JDS14	++	+++	+	+	+++	++	+	+	+	+
BDS136	+	+++	+	++	+	++	+	+	+	++
JCS32	-	+++	++	+	+	++	+	+	+	+
JCS33	+	+++	++	+	+	++	+	+	+	+
MCS4	+	++	+	+	+	+	+	+	+	+
MCS12	+	+++	+	+	+	+	+	+	+	+
MCS25	+	++	+	+	+	++	+	+	+	+
KCS50	-	++	+	+	+	++	+	+	+	+
KCS73	+	+++	+	+	+	++	+	+	+	+
BCS409	+	+++	+	+	+	++	+	+	+	+
KCS79	+	++	+	+	+	++	+	+	+	+
BCS410	+	+++	+	+	nd	++	+	+	+	+
BDS50/0	+	+++	+	+	+	++	+	+	+	+
BDS121	-	++	+	+	+	++	+	+	+	+
BDS122	-	++	++	++	+	++	+	+	+	+
BDS123	+	+++	+	+	+	++	+	+	+	+
BDS124	+	+++	+	+	++	++	+	+	+	+
BP16/0	+	+++	+	+	+	++	+	+	+	+
BP17/0	+	+++	+	+	+	+	+	+	+	+

+++: strong reaction, ++: moderate reaction,
+: weak reaction and -: non-binding reaction.

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