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Seroprevalence of Newcastle Disease in Kampong Chickensin Gianyar Regency Bali

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Abstract. Newcastle disease (ND) or *Tetelo* is one of the viral diseases in poultry that causes high mortality and significant economic losses in chicken farms. Newcastle Disease is endemic in Bali as well as in Gianyar. Kerta village is one of the villages in Payangan Subdistrict at Gianyar regency where its location is nearby to the centre of hi breed chicken breeding industry in Kintamani Subdistrict at Bangli Regency and is the poultry trade traffic way from Kintamani to Denpasar. This study aimed to determine the seroprevalence of ND in kampong chickens in the village of Kerta at Gianyar regency. Purposive serum samples were collected from 80 unvaccinated backyard chickens from four (4) *banjar* (Kerta, Pilan, Buhu, and Marga Tengah) in Kerta village. Serological tests using Hemagglutination (HA) and Hemagglutination Inhibition (HI) were performed to the sera samples. Twenty out of the 80 serum samples (25%) were ND positive. The proportion of ND seropositive in each banjar was: 35%, 30%, 30% and 5% at Kerta, Pilan, Buhu, and Central Marga *banjar*, respectively. Twelve out of the 20 seropositive samples (12/20 = 60%) had protective antibodies, whilst the remains were negative. It is concluded that the seroprevalence of ND disease in the kampong chickens in Kerta village was 25% with antibody titres of 2^2 to 2^9 HI units which derived from natural infection.

***Keywords*: seroprevalence, Newcastle disease, chicken, serology**

I. INTRODUCTION

Newcastle disease (ND) is one of the viral diseases that cause deaths in poultry including native chickens. The etiology agent is a virus from Familia Paramyxoviridae, genus of Avian Paramyxovirus type-1 (APMV-1) [22]. The disease can be transmitted from infected poultry through their feces, exudates from eyes and nose which then spread to the environment and infect the surrounding healthy chickens. Within poultry, layers and broilers are sensitive to ND disease. Therefore, vaccination is routinely performed by the farmers to prevent the disease.

In Indonesia, ND also known as Tetelo. In Bali, the disease is also known as Gerubug. Newcastle disease was first discovered in the Newcastle area in 1926 and then spread throughout the world [3]. In the same year, ND in Indonesia is also found in Bogor, West Java. Various types of poultry can be infected by ND including chickens, birds and waterfowl.

The disease infects the gastrointestinal and respiratory tract of poultry causing severe, moderate, mild, or subclinical and clinical symptoms depending on the attacking virus pathotype. Clinically, ND is characterized by diarrhea, sneezing, shortness of breath and snoring. Neurological symptoms are also common in ND with symptoms of tremor to torticollis [16]. Newcastle disease is often confused with Avian Influenza, both diseases have similar clinical symptoms and are endemic in Indonesia [6];[10]. Vaccination in native chickens is rarely done by farmers, however native chickens tend to be more resistant to ND compared to the hi bred chickens.

Unlike the layer and broilers in commercial farms, where the farming system is intensive, the native chickens usually raise as a sideline business for the farmer. The chicken farms in rural area are maintain in traditional system. The chickens are raise as a backyard poultry without proper housing, no vaccination program, and feeding with household waste or kitchen left over. Such farming system plays a potential risk in spreading viral diseases including Newcastle disease since the disease is endemic in Indonesia as well as in Bali.

The native or kampong chickens are mainly raised by farmers in rural areas. This is also commonly found in the community in Kerta Village, Gianyar regency. The location of Kerta Village is adjacent to Kintamani which is the

center of layer farms in Bangli district and is reported to have been experiencing outbreak of ND so that vaccination towards ND was performed in this area [12]. In Kerta village there are several Hindu temples and the community frequently performs Hindu ceremony. This is one of the explanation why the community in Kerta village raise native chickens is to meet the needs for religious ceremonies. Most of the population of Kerta village is crop or livestock/poultry farmers. Almost every family head in Kerta Village own three to ten native chickens. Nevertheless, in general they keep their native poultry as an additional investment. Geographically Kerta Village is the crossing area of trading of laying hens as well as the commodities of layer farms from Kintamani to Denpasar. These conditions make Kerta Village prone to become exposed to poultry infectious diseases including ND [5].

Epidemiology study is necessary in order to map the extent of ND virus in kampong chickens in the field [2]. Surveillance of ND in rural area is important since mostly native chicken farms is found in this area. Sero-surveilance studies need to be conducted to determine ND antibodies titer in native chickens. This study was conducted in Kerta Village, Payangan District, Gianyar Regency. The results of this study will be recommended to the local government for planning, monitoring, and evaluation of ND outbreak prevention program.

II. RESEARCH METHODS

Determination of minimum samples

Kerta Village consists of 7 (seven) *banjar*: (i) Kerta; (ii) Penyabangan; (iii) Marga Tengah; (iv) Saren; (v) Pilan; (vi) Bunteh, and (vii) Seming, respectively. Purposive blood samples were collected from kampong chickens located in four different *banjar* which were selected randomly. Eighty sera samples from unvaccinated native chickens were used in this study. The numbers of minimum samples is calculated using the Thrusfield formula [20]:

$n = 1.92^2 P_{exp} (1-P_{exp}): d^2.$

n = required samples size

P_{exp} = expected prevalence

d = desired absolute precision

Serum preparation

Two ml of blood samples were withdrawn aseptically from the brachial vein using a 3 ml syringe, then left stand in room temperature for collection of serum. The samples were further centrifuged to clarify and remove contaminating red blood cells. Each sera sample were placed in a sterile micro tube [14].

Hemagglutination Test

Hemagglutination test (HA) is performed following the standard recommendation test by OIE [16]. This test is to determine the viral titer required for the preparation of 4 HA units to be used in the HI test. Procedure: Dispense 0.025 ml of PBS in each well of 96 well micro-plate. Then place a standard ND antigen suspension to wells in the 1st and 2nd well, then two-fold serial dilution was performed in wells from the 2nd to 11th. Then added 0.025 ml PBS into each well of micro-plate (starting from the 1st to the 12th), stirred with a micro shaker. Added to each well 0.025 ml 1% red blood cells then gently tap sides of the plate for 30 seconds to mix. Place a cover on the micro-plate and allowed the plate to stand at room temperature and observed every 15 minutes for the formation of agglutination for one hour. The last well that showed complete hemagglutination contains one hemagglutinating unit in 25 µl indicated the virus titer. Following this the virus titer was diluted to equal to 4 HA unit [9].

Hemagglutination Inhibition Test

Hemagglutination inhibition (HI) test was performed following the method of OIE [16]. Serum was tested using HI, a serological test in order to detect the presence of antibodies to ND. The basic principle of HI test is the bond between antibodies and homologous antigens will prevent the attachment of the Paramyxo virus to RBC. Therefore hemagglutination is inhibited when antibodies are present. Procedure of Rapid HI: 0.025 ml of serum was reacted with 0.025 ml of ND 4 HA units and 0.025 ml of 1% erythrocyte. Positive HI test is characterized by the presence of red blood cell deposits at the bottom of the microplate. The HI antibody titer is the highest serum dilution that inhibits hemagglutination [15].

Seroprevalence of ND in Kerta Village Gianyar is the total number of HI test positive samples divided by the total samples collected from native chicken farms in Kerta Village multiplied by 100%.

III. RESULTS AND ANALYSIS

The prevalence of seropositive ND in Kerta village Gianyar regency varied from 5.0% to 35%. Of the 80 kampong chicken sera samplescollected 20 (25%) were HI positive while the remaining 60 samples (75%) were negative (Table 1).

TABLE 1.
DISTRIBUTION OF HI TEST RESULTS FROM 80 KAMPONG CHICKENS AT 4 *BANJAR* IN KERTA VILLAGE, PAYANGAN DISTRICT, GIANYAR REGENCY

No	Name of *Banjar*	HI test		Number of samples	Percentage (%)of seropositive
		Seropositive	Seronegative		
1	Pilan	6	14	20	30%
2	Kerta	7	13	20	35%
3	Buhu	6	14	20	30%
4	Marga Tengah	1	19	20	5%
	Total	20	60	80	25%

Of the 20 HI positive samples, the highest prevalence was found in *Banjar* Kerta (35%, 7/20), followed by *Banjar* Pilan and *Banjar* Buhu (30%, 6/20) and the lowest prevalence was in *Banjar* Marga Tengah (5%, 1/20), respectively.

Based on the results finding where the prevalence of seropositive ND was detected in the native chickens in Kerta village which have never been vaccinated against ND virus indicated that the chickens might have been naturally infected with ND. Kerta village is located in crossing area of shipping poultry and poultry products from Kintamani to Denpasar. This condition might increase the risk of chicken farms in Kerta village being exposed to ND. Newcastle disease can spread directly from infected poultry to the surrounding healthy poultry; or indirectly, via contaminated feed and drinking water [3][8].

The spread of Newcastle virus can also occur through mechanical vectors i.e. mice and insect intermediates [21]. Other risk factors of ND in native chickens in Kerta village including the traditional poultry markets in the transmission of ND viruses within poultry species have been documented [19]. In addition, sub-clinically infected poultry also play a role in transmission of the virus. The relatively low proportion of seropositive ND found in *banjar* Marga Tengah (5%), possibly because of the relatively far distance between the farmer's house. Therefore, it is less supportive in the spread of ND in *banjar* Marga Tengah compared to the others. In general, the seropositive of ND in Kerta village might be due to naturally infection with ND virus in the kampong chickens, since all the chickens sampled in this study have never been vaccinated against ND virus.

The relatively low of the overall seroprevalence of ND virus antibodies in kampong chickens resulted from the uncommonly practice of vaccination against ND virus in traditional farms. Lack of awareness of the farmers regarding the importance of vaccination is close distance between each farmer house which might accelerate the spread of ND. In addition, people's habits of buying live chickens from the market for ceremonial and consumption purposes also play a role in spreading ND. Wild birds have been reported to contribute to the spread of ND, and even rats may also act as reservoirs of the virus [17].

The highest proportion of seropositive ND levels was found in *banjar* Kerta (35%), followed by *banjar* Pilan and Buhu (each 30%). This condition possibly due to naturally infected chickens since the farmer's house in *banjar* Kerta, Pilan and Buhu were located close to the poultry traffic from Kintamani to Denpasar. The people's habits of selling poultry products i.e. eggs and rejected laying hen in market in Kintamani area which were then distributed to Denpasar through the Kerta village also contributed to the chances of air borne spreading of ND virus. The role of also a major threat towards the disease. The traditional farming systems of raising kampong chickens also play a role in the transmission of ND. The pattern of raising kampong chicken significantly affects the risk of becoming infected with ND virus [18].

The limitedness of conducting epidemiological surveys in kampong chickens mainly due to the farmers way of raising freely their chickens in the backyard. Of the 25% (20 of 80 chickens sampled) seropositive to ND mostly (12 of the 20 chickens) were having protective ND antibody titer ($\geq 2^4$ HI titer), whilst the remains (8 of 20 chickens) below 2^4 HI titer.

The range of ND antibody titers of kampong chickens in Kerta village was varied between 2^2 to 2^9, as shown in Table 2. Some of the kampong chickens had protective ND virus antibody titer, the highest titer (2^9 HI units) was found only in two kampong chickens from *banjar* Pilan. Five of the 6 seropositive chickens in banjar

Pilan had ≥ 2^4 HI titer units, where two of the chickens had the highest titer (2^9 HI unit), followed by one and two chickens had titer 2^8 and 2^4 HI unit, respectively. In *banjar* Kerta, 4 of the 7 seropositive chickens had antibody titer ≥2^4 HI unit. In *banjar* Buhu, 3 of the 6 seropositive kampong chickens had protective antibody titer (≥2^4 HI unit). Whereas in *banjar* Marga Tengah, none of the kampong chickens had a protective antibody titer (<2^4 HI unit). Thus, *banjar* Marga Tengah had the highest risk of getting infected with ND virus; on the other hand *banjar* Pilan had the lowest risk of getting ND virus infection when there is an outbreak of ND in the Kerta village.

TABLE 2.
THE NEWCASTLE DISEASE VIRUS ANTIBODY TITER OF KAMPONG CHICKENS IN KERTA VILLAGE PAYANGAN DISTRICT, GIANYAR REGENCY

No	The name of Banjar	Titer of ND virus antibody in seropositive samples						
1	Pilan	2^2	2^4	2^4	2^8	2^9	2^9	
2	Kerta	2^2	2^2	2^2	2^4	2^4	2^7	2^8
3	Buhu	2^2	2^3	2^3	2^4	2^4	2^6	
4	Marga Tengah	2^3						

The protective antibody titer of ND is 2^4 HI unit [5]. Of the 20 seropositive ND samples, 12 (60%) had protective antibodies, while 8 samples (40%) had low ND virus antibody titer (< 2^4 HI unit). Although 60% of the chickens in Kerta Village had a protective ND virus antibody titer, this is considered milt to combat ND virus infection when an outbreak of the disease occurred in the village. Therefore, vaccination is a must in order to prevent ND virus infection in Kerta Village.

Chickens infected with the lentogenic strain virus [11], and the relatively small numbers of antigen that infect the animals resulting in incapability of the body to produce protective antibodies [5] are among the factors contributed to the low antibody titer. Moreover, the possibility of getting a chronic ND infection, which resulted in low ND virus antibody titer. Antibody titers usually can be detected by serum examination at 6-10 days post infection which will reach its peak at 3-4 weeks post infection. Afterwards at approximately 3-4 months later, antibodies will decrease and will not be detected at 8-12 months post infection [1]. This study found that 60% of the kampong chicken had ND virus antibody titer, which suggested that they had been infected with lentogenic strains of ND virus. Chickens infected with lentogenic strains of ND virus usually show mild clinical signs. This explain the likelihood of undetected and unreported ND cases in the Kerta village. In addition, recuperate chickens following infection with the mesogenic strain of ND virus also have ND virus antibody. However, when infected with the velogenic strain the animals will not survive prior to the formation of antibody itself.

The antibody of recuperate chickens following ND virus infection is an active adaptive immunity. The protection given by this immunity is specific and is often referred to as humoral immunity. In addition to adaptive immunity, chickens also have a non-specific immunity obtained naturally and the protection provided is not very strong [7]. When the antigen can pass the non-specific immune system, it will face a macrophage that serves as Antigen Presenting Cells (APC). Macrophages will then present the antigen to T-lymphocytes through the Major Histocompatibility Complex (MHC) molecule. Helper T cells (Th) recognize antigens that bind to MHC II, whereas cytotoxic T cells will recognize antigens that bind to MHC I. The interaction of Th cells with APC plays a role in humoral immunity by inducing the release of cytokines which are soluble factors intercellular communication. This interaction ability can

induce B lymphocyte cell maturation into plasma cells that produce antibodies [13].

Newcastle disease is endemic in Indonesia [5]. Therefore, vaccination strategies should not only be programmed in commercial chicken farms, but would be of significant beneficial if it is implemented in traditional household scale chicken farms

IV. CONCLUSIONS

The seroprevalence of ND in kampong chickens in Kerta Village, Payangan District, Gianyar Regency was 25% with antibody titers varied from 2^2 HI units to 2^9 HI units. The ND virus antibody titer in kampong chickens in Kerta village Gianyar is not derived from vaccination against ND virus but from natural ND virus infection.

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