Effect of oral and intra-rectal Infectious Bursal Disease Vaccination on immune response against Newcastle Disease in chicks

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Abstract: The aim of this study was to ascertain the best vaccine regimen for infectious Bursal Disease virus (IBDV) vaccination. It also determined the best regimen for IBDV vaccination using oral and intra-rectal routes, which will minimize the effect of IBDV on Newcastle Disease virus (NDV) vaccine response. One hundred and twenty Isa-brown day old cockerels were divided equally into ten groups, which were each subdivided to two subgroups of six birds each. The groupings were based on the ages in days pre-inoculation. All the birds were given normal Newcastle disease vaccination of Hitchner B1, Lasota and Komarov vaccines. Six groups were given single inoculation of IBDV vaccine, while four groups were given double inoculation of the IBDV vaccine. Each group received the IBDV vaccine by two different routes of oral and intra-rectal. Single IBDV inoculation using oral route was observed to be the best for IBDV vaccination, which will minimize the effect of IBDV on NDV vaccine response. Serologically, double intra-rectal IBDV inoculation on the 14th and 28th days against IBDV infection was the best vaccine regimen. Histological, the bursa of fabricus of the birds with single intra-rectal IBDV inoculation showed severe disintegration of follicles, necrosis of cells and loss of lymphoid cells.
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1. Introduction

Newcastle disease (ND) is caused by specified viruses of the avian paramyxovirus type 1 (APMV-1) serotype of the genus *Avulavirus* belonging to the sub-family paramyxovirinae, family paramyxoviridae. The paramyxoviruses isolated from Avian species have been classified by serological testing into nine serotypes designated APMV-1 to APMV – 9; ND virus (NDV) has been designated APMV-1 (Alexander, 2003). The disease is spread worldwide affecting various species of poultry and other birds (Alexander, 2003; Alexander 1995; Kouvenhoven, 1993).

Since its recognition in 1926, ND is regarded as being endemic in many countries. Cases of ND has been variously reported in Nigeria (Iroegbu and Amadi, 2004; Musa et al., 2009), Iran (Hassanzadeh and Bozorgmeri Fard, 2004) and South Africa (Ananth et al., 2008). Prophylactic vaccination is practiced in all but a few of the countries that produce poultry on a commercial scale.

NDV differs in virulence and has been grouped into 5 pathotypes: velogenic viscerotropic, velogenic neurotropic, mesogenic, lentogenic and asymptomatic (Beard et al., 1981). Iroegbu and Amadi (2004) reported the recovery of lentogenic, mesogenic and velogenic pathotypes of NDV from avian species as well as pond water sample in Nsukka, Nigeria.

Infections bursal disease (IBD) is a contagious disease of fowl caused by double stranded RNA Virus. Infectious bursal disease virus (IBDV) is of major importance in all poultry producing regions of the world. It is highly infectious in young chickens and causes severe damage to the bursa, resulting in suppression of the immune system as well as the possibility of significant morbidity and mortality (Van Den Wijngaard et al., 2001). Only infected chickens show clinical signs but no clinical signs had been found in other infected avian species (Wyeth, 2000).The clinical signs of infected chickens relate to the age of the chickens and the virulence and the strains of virus. The classical strain of IBD virus (IBDV) belonging to serotype 1, shows the ability to cause clinical disease in infected chickens (Rautenschlein, et al, 2003).The clinical signs are depression, reluctance to move, ruffled feather, loss of appetite, watery diarrhea and death 2-3days after the clinical disease has been diagnosed (Chansiripornchai and Sasipreeyajan, 2005). The major symptoms of clinical diseased chickens are dehydration, petechial hemorrhage at the thigh and breast muscles or juncture between the proventriculus and the ventriculus. Bursa of fabricus of infected chickens reveals inflammation, edema, and hemorrhage or atrophy depending on the course of the infection (Chansiripornchai and Sasipreeyajan, 2009; Sharma et

al, 2000). The infection caused by the variant strain of serotype 1 reveals non clinical disease (Elankumaran, et al, 2002). Although Heamagglutination inhibition screening using chick embryo has been the age long traditional method of testing for NDV, some works has shown that the use of Vero cell line, ELISA and/or reverse transcriptase polymerase chain reaction are more rapid, specific and sensitive (Mehedi et al., 2002; Tabidi et al., 2004; Mohammed et al., 2005). Important methods of IBD prevention and control in the poultry industry are disinfection, biosecurity and vaccination at the appropriate time (Chansiripornchai and Sasipreeyajan, 2009). The present study aimed at determining the best regimen of IBD vaccination using intra-rectal and oral routes which will minimize the effect of IBD on NDV vaccine response. It also assessed the degenerative changes in the bursa of fabricus induced by inoculation of infectious bursal disease vaccine given.

2. Materials and Methods

Experimental Birds

One hundred and twenty Isa-brown day old cockerels were brought for the research work. They were fed with starter and growers mash respectively. The birds were also given vitamins as food supplements. The birds were reared intensively in deep litter systems in an animal house.

Grouping

The one hundred and twenty day old cockerels were grouped into ten groups consisting of 12 birds per group. Each group consisted of two sub-groups with each of the sub-group consisting of six birds. These groups included six groups for single vaccination which were groups (7), (14), (18), (21), (24), (28) and four groups for double vaccination which were groups (1+14), (7+21), (10+24) and (14+28). The groupings were based on the ages in days pre-inoculation.

Vaccine source

Newcastle disease vaccines (NDV) – Hitchner B1, Lasota, komarov and infectious bursal disease vaccine (IBDV) produced by the National veterinary Research institute (NVRI), VOM in Plateau State, Nigeria, were used for the research work. Each of the vaccines were reconstituted as recommended by the manufacturer in phosphate buffered solution using a sterile syringe and needle. These constituted vaccines were given to the 120 birds. The vaccines were reconstituted just before inoculation.

Inoculation of Experimental Birds

Inoculation with Newcastle Disease Vaccine

NDV Hitchner BI was given intraocularly to all birds on the first day, NDV Lasota vaccine on the 14th day intraocularly and NDV Komarov vaccine intramuscularly on the 28th day of the birds arrival.

Inoculation with Infectious Bursal Disease Vaccine (IBDV)

Reconstituted Gumboro vaccine was inoculated by intrarectal and oral routes giving two different routes for the two different sub-groups in each group (one route per sub-group).About 0.2ml of reconstituted Gumboro vaccine was inoculated into each of the birds according to specification of ages in days. The groups inoculated were as follows: (7), (14), (18), (21), (24), (28) for single inoculation and groups (1+14), (7+21), (10+24) and (14+28) for double inoculation. The groupings were based on the ages in days pre-inoculation.

Collection of Blood samples from Birds

Using 2 mls syringes and 26G needles, blood samples were collected from all the birds through the jugular veins. The zone of insertion was properly swabbed with cotton wool, soaked in methylated spirit before collecting the blood. The syringe containing the blood sample was slanted and allowed to clot and retract for a good yield of serum. The serum was then aspirated from the syringes into sterile labelled vials and stored in a freezer at – 20^{o}C. The serum samples were used to test for antibodies against infectious bursa disease virus using Agar Gel immuno-diffusion test (AGIDT). Blood collection was at weekly intervals for 5 weeks post IBD virus inoculation.

Harvesting of Bursa of fabricus

On the 5th and 10th days post infection respectively, one bird from each group was slaughtered and bursa of fabricus surgically obtained for gross and histopathological examination. The bursa tissues were preserved in 10mls of 10% formalin (formal saline). Uninfected bursa of fabricus was also collected and preserved. The gross examination was made before fixing in 10% formal saline. The bursa samples were histologically processed, embedded in paraffin wax sectioned at 5µ thick using rotary (Glick, 1983).

Serology

Haemagglutination inhibition technique was used to detect Newcastle antibody (Allan and Gough, 1974) and Agar – gel immuno-diffusion technique (AGIDT) was used to detect antibody production to IBD virus by the experimental birds. (Culler and Wyeth, 1975, Marguardt et al 1980; Thayer and Beard 1998).

3. Results

3.1. Effects of single oral IBD inoculation

3.1.1 Response to Newcastle Disease

Newcastle disease HI antibody was detectable throughout the five week post IBD inoculation assay at between $\log_2$ values of 2 to 10 but 2/3 between 5 and 10. Newcastle disease antibody titre generally increased from the 7th day post vaccination to the fourth week before declining. (FIG. I).

3.1.2. Response to Infectious Bursa Disease

IBD inoculation at days 7 and 14 failed to produce detectable precipitating antibody till the fifth week post inoculation. Except for inoculation on day 28 which produced IBD antibody from week 2,all other single IBD inoculation produced antibody from the third week post inoculation and antibody persisted till the end of assay in the fifth week. (TABLE 1)

3.2 Effects of double oral IBD inoculation

3.2.1 Response of Newcastle Disease

Newcastle disease antibody was also detectable throughout the five weeks following the booster IBD vaccination. However ND antibody titre were generally lower than those of single inoculation ranging between 2 and 8 but with ¾ being between 2 and 5. (FIG. 1)

3.2.2. Response of Infectious Bursa Disease

All the booster inoculation regimen did not produce precipitating IBD antibody after one week of inoculation. Inoculation given on days I and 14 did not give antibody response throughout the five weeks of post – inoculation assay. All other double inoculation produced antibodies from the second week which lasted till the end of assay in the 5th week. (TABLE 1)

3.3. Effects of single intra-rectal IBD inoculation

3.3.1. Response of Newcastle Disease

HI antibody against ND were present throughout the period of post IBD vaccination assay ranging from log2 value 1 to 10 with about 2/3 ranging between 4 and 10. ND antibody titre rose from 4 at age of 7 days to 10 at 3 weeks and declined to 5 in the 5th week (FIG. 1)

3.3.2. Response of Infectious Bursa Disease

Except for the 28th day inoculation which showed detectable antibody only at the 4th and 5th weeks post vaccination, no other single inoculation showed evidence of seroconversion. (TABLE 1)

Effects of double intra-rectal IBD inoculation

Response to Newcastle Disease

Newcastle disease HI antibody was detectable throughout the five weeks post IBD inoculation assay at between $\log_2$ value of 1 to 8 with 4/5 between 3 and 8. The antibodies were generally lower than those of single inoculation.

3.4. Response to Infectious Bursa Disease

Only the 14+28 days inoculation produced antibody from one week after vaccination and persisted till the end of assay at the 5th week. Inoculation made on days 10 and 24 produced detectable antibody from the third week whereas those made on days 1+14 and 7+21 showed detectable antibody in the 5th week post inoculation (TABLE 1).

3.5. Gross Pathology of Bursa of Fabricus

Bursa of uninfected birds

The Bursa of fabricus of uninfected birds appeared whitish, ovoid shaped and measuring up to 0.5cm in diameter. The cut surface displayed several lobulation.

3.6. Bursa of Experimental birds

The colour of all the bursa in the experimental birds had changed from creamy white colour by the 5th day to grey colour by the 10th day.

Reductions in size were noted from the 5th to the 10th day post infection. Gelatinous surface was evident on the surface of all infected bursa. All displayed several lobulation on sectioned surfaces.

3.7. Histopathology Examination of Bursa of Fabricus of uninfected birds (Normal control)

Bursa of uninfected bird displayed large active follicles separated by clear stroma with little inter-follicular tissue. Lymphoid cells were distinguished within the follicles.

3.8. Bursa of infected birds

Three types of histological lesions were distinguished among the bursa of infected birds (TABLES 2 & 3). Patchy infiltration of the stroma by inflammatory cells.. This was noted in groups 28, (14+28) inoculated intra-rectally (TABLE 3) and groups 18, 24, 28, (17+21) (10+24) and (14+28) inoculated orally (TABLE 2).

Generalized lesion with mild necrosis and minimal disintegration of follicle . This was noted in groups 7, (1+14), (7+21) inoculated intra-rectally (TABLE 3) and groups 7, 14, (1+14) inoculated orally (TABLE 2).

Severe disintegration, necrosis and loss of follicles . There were noted in groups 14, 18, 21, 24 that received infectious bursal disease virus single dose intra-rectally (TABLE 3) and group 21 orally (TABLE 2).

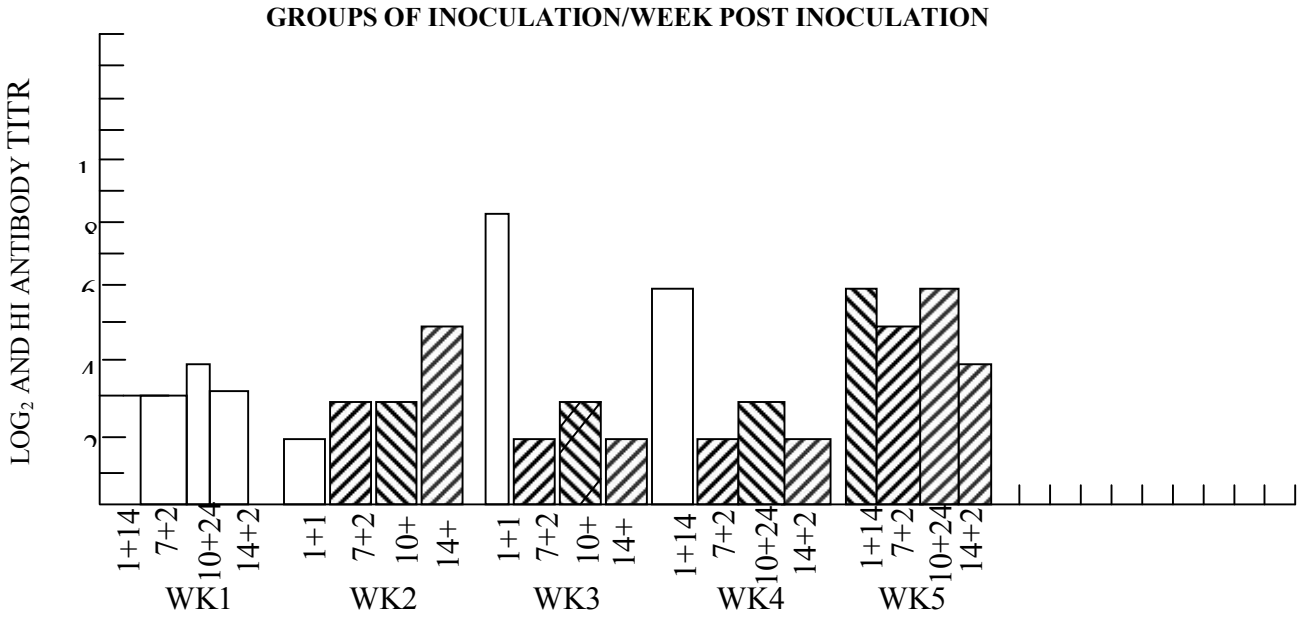

FIG1: NEWCASTLE HAEMAGGLUTINATION INHIBITION TITRE AT WEEKLY INTERVAL POST INOCULATION FOR THE VARIOUS GROUPS OF BIRDS INOCULATED WITH IBDV VACCINE BY ORAL ROUTE

KEY: PLAIN – NEGATIVE IBD ANTIBODY
STRIPED – POSITIVE IBD ANTIBODY

Table 1: Agar Gel Inmmuno-Diffusion Test (Agidt) At Weekly Interval Post Inoculation For The Various Groups Of Birds Inoculated With Ibdv Vaccine By Oral And Intra-Rectal Routes.

AGESIN DAYS PRE-INOCULATION	ORAL	INTRA-RECTAL	ORAL	INTRA-RECTAL	ORAL	INTRA – RECTAL	ORAL	INTRA – RECTAL	ORAL	INTRA-RECTAL
GRP (7)	_	_	_	_	_	_	_	_	+	_
GRP (17)	_	_	_	_	_	_	_	_	+	_
GRP (1+14)	_	_	_	_	_	_	_	_	_	_
GRP (18)	_	_	_	_	+	_	+	_	+	_
GRP (21)	_	_	_	_	+	_	+	_	+	_
GRP (7+21)	_	_	+	_	+	_	+	_	+	_
GRP (24)	_	_	-	_	+	_	+	_	+	_
GRP (10+24)	_	_	+	_	+	+	+	+	+	+
GRP (28)	_	_	+	_	+	_	+	+	+	+
GRP (14+28)	_	+	+	+	+	+	+	+	+	+

Table 2: Histopathological Examination of Bursa of Birds Inoculated Orally with Ibd Virus

DESCRIPTION OF BURSAL LESIONS	GROUPS OF EXPERIMENTAL BIRDS SINGLE DOSE IBDV	DOUBLE DOSE IBDV
Patchy infiltration of the stroma by inflammatory cells.	18,24,28	(7+21), (10+24)
Generalized lesions with mild necrosis and minimal disintegration of follicles	7, 14	(14+28), (1+14)
Severe disintegration of follicles necrosis of cells and loss of lymphoid cells and follicles.	21	None

Table 3: Histopathological Examination of Bursa of Birds Inoculated Intra-Rectally with Ibd Virus

DESCRIPTION OF BURSAL LESIONS	GROUPS OF EXPERIMENTAL BIRDS SINGLE DOSE IBDV DOUBLE DOSE IBDV	
Patchy infiltration of bursal stroma by inflammatory cells.	28	(10+24), (14+28)
Generalized lesions with mild necrosis and minimal disintegration of follicles	7	(1+14), (7+21)
Severe disintegration of follicles necrosis of cells and loss of lymphoid cells.	14, 18, 21, 24	None

4. Discussion

In this study, it was observed that ND HI antibody was detectable throughout the five weeks in both oral and intra – rectal routes, post IBD inoculation assay. ND antibody titre were generally lower in birds with double oral IBD inoculation and also in birds with double intra – rectal IBD inoculation when compared to those with single IBD inoculation. This may be as a result of the interference of the second IBD inoculation, which may have led to necrosis of the lymphocytes in the medullary area of the lymphoid organs resulting in the suppression of both humoral and cell mediated immune response (Lukert, 1992,Ritter 1982, Park, et al, 2009). Single oral IBD inoculation showed longer NDV antibody response in birds when compared with double intra-rectal IBD inoculation post vaccination. This was observed to be the best for IBV vaccination which will minimize the effect of IBV on NDV vaccine response.

On the response to IBD, it showed no detectable antibody in the first few weeks of both oral and intra – rectal routes. This may be the effect of maternal derived antibody. The maternal derived antibody of chickens can impede the virus in vaccine infected to the target cells and also reduces the ability of the virus in vaccine to stimulate the chicken's immune system (Chansiripornchai and Wanasawaeng, 2009).

However the maternal derived antibody is of the benefit to IBDV infection (AL – Natour et al 2004). Detectable antibody were noticed in double oral IBD inoculated birds from the 2nd week to the 5th week and in single oral IBD inoculated birds from the 3rd week to the 5th week. Murphy et al (1999), reported that the half – life of maternal antibodies is approximately 3 days, while Engstrom et al, (2003) reported that maternal protection often last 2 – 4 weeks after hatching. This explains why IBD antibodies were notice from the 2nd week.

Serologically, double intra-rectal IBD inoculation of the 14th and 28th days of the ages in days pre-inoculation was observed to be the best IBD regimen. The gross examination of bursa from the infected birds showed changes in colour from white to grey and decreased in size as diseased progressed from 5th to the 10th day post inoculation. Atrophy was noted in 14, 18, 21, 24 ages in days pre-inoculation that received IBD intra-rectally and 21 ages in days pre-inoculation (Orally). Atrophy is a sign of serve damage to bursa of fabricus by infectious bursal disease. Atrophied bursa often have intense loss of the bursal stroma and of the follicular micro-environment that sustains B cells differentiation. This probably underscores the consequent severe immune- depression.

The histopathology of bursa of fabricus infected by IBD have been studied by Peters, (1967). Earlier workers regarded IBD as a lymphocidal disease with histologic lesion on lymphoid structure, especially the bursa of fabricus. In the present study, the bird that was not infected by IBD virus displayed bursa with large active follicle separated by clear stroma. The lymphoid cells form discrete follicles. The infected bursa on the other hand showed various degrees of lesions according to the severity of the infection on bursa. Patching infiltration of the bursa stroma by the inflammatory cells was a mild inflammatory lesion noted in all. The histopathology of bursa is related to the bursal antibody response (Abdel et al,2001; Maas et al,2001; Nielson et al,1998;). IBD virus is tolerated more as the birds get older. Also (10+24) (14 + 28) and (7+21) ages in days pre-inoculation that received IBD orally had mild lesions. These ages in days pre-inoculation received double dose IBD. Although bursal lesion is commonly the same in all, their response to antibody depended in ages at IBDV application. Response is earlier in those that received IBDV later in age. Moderation lesions described in this work as generalized infiltration of inflammatory cells were noted in 7, (1+14), (7+21) ages in days, pre-inoculation that received IBDV intra-rectally.

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References

1. Abdel–Alim,G.A.,Saif,Y.M. Immunogenicity and Antigenicity of very virulent strains of infectious bursal disease viruses. Avian Disease 2001; 45(1): 92 – 101.
2. Alexander,D.J.Newcastle disease, other avian Paramyxoviruses and Pneumovirus infections:Newcastle disease.In: Diseases of poultry, Saif,Y.M., ed. Iowa State University Press, USA, 2003; 64 – 87.
3. Alexander,D.J. The epidemiology and control of avian influenza and Newcastle disease.J.Comp. Pathol, 1995;112, 105 – 126.
4. Al–Natour,M.Q.,Wrad, L.A., Brown, B.S.,and Keck, L.D. Effect of different level of maternally derived antibodies on protection against infectious bursal disease virus. Avian Dis. 2004; 48(1): 177 – 182.
5. Ananth, R., John Kirubaharan, J., Priyadarshini, M.L.M and Albert, A. Isolation of NDV of high virulence in uninnoculated village chickens in South Africa. Intl Jour Poultry Sci, 2008;7(4): 368-373
6. Beard,C.W., and Hanson,R.P. Newcastle disease. In Disease of poultry, Eighth Edition, Hofstad, M.S., Bames, H.J., Calnek,B.W., Raid, W.M. and Yoder, H.W., eds. Iowa state University Press, Ames, Iowa, U.S.A, 1981; 452 – 470.
7. Chansiripornchai,N. and Sasipreeyajan, J. The efficacy of intermediate and intermediate plus infectious bursal disease vaccine for the preventions of infections bursal disease in boilers. Thai J. Vet. Med. 2005; 34(4): 91 – 96.
8. Chansiripornchai,N. and Sasipreeyajan, J. Comparison of the efficacy of the immune complex and conventionally live vaccine in boilers against infectious bursal disease infection. Thai. J. Vet. Med. 2009; 39(2): 115 – 120.
9. Elankumaran,S.,Heckert,R.A. and Moura,L. Pathogenesis and tissue distribution of a variant strain of infectious bursal disease virus in commercial boiler chickens. Avian Dis. 2002; 46(1): 169 – 176.
10. Engstrom,B.,Eriksson, H. Fossum, O., and Jansson, D. Fjaderfasjukdomar, Sveriges Veterinarmedicinska Anstalt. 2003.
11. Hassanzadeh, M and Bozorgmeri Fard, M.H. A serological study of Newcastle Disease in pre- and post vaccinated village chickens in North of Iran. Intl Jour Poultry Sci, 2004; 3(10): 658-661
12. Iroegbu, C.U and Amadi, E.S. Ecological and Serological evidence of enzootic circulation of Newcastle Disease virus strains among free-roaming avian species in Nsukka environment, Nigeria. Bull Anim Hlth Prod Afr. 2004; 52: 13-20.
13. Konvenhover,B. Newcastle disease. In: Virus infections of Birds, Elsevier Science Publishers B.V. 1993; 341 – 361.
14. Lukert, P.D. Infectious bursal disease. In : A.E. Castro and W.P. Henschelle (Ed) Veterinary diagnostic Virology : a practitioners guide. Mosby year book publishers, Missouri., 1992; 35 -36.
15. Maas, R.A., Venema, S., Ori, H.L., Pol, J.M.A. Efficacy of inactivated infectious bursal disease (IBD) Vaccines, comparison of serology with protection of progeny against IBD virus strains of varying virulence. Avian Pathol. 2001; 30 (4); 345 – 354.
16. Mehedi, M., Hossain, K.M., Taimur, M.J.F.A., Sil, B.K and Islam, M.R. Heamagglutination antigen preparation of Newastle Disease Virus on Vero cell line. Jour Biol Sci, 2002; 2(10): 648-649.
17. Mohammed, A.M., Aradaib, I.E., Khairalla, K.M.S., Abdalla, M.A., Karrar, A.R.E and EIHussein, A.R.M. Evaluation of RT-PCR for rapid detection of Sudanese isolation and vaccine strains of NDV. Pakistan Jour Biol Sci, 2005; 8(3): 418-420
18. Murphy, F.A., Gibbs, E.P.J., Horzinek, M.C., and Studdert, M.J. Veterinary Virology, 3rd Academic Press.1999.
19. Musa, U., Abdu, P.A., Dafwang, I.I., Umoh, J.U., Sa'idu, L., Mera, U.M and Edache, J.A. Seroprevalence, seasonal
20. Occurrence and clinical manifestations of Newscastle Disease in rural household chickens in Plateau State, Nigeria. Intl Jour Poultry Sci, 2009; 8(2): 200-204.
21. Nielson,O.L.,Saerensen,P.,Hedemand,J.E.,Laursen,S. B. Inflammatory response of different chicken lines and B haptotypes to infection with infectious bursal disease virus. Avian Pathol. 1998; 27; (2); 181 – 9.
22. Peters,G. The histology of Gumboro disease infected birds. Berlin. Muench. Tieraertel. Wochschr, 1967; 80; 394 – 96.
23. Rautenschlein,S.,Yel,H.Y., and Sharma,J.M.,Comparative immuno-pathogenesis of mild intermediate, and Virulent Strain of classic infectious bursal disease virus. Avian Dis. 47(1); 66 – 78.
24. Ritter,J.On vaccination against Gumboro. Poultry international, 1982; 68 – 70.
25. Sharma,J.M.,Kim,I.J.,Rautenschlein,S., and Yeh,H.J. Infectious bursal disease virus of chicken: Pathogenesis and immuno-suppression. Dev. Comp. Immunol. 2000; 24 (2-3): 223 – 235.
26. Tabidi, M.H., Makkawi, A., Mahasin, E and Ali, A.S. Comparative evaluation of HA test and ELISA for detection of antibody against ND vaccine in broiler chicks. Intl Jour Poultry Sci, 2004; 3(10): 668-670.
27. Van den Wijngaard,J.K.,Avakian,A.,Whutfill,C., and Haddad, E. In: IBD vaccinations surpass conventional approach. World Poult. Elsev., 2001;16 – 17.
28. Wyeth,P.J. Infectious bursal disease (Gumboro disease). In: Manual of standard for Diagnostic Test and vaccines, 4th ed, Office International Des. Epizooties, Paris, France.2000; 647 – 656.

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