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Raising Hyperimmune Serum Against Avian Paramyxo Virus (APMV-1) and Pigeon Paramyxovirus (PPMV-l) in Rabbits and Their Cross Reactivity

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Abstract: Polyclonal antibodies against avian paramyxovirus-1 (APMV-1) and pigeon paramyxovirus-1 (PPMV-1) were raised in rabbits to examine their diagnostic efficacy against APMV-1 and PPMV-1 infections in birds. Rabbits were divided into two groups A (immunized with APMV-1) and group B (immunized with PPMV-1). An antibody titer of 1:1024 group A against APMV-1 antigen and group B against PPMV-1 antigen were found 1:1024. While final titer of group A was found 1:256 against PPMV-1 and for group B, 1:512 against APMV-1. This study suggests that it is possible to diagnose Newcastle disease and its type by the use of these polyclonal antibodies from the field out breaks. However, the subjected serum must be examined with both antiseras. This will only suggest the type of infection. By the use of these polyclonal antibodies screening at large scale can be done and samples can be selected for further diagnosis using advanced techniques. This will in turn save time and expensive foreign exchange.

Key words: PPMV-1, APMV-1, pigeons, hyperimmune serum, rabbits, cross reactivity

INTRODUCTION

Newcastle disease virus, belonging to the family paramyxoviridae, has a wide host range. It has been reported in chickens, pigeons, turkeys, partridges, pheasants, dove, sparrow, gees, starling and other free flying birds (Vindevogel *et al*., 1982). Newcastle disease is havoc for poultry industry due to high mortality and morbidity rate through out the world. Along with chicken, Newcastle disease is a serious problem in pigeon in Pakistan (Arshad, 1984). There are many strains of ND virus out of them some cause 100% mortality while other produces moderate disease, leading serious reduction in egg production (Alexander, 1997). Newcastle disease in pigeon is caused by Pigeon paramyxovirus serotype-l (PPMV-1) that is a variant of paramyxo viruses causing Newcastle disease in poultry (APMV-1) (Alexander *et al*., 1985; Kaleta *et al*., 1985).

Due to its worldwide occurrence, the disease drew the attention of research workers for an effective control. For monitoring and diagnosis purposes H.I. tests are quite effective (Brugh *et al*., 1978; Cernik *et al*., 1985). The diagnosis of any disease is the first and foremost requirement which requires known serum against this disease. Cross-reaction between pigeon PMV-l and chicken PMV-1 occures in hemagglutination inhibition tests using polyclonal antisera. However, pigeon PMV-l and NDV are readily distinguishable using NDV monoclonal antibodies (Gelb *et al*., 1987).

Keeping in mind the fact that imported antiserums and monoclonal antibodies are very expensive, a need was felt to produce a quality antiserum (polyclonal antibodies) against both (APMV-l and PPM V-I) and to compare their cross reactivity alongwith their diagnostic efficacy.

MATERIALS AND METHODS

This study was conducted at the Department of Veterinary Pathology, University of Agriculture, Faisalabad in 2005.

Production of antigen: For production of polyclonal antibodies against chicken origin Newcastle disease (APMV-I), La Sota strain of Newcastle disease vaccine (Fort dodge, USA) was used by inoculating in 10 days old chicken embryonating eggs via allantoic route and was designated as group A. While pigeon origin virus (PPMV-l) was collected from field outbreak in pigeons and was designated as group B as described above. The Allanto-amniotic Fluid (AAF) of the embryos died after 36 h from both groups (A and B) was collected (Graham *et al*., 1989). Pigeon origin virus (group B) was further confirmed by using monoclonal antibodies as described by (Pedro, 1986). The viruses having HI activity 1:128 or more were selected from both groups. Selected virus suspensions were centrifuged at 8000 RPM for 10 min, supernatant was collected and debris was removed.

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Table 1: Inoculation schedule

Injection (day)	Inoculum type	Quantity of inoculum (mL) Group A	Group B
0	AAF[1]	0.5	0.5
14	AAF	0.5	0.5
21	AAF	0.5	0.5
28	AAF+IFA[2]	0.2	0.2
42	AAF+IFA[2]	0.2	0.2

1 Allanto-amniotic fluid, 2 Allanto-amniotic fluid+Incomplete Freund's adjuvant

Supernatant thus collected was used for priming of the rabbits and also for the production of booster antigen (Iqbal *et al*., 2003) for both groups. Booster dose was prepared by mixing equal quantity of incomplete Freund's adjuvant (Sigma) to the antigen. The inoculum prepared was given 0.2 mL per rabbit (Jurd and Hansen, 1990).

Inoculation to rabbits: Ten adult rabbits were procured and dewormed to rule out any parasitic infestation. Serum from all animals was subjected to observe any heme agglutination activity after inactivation at 56°C for 30 min. Then they were divided into two groups randomly A and B (five rabbits in each group). The virus preparation was injected as in Table 1.

Blood samples from the rabbits were taken periodically to observe their antibody titer against antigens given by H.I. test.

RESULTS AND DISCUSSION

Antibody production is a complex biological process. It is not always possible to obtain same results described by others. For some purpose a single injection may be sufficient but in general, higher antibody titers are obtained by administering a series of injections (Cruickshank *et al*., 1968). A number of vertebrate species ranging from farm animals to rabbits, small laboratory rodents and chickens have been used over the years (Carpenter, 1975). Rabbits are the single most frequently used species because of convenient size, easy to bleed, relelatively long life span and produce adequate quantities of antisera. Moreover, they are diverged significantly from avian species so they are naturally best choice (Leeuw de and de Greeve, 1996).

Immune response of rabbits of both groups (A and B) immunized against Avian Paramyxovirus-I (APMV-I) and Pigeon Paramyxovirus-1 (PPMV-I) respectively is given in Table 2.

Antibody titer of rabbits against both groups was found nil at zero and seven days post inoculation. However, it gradually increased by the passage of time to a level of 1:1024 in both groups, i.e., group A (immunized with APMV-1) against APMV-1 and group B (immunized with PPMV-1) against PPMV-1 after 91 and 77 days post challenge, respectively. The increase in antibody level after 77 days was considerable due to repeated injections of two booster doses having Incomplete Freund's Adjuvant (IFA). This is also described by other workers as antibody formation is enhanced by use of certain adjuvant substances. They are supposed to prolong the exposure of antigen to the immune system, protecting it from degradation and enhance the immune response by attracting and stimulating the immune system cells (Jennings, 1995). One has to always consider the potential of adjuvants to cause pain and stress to the animal. Many adjuvants can be toxic to animals and can cause significant pathologic lesions. Incomplete Freund's Adjuvant (IFA) is a water-in-oil emulsion of mineral oil and surfactant. Clinically it appeared that the adjuvant did not cause considerable pain in the rabbits. It has been strongly recommended by Jurd and Hansen (1990). These findings are also supported by Iqbal *et al*. (2003) and Kaeberle (1986), who observed that antiserum plus adjuvant permits much smaller use of antigen and greatly enhances the antibody titer compared with antigen without adjuvant.

Table 2: Immune response against respective antigen

Injection (day)	Geometric Mean Titer Group A with APMV-I	Group A with PPMV-I	Group B with APMV-I	Group B with PPMV-I
0	NIL	NIL	NIL	NIL
7	NIL	NIL	NIL	NIL
21	1:64	NIL	NIL	1:64
35	1:64	1:16	1:16	1:128
49	1:128	1:64	1:32	1:256
63	1:256	1:64	1:64	1:512
77	1:512	1:128	1:256	1:1024
91	1:1024	1:256	1:512	1:1024

Immune response of rabbits from group A, when titrated against PPMV-1 antigen remained zero till 21 days and it went upto 1: 256 after 91 days post challenge, while immune response of the rabbits of group B against APMV-1 remained zero till 21 days and it went upto 1: 512 after 91 days post challange (Fig. 1). This finding is suggestive of the fact that although there is a cross reactivity among the both groups but the extent of reaction was not similar in both groups when examined with different antigens. This fact is suggestive of the reason that there might be some antigenic differences among the both antigens. This is also supported by Gelb *et al*. (1987).

This study suggests that it is possible to diagnose Newcastle disease by the use of these polyclonal antibodies from the field out breaks. To confirm the type of infection, (AMPV-1 or PPMV-1) the subjected antigen must be reacted with both type of antibodies at same time.

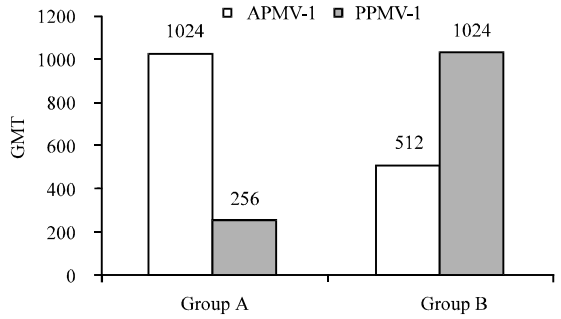

Fig. 1: Final immune response in rabbit serum against APMV-1 and PPMV-1 antigens

This will only suggest the type of infection, but for confirmation one has to go for the use of monoclonal antibodies or other advanced diagnostic techniques. However, by the use of these polyclonal antibodies screening at large scale can be done and samples can be selected for further diagnosis using advanced techniques. This will in turn save time and expensive foreign exchange.

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