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Determination of haemagglutination-inhibition antibody titer of newcastle disease vaccinated poultry

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ABSTRACT

New castle disease is a highly contagious viral disease of poultry and other birds causing economic impact with flock mortality rates up to 100%. Antibodies were produced as a result of vaccinating the birds. The study aimed at collecting both whole blood and dried blood (collected on filter paper) samples from vaccinated poultry in different post vaccination period, determining the HI (Haemagglutination-Inhibition) titer of the samples and consequently the efficacy of vaccination, standardization of the filter paper technique for an easier, cheap method of sample collection for HI titer determination, comparing the HI titer of serum and dried blood samples and the influence of post-vaccination period on HI titer.

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KEYWORDS

ND;
Haemagglutinin;
Inhibiting antibody;
Poultry.

INTRODUCTION

The viral disease known as newcastle disease (ND) causes devastating loss to commercial poultry. Newcastle disease first came into international attention in Newcastle Upon Tyne, England, 1926. Later it spread to Indonesia and then to the whole of european and asian countries.

The causative agent-Newcastle Disease Virus (NDV) is a negative, single stranded RNA virus. It belongs to the family Paramyxoviridae. The members of this family possess two surface proteins, Haemagglutinin-neuramidase (HN) which is important for attachment and release of the virus from the host cells in addition to its serologic identification and fusion protein (F) that has a role in pathogenesis. Haemagglutinin neuramidase enzyme is the target for various drug-designing methodologies.

Transmission occurs by exposure to excretions from infected birds and through contaminated food, water, equipments, etc., The incubation period is usually 5-6 days. Digestive, respiratory and/or nervous signs characterize the disease. The severity of the infection is influenced by the strain of the virus^[1] and the age, condition and species of the bird. Clinical signs in poultry range from mild, almost unapparent respiratory disease to very severe depressions in egg productions, increased respiration, profuse diarrhea followed by collapse or long term nervous signs if the bird survives. Mortality rate can be up to 100% in the severe form of disease. Chicken that survive the infection develop a long lasting immunity to further infection by newcastle disease virus. Vaccination for protecting chickens from ND is routinely practiced throughout the world^[2]. Vaccinated birds produce antibodies specific to the virus. The presence of antibodies is detected by serological testing.

Review

The results serve two purposes of assessing the level of circulating antibodies and determining the efficacy of vaccination. Two assays commonly used for serological testing of antibodies are haemagglutination-Inhibition (HI) and enzyme linked immuno sorbant assay (ELISA).

Conventionally whole blood samples are collected using needles and syringes. However, lately alternate methods of sample collection have been developed. One of them is the filter paper technique, which was standardized by this work.

MATERIALS AND METHODS

Sample collection

Whole blood samples were collected from the brachial vein, serum was separated and 1% RBC suspension was prepared using phosphate buffer saline (PBS). Dried blood samples (DBS) were prepared by soaking one end of Whatman No.1 filter paper in blood collected from vein or heel puncture.

Antigen preparation

Live vaccine of NDV-RDVK and *Lasota* strain (1ml of diluent was added to 100 doses of vaccine) was used as antigen in the test. The standard amount of antigen used in the HI tests was 4HA units. This was done by haemagglutination (HA) tests. The dilution factor required to produce 4HA units of antigen was calculated by dividing the dilution factor by 4.

Laboratory Standards

Serum from an unvaccinated chick was used as negative control and the positive control was serum that showed high HI titer.

Elution from filter paper dried blood samples

The end of the filter paper with DBS was punched to get four paper discs (1mm diameter). Different elution methods were employed.

On the basis of amount of PBS used

- Putting two filter paper discs in 0.5ml of PBS (pH 7.2) and agitating vigorously to get a light brown eluate.
- Putting four discs in different amounts of PBS-50 μ l, 150 μ l and 200 μ l of PBS respectively.

On the basis of temperature

- The PBS with four strips were subjected to agita-

tion for 1 hour and incubated overnight at 4°C.

Approximate dilution was calculated by

- **Empirical method-** According to previous works done on filter paper dried blood samples, an approximate dilution was calculated.
- **Globulin quantitation method-** Eluates were treated with ammonium sulfate (final concentration 35%) to precipitate globulin, which was assayed by Lowry's method.
- **Volumetric method-** The volume of whole blood required to load 1mm of filter paper was determined using a micropipette.

Haemagglutination inhibition test

The HI test method was standardized by Hierholzer^[3]. In the wells where antibodies are present there will be haemagglutination inhibition and the red blood cells will settle as a button, which will agglutinate in case of absence of antibodies. The end point of the titration is the well that shows complete haemagglutination inhibition. The antibody levels recorded is expressed as a log base for convenience. For example, a titer of 2⁶ would be 6.

RESULTS

Whole blood and dried blood samples were collected from chickens in different post vaccination period (30, 45, 90, 135 and 150 days). Serum was separated from whole blood samples and 1% RBC (red blood cells) suspension was prepared using phosphate buffer saline (PBS).

HA test was performed and the titer of antigen was found to be 64. From this the diluent to be added was found to be 0.5ml to 30 μ l of antigen. It was back titrated to confirm that the antigen is in 4 HA units.

All the elution methods were used. The only method, which gave reliable results, was by punching 4 discs (each 1mm in diameter), added into 200 μ l of PBS, agitated at room temperature for 1 hour and kept for incubation overnight at 4°C.

Beard et al.^[4] obtained results from dried blood sample using 2 discs of 4.8mm in 200 μ l of PBS and the serum dilution was 1:10. So empirically, using 4 discs of 1mm each (total 4mm) should have a dilution closer to 1/10.

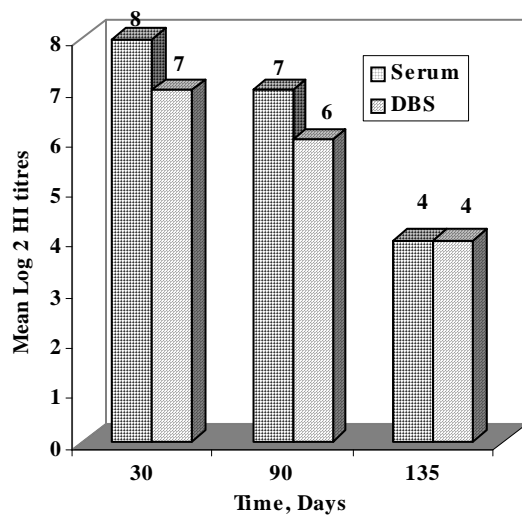
By Lowry's method, globulin in whole blood was 43.3 mg/ml and globulin of dried blood samples was

5.2 mg/ml approximately. The ratio of globulin of the eluate to that of whole blood is 1/8.2. Therefore, serum dilution is 1/8.2.

The volume of whole blood required to load 1mm filter paper disc under stimulated conditions was 10 μ l. So 4 discs in 200 μ l PBS will give an eluate with a whole blood dilution of 40 to 200, i.e., 1/5. Hence volumetrically, if serum portion of whole blood is 60% the dilution of serum will be 0.05-0.06 or 1/8.3.

Antibody dilution by the 3 methods gave values between 1/8.2 to 1/10. So 1/8 was chosen as approximate dilution.

HI test of both serum and eluates were done. HI titers ranged from 2-9 in serum samples and 1-8 in dried blood samples.



Graph showing comparison of both serum and DBS HI titers at different post vaccination period

DISCUSSION

Newcastle disease virus or avian paramyxovirus I may result in substantial loss to poultry industry^[5]. Newcastle disease is now present in almost all continents. Although the virus is currently controlled effectively by vaccination and mass slaughtering, it remains a potential threat to commercial production as it was proven by recent outbreaks in Australia^[6] and Malaysia resulting in mass eradication.

Haemagglutination-inhibition tests are important for both diagnosis and serological studies^[7]. The standardized method for HI test was established long back by Hierholzer^[3]. This serological method of determining HI titer presents difficulties of using needles, syringes and collection tubes and transport, storage, refrigeration, etc.^[8].

The use of filter paper for blood specimen collection was experimented as an alternate for whole blood sample collection in serological method by Brody^[8]. The first work using filter paper in Newcastle disease was by Beard and coworkers^[4].

The antibody dilutions estimated by the empirical, globulin quantitation and volumetric methods ranged from 1/8.2-1/10. This was similar to the work of Beard et al.^[4], where 2 discs of different sizes were used.

The graph of HI antibody titer shows that the antibody level after 30 days is enough to give protection to the birds. This continued until 3 months after which the antibody levels slowly decrease by 5 months but still in the protective range. This is in accordance to the report by Allan and coworkers^[9] that says that the antibody level increases only after 2-3 days of vaccination and stays stationary up to 3 months and HI titer of 3 or more has the capacity to protect poultry against the disease.

Comparing the HI titer of whole blood and dried blood samples, the HI titer of dried blood samples was usually one dilution less but still it could detect the protective levels of antibody in the sample. Hence, the filter paper technique can serve the 2 purposes of assessing antibody level and vaccine efficacy studies.

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