

Full Length Research Paper

Oral Vaccination of Chickens against Infectious Bursal Disease (IBD) Using Parboiled Rice as Vaccine Vehicle

J. R. Lawal¹, A. G. Balami¹, A. M. Bello¹, Y. Wakil¹, S. Y. Balami², Y. Audu¹, Y. M. Lekko¹ and U. I. Ibrahim¹

¹Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, P M B 1069, Maiduguri, Borno State, Nigeria.

²Poultry Unit, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Maiduguri, P M B 1069, Maiduguri, Borno State, Nigeria.

Accepted 4th July, 2016

Abstract

Chickens were vaccinated against infectious bursal disease (IBD) by feeding them with IBD vaccine applied on parboiled rice at different time intervals (30 min, 1 hour, 2 hours, 3 hours and 4 hours) post reconstitution of the vaccine. There was no significant variation ($P>0.05$) in the antibody titre between those vaccinated at different time intervals of 1, 2, 3 and 4 hours post reconstitution of the vaccine and those vaccinated through drinking water. The vaccine virus also spread to the in-contact chickens. Thus, IBD vaccine delivered on parboiled rice could provide a practical method of protecting chickens especially village chickens against the disease in Nigeria.

Key words: Infectious bursal disease, Infectious bursal disease vaccine, Village chickens, parboiled rice.

INTRODUCTION

Village chickens (*Gallus gallus domesticus*) are the only livestock species that are widely accepted by people from a wide variety of cultures and religious background (Echeonwu *et al.*, 2007; Meseret, 2010; Dontwi *et al.*, 2011). They also serve as a source of good protein which supplement human protein requirement (Copland and Alders, 2005; 2009; Dontwi *et al.*, 2011; Nyoni and Masika, 2012). The rural household keeps a few chickens of local breeds mainly on free range system of management but sometimes simple housing and supplementary feeding is provided to safeguard their health and improve production (Ideris *et al.*, 1990; Sonaiya, 2007). In most African countries, the village chickens kept in the villages has no regular health control

program, may or may not have shelter and scavenge for most of their nutritional needs (Pedersen *et al.*, 2002; Aboe *et al.*, 2006; Lawal *et al.*, 2014).

According to Alganesh *et al.* (2003) and Negussie *et al.* (2003) the low productivity of the local scavenging hens is not only because they are low producers of small sized eggs and slow growers but also the system is characterized by its high chick mortality before they reach around 8 weeks of age. Under the free range system of management, village chickens are maintained virtually without much cost and they provide meat and eggs which serve as source of income to the farmers because of good price paid for them (Leony and Jalaludin, 1982; Yakubu, 2010).

The enormous increase in human population especially in developing countries such as Nigeria has necessitated the need for increase in animal production more especially poultry to meet the increasing need for protein requirement (FAO, 2010). However, several factors limit poultry production in Nigeria especially those of diseases,

particularly coccidiosis, helminthiasis, bacterial and viral diseases resulting in losses due to mortality and morbidity (Biu and Etukwudo 2004; Luka and Ndams, 2007).

In Nigeria, the poultry population outnumbered all other forms of livestock and not surprisingly, the village chickens are found throughout the country where ever there is human settlement. Although other birds such as pigeons, ducks, guinea fowls and sometimes turkeys are also widely kept, chickens are by far the most common (Acamovic *et al.*, 2005; Ajala *et al.*, 2007). Typically, they are maintained under traditional low input, free range system of management but substantial numbers are also reared intensively on a commercial basis, particularly in the southern states (Ibrahim and Tanya, 2001) where commercial holdings account for some 10 million chickens or 11% of the total estimated population of 82.4 million (Duru *et al.*, 2008; Nnadi and George, 2010).

Infectious bursal disease (IBD) is a viral disease of young chickens between the ages of 3 – 6 weeks which in its acute form, is characterized by sudden onset, short course with a sharp mortality, peak and extensive destruction of lymphocytes in the Bursa of Fabricius and other lymphoid tissues (OIE, 2004; Thrusfield, 2005; Woldemariam and Wossene, 2007; Khan *et al.*, 2007). Mild forms of the disease also occur, and inapparent infection has been reported (Gordon, 1979, Mai *et al.*, 2004; Tesfaheywet *et al.*, 2012). Vaccination is the most practical procedure for prevention of the disease (Mai *et al.*, 2004; Sule *et al.*, 2013; El-Yuguda *et al.*, 2014). A variety of attenuated vaccines made of classic and variant, and propagated in embryos or tissue cultures are commercially available. Live viruses have varying degrees of attenuation and are recognized as mild, intermediate or hot. Also available are inactivated vaccines made of classic and variant viruses and propagated in chicken embryos, tissue culture or bursa of fabricius. The primary objective of this work was to assess the efficacy of parboiled rice as a potential carrier for the IBD vaccine and also to determine the effect of time post- reconstitution on the responses of chickens to IBD immunization using feed as vaccine vehicle.

MATERIALS AND METHOD

Sources of Vaccines

The vaccines used for this study was an avirulent IBD virus vaccine which was obtained from the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria with Batch Number 62/2005 and 61/2005.

Experimental Birds

A total of 71 chicks comprising of 18 rural chicken and 53 exotic cockerels, chicks were used for the experiment.

The rural chicks were sampled from different households within Maiduguri Metropolis while the exotic chickens (Cockerel chicks) were obtained at day-old from ECWA hatcheries Jos, Plateau State.

Housing and Feeding

The village chickens were on semi – intensive farming system with little or no feeding supplementation. The cockerel chicks were housed in cages measuring 99cm length x 84 cm width by 61 cm height with 8 birds per cage. They were fed chick mash within their first week of arrival and later introduced to local par-boiled rice mixed at different proportions with chick mash. The parboiled rice gradually replaced the chick mash by 2 weeks of age.

Experimental Procedure

Vaccination

The village chickens were bled once prior to vaccination to obtain a baseline data. The IBD vaccine with batch No. 61/2005 was diluted in 2 litres of chlorine free drinking water and was administered orally at 10 ml / bird. The cockerel chicks were bled at days 7 and 17 prior to vaccination to obtain a base line. Using the protocols described for thermos table Newcastle disease Vaccination using feeds (Spradbrow and Samuel, 1991), 60 ml of normal saline was used to reconstitute IBD vaccine with batch No. 62/2005. Out of the reconstituted vaccine, 10 ml was used to mix with 100 grams of par-boiled rice for each of the experimental groups. The par-boiled rice mixed with the vaccines was kept at room temperature for 30 minutes, 1 hour, 2 hours, 3 hours and 4 hours respectively before administering to the respective groups. The in-contact were not vaccinated but were kept with each group of the vaccinated birds.

Sample Collection

The experimental birds were bled on days 7, 14, 21, 28, 42 and 56 post vaccinations. The blood samples in each case were centrifuged at 1500 rpm for 10 minutes to separate the sera. All the serum samples were kept in sterile nunc tubes at -120°C until tested.

Agar Gel Precipitation Test (AGPT)

Preparation of the Agar

The agar was prepared by mixing agarose (1g) sodium chloride (8g) and sodium azide (0.1g) in distilled water (qrs 100ml). The mixture was heated until there was complete dissolution. The molten agar was then dispensed into petri dishes and allowed to solidify. Well were cut on each plate using the ohcterlony template.

Table 1. Precipitating antibodies to IBDV in chickens vaccinated with IBD vaccine using parboiled rice as vaccine vehicle at different time intervals

	Time interval post reconstitution									
	30min	1hr	2hrs	3hrs	4hrs	30i	1i	2i	3i	4i
Day 0	-	-	-	-	-	-	-	-	-	-
Day 7	-	-	-	-	-	-	-	-	-	-
Day 14	-	-	-	-	-	-	-	-	-	-
Day 21	-	-	-	-	-	-	-	-	-	-
Day 28	1:64	1:64	-	1:8	1:4	1:8	-	1:8	-	-
Day 42	1:8	1:64	1:64	1:64	1:64	1:64	-	1:64	1:64	1:64
Day 56	-	-	-	-	-	-	-	-	-	-

Key: 30i: = In contacts for 30 minutes post reconstitution

1i = " " 1 hour "

2i = " " 2 hours "

3i = " " 3 hours "

4i = " " 4 hours "

Table 2. Antibody response of chickens to vaccination against IBDV vaccine administered through drinking water to different age groups of chickens.

	Adult	Growers	Chicks
Day 0	-	-	-
Day 14	1:32	1:32	1:32
Day 21	1:4	1:8	1:16
Day 42	-	-	1:2
Day 56	-	-	-

The bottom of the wells were sealed using some of the molten agar.

Antigen

The antigen used was a macerated bursa of an IBD virus infected chicken. The antigen was used to screen for antibodies against IBD virus.

Test Procedure

The sera collected from the experimental birds were assayed for the presence of antibodies against IBD virus using Agar Gel Precipitation Test (AGPT) as described earlier by Hirai *et al.*, (1972). The AGPT was performed by placing the sera into surrounding wells and the IBD virus then inoculated at 37°C in a humid chamber. The plates were observed daily for lines of precipitation for 72 hours.

RESULTS

The response of chickens to IBD vaccine administered through par-boiled rice at different time intervals following reconstitution of the vaccine is presented in Table 1. The result showed that the vaccine stimulated a protective

immunity against IBD at day 28 post vaccination (PV).

All the groups showed a latent period of immune response between days 0 and 21 P.V. At day 28 PV, the highest antibody titre of 1:64 was obtained from the group that received the reconstituted rice vaccine at 30 minutes and hour post reconstitution (PR) respectively, while the lowest titre of 1:4 was obtained from the group that received the rice vaccine at 4 hours PR. Both the in contacts of 30 minutes and 2 hours PR also developed antibody (Ab) titres of 1:8 respectively.

At day 42 PV, the lowest Ab titre of 1:8 was obtained from chickens that received vaccination with parboiled rice at 30 minutes PR. The highest Ab titre of 1:64 was obtained in all the groups with the exception of the in contact group for 1 hour which recorded no Ab titre (Table 1). The chicken vaccinated via drinking water exhibited an Ab titre of 1:32 at day 14 PV for all the age groups. At day 28, the highest Ab titre was obtained from the chicks while the lowest Ab titre of 1:4 was obtained in the adult group. By day 42 PV, Ab titre of 1:2 was exhibited by the chicks while the adult and the growers developed no Ab to the vaccination (Table 2).

DISCUSSION

Vaccination of chickens with IBD vaccine has been reported

to produce satisfactory immunity against the disease (Abdu *et al.*, 1986; El-Yuguda *et al.*, 2014). However, the vaccines have several limitations especially in village poultry where individual handling of birds is not practicable. Spradbrow *et al.*, (1987) demonstrated the practical application of using food pellets as vehicle for Newcastle Disease Vaccine. Earlier studies, (Spradbrow *et al.*, 1991; Ibrahim *et al.*, 2001) have shown that chickens vaccinated against Newcastle Disease (ND) using parboiled rice as vehicle developed HI – antibodies comparable to other chickens that received the same vaccination through drinking water. This suggests that feeds could be used as alternative means of administering poultry vaccine more especially to village chickens.

The result of this study has shown that feeds such as parboiled rice could be used as vehicle for administering IBD vaccine to chickens. Although, the latent period of response to the vaccination by chickens was about 21 days, however, a higher titre of precipitating antibodies was obtained between days 28 and 42 post vaccination (PV) with the in-contacts developing antibody titre of 1:64 which is comparable to those that ate the vaccine coated parboiled rice. The relatively prolonged latent period of antibody response observed in this study could be due to some factors which are not yet understood. However, other workers (Tantaswasdi *et al.*, 1992; Ibrahim *et al.*, 2000) have reported that factors responsible for differences in ND HI antibody response stimulated by ND vaccines on different feed types including parboiled rice could be due to the differences in their rate of digestion, and absorption due to hard and smooth surfaces of the feeds which may not be compatible with viral adhesion. The use of other grains as vaccine vehicles for administering IBD vaccine may be tried in other to establish whether the longer latent period of immune response developed by parboiled rice could be bridged.

Challenge experiments are also needed to ascertain the protectiveness of IBD vaccine on feeds. This study demonstrated the potentials of IBD vaccine administered on parboiled rice for chickens and the great promise the methods hold for the village chicken. The use of parboiled rice as vehicle for administering IBD vaccine could be more advantageous over the use of drinking water because most village chickens are used to early morning feeding and this would make it easier for the vaccine incorporated feed to be consumed early before the vaccine virus in the feed gets inactivated, whereas if the vaccine is given in drinking water, only a small percentage of the chicken may take the water within the required time because of availability of surface water in many areas (Tantaswasdi *et al.*, 1992).

CONCLUSION

IBD has been one of the most economically important diseases of poultry worldwide. The economic losses due

to IBD mortalities and immunosuppression in Nigeria specifically amounts to billions of Naira annually. The outbreak of the disease has remained the biggest nightmare of poultry production. Amongst which are the lower economic class; the small scale holder families and poultry producers who are predominantly the poorest rural women and their families.

Hence, to forestall future outbreaks, the result of this study could go a long way in reducing the gigantic loss due to the devastating effect of the disease.

RECOMMENDATION

Other grains such as maize, millet, guinea corn etc should be tried as vehicles for administering IBD vaccine in chicken so as to ascertain their effectiveness and to establish whether the latent period of immunity observed in this study could be bridged.

REFERENCES

- Abdu, P. A. (1986), An Out break of Gumboro Disease in Vaccinated flocks in Zaria. *Zariya Vet.* 1:40 –41.
- Aboe, P. A. T., Boa-Amponsem K., Okantah, S. A., Butler, E. A., Dorward, P. T. and Brant, M. J. (2006). Free-range village chickens on the Accra Plains, Ghana: Their husbandry and productivity. *Tropical Animal Health and Production*, 38: 235-248.
- Acamovic, T., A. Sinurat, A. Natarajan, K. Anitha, D. Chandrasekaran, D. Shindey, N. Sparks, O. Oduguwa, B. Mupeta and A. Kitalyi, (2005). Poultry. In: Owen *et al.* (Ed.), *Livestock and Wealth Creation: Improving the husbandry of animals kept by resource-poor people in developing countries.* Nottingham University Press, Nottingham, pp: 301- 322.
- Ajala, M. J., Nwagu, B. I. and Otchere, E. O. (2007). Socioeconomics of free-range poultry production among agro pastoral women in Giwa Local Government Area of Kaduna State, Nigeria. *Nig. Vet. J.*, 28: 11-18.
- Alganesh Tola, Matewos Belisa and Gizaw Kebede (2003), Survey on traditional livestock production system. pp. 141-150. *Proceeding 11th Annual Conference of Ethiopian Society of Animal production, Addis Ababa, Ethiopia, August 28-30, 2003.*
- Allan, G. M. McNutty, M. S., Connor, T. J., Mccracken, (1984), Rapid diagnosis of IBD by Immunoflorescent. *Avian Pathology* 13: 419-429.
- Benton, W. J, Cover M.S, Rosenberger, J. K. and Lake, R. S. (1967). Studies on the transmission of IBD of chicken. *Avian disease* 14:430-438.
- Biu, A. A. and Etukwudo, J. (2004): Cestodes of the guinea fowl (*Numida meleagris geleata*) in Borno State Nigeria. *Nigerian Journal of Experimental and Applied Biology*, 5: 2.
- Cernik, R. (1983). Thermostability of a vaccine strain of Avian infections bursitis virus, Philadelphia. Pp. 56 – 81.
- Chettle, N. J. C., Stout, J. C. and Wyeth, P. J. (1989). Outbreak of virulent infectious bursal disease in East Anglia. *Vet. Rec.*, 125: 271 – 242.
- Copland, J. W. and Alders, R. G. (2005): The Australian village poultry development Programme in Asia and Africa. *World's Poultry Science Journal*, 61: 31 – 37.
- Copland, J.W. and Alders, R.G. (2009). The Comparative Advantages of Village or Smallholder Poultry in Rural Development. In: *Village Chickens, Poverty Alleviation and the Sustainable Control of Newcastle Disease.* Proceedings of an international conference, Dar es Salaam, Tanzania, October 5-7,

2005. Proceedings No 131. 11-14. Canberra: Australian Centre for International Agricultural Research (ACIAR).
- Cosgrove, A. S. (1962). An apparently new disease of chickens-Avian necrosis, nephritis and Gumboro disease L and M news views 3:103.
- Dontwi, I. K., Odoro, F. T., Amponsah, S. K., Owusu-Ansah, E. and Appiah, D. (2011). Dynamics and Control of Bacteria Poultry Diseases: A Case Study of Ashanti Region in Ghana. *American Journal of Scientific Research*, 14: 40-46.
- Duru, S., Saidu, L., Akpa, G. N., Jokthan, G.E., Kabir, M., Olugbemi, T. S., Abdu, S. B., Yashim, S. M. and Hamman, I. (2008): Prevalent disease in Local Poultry: A case study of Zaria area, Kaduna state. In: *Proceedings of the 13th Annual Conference of the Animal Science Association of Nigeria (ASAN)*, pp. 683– 686.
- Echeonwu, G. O. N., Iroegbu, C. U., Echeonwu, B. C., Ngene, A., Olabode, A. O., Okeke, O. I., Ndako, J., Paul, G., Onovoh, E. M., Junaid, S. A. and Nwankiti, O. (2007). Delivery of thermostable Newcastle disease (ND) vaccine to chickens with broken millet grains as the vehicle. *African Journal of Biotechnology*, 6 (23): 2694-2699.
- El-Yuguda, A. D., Baba, S. S. and Geidam, Y. A. (2014). Specific antibody response of village chickens to single or combined Newcastle disease and infectious bursal disease vaccines. *J. Adv. Vet. Anim. Res.*, 1(1): 16-20.
- FAO, (2010). Poultry meat and eggs: Agribusiness Handbook. Director of Investment Centre Division, FAO., Rome, Italy, pp: 77.
- Faragher, J. T. (1972). Infectious Bursal disease of chickens. *Vet. Bull.*: 361 – 369.
- Hirai, K. and Calnek, B. W. (1979). In vitro replication of infectious bursal disease virus in established Lymphoid cell lines and chicken B-lymphocytes infect. *Immun.* 25: 964-970.
- Ibrahim, U. I., El-Yuguda, A. D., and Tambari, P. S. (2000). Trial of feed-Base 1 Newcastle Diseases "Lasota" Vaccine in Chickens Using feeds as vaccine vehicle. *Nigerian Journal of Experimental and Applied Biology*, 1(2): 83-86.
- Ideris, A., Latif Ibrahim, A. and Spradbrow, P. B (1990). Vaccination of chickens against Newcastle disease with a food pellet vaccine. *Avian Pathology* 19:2, 371 – 384.
- Jordan, F. T. W. and Pattison, M. (1996). Poultry disease fourth edition. Pp 199-203.
- Khan, M. A., Siddique, M., Hassan, I., Rahman, S.U. and Arshad, M. (2007). Adaptation of local wild infectious bursal disease virus in chicken embryo fibroblast cell culture. *Int. J. Agric. and Biol.*, 9: 925-927.
- Komolafe, O. O., Anazodo, N. I and Aiyenogun, J. A (1990). A comparative prophylactic and therapeutic efficacy of groundnut oil/penicillin and palm oil/penicillin mixtures in treatment of infectious bursal disease of chickens. *Zariya Vet* 5(2): 78-83.
- Lawal, J. R., Jajere S. M., Bello, A. M., Mustapha, M., Wakil, Y., Ndahi, J. J., Mustapha, F. B., Paul, B. T., Gulani, I. A., Ibrahim, U. I., Geidam, Y. A., Ambali, A. G. and Waziri, I. (2014). Prevalence of Infectious Bursal Disease (Gumboro) Antibodies in Village Chickens in Gombe State, Northeastern Nigeria. *International Journal of Poultry Science* 13 (12): 703 – 708.
- Leony, E. and Jalaludin, S. (1982). The poultry industries of S.E Asia. The need for an integrated farming system of small poultry producers. *World's poultry science journal*, 38: 213-219.
- Luka, S.A. and Ndams, I.S. (2007): Gastrointestinal parasites of domestic chicken *Gallus gallus domesticus* Linnaeus 1758 in Samaru, Zaria, Nigeria. *Science World Journal*, 2(1):27 – 29.
- Mai, H. M., Ogunsola, O. D. and Obasi, O. L. (2004). Serological Survey of the Newcastle Disease and Infectious Bursal Disease in Local Ducks and Local Guinea Fowls in Jos, Plateau State, Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 57 (1-2): 41-44
- Mellroy, S. G., Gordall, E. A., Rice D. A. McNutty, M. S. and Kenedy, D. G. (1993). Improved performance in commercial broilers flocks with subclinical infectious bursal disease when fed diets containing increased concentration of vitamin E. *Avian disease*. 22: 81-94.
- Meseret, M. B. (2010). Characterization of village chicken production and marketing system in Gomma Wereda, Jimma zone, Ethiopia. M. Sc. Animal Sciences (Specialization: Animal Production) Thesis Submitted to the Department of Animal Science, Jimma University, College of Agriculture and Veterinary Medicine, School of Graduate Studies Jimma, Ethiopia, August, 2010.
- Meulemans, G. and Halen, P. (1981). Efficacy of some disinfectants against infectious bursal disease virus and avian retrovirus. *Veterinary record* 111:112-413.
- Negussie D., Alemu Y., Tadelle D. and Samuel W. (2003). On farm and on station evaluation of the "hay box chicken brooder" using different insulation materials at the Debrzeit Agricultural Research Center and Denbi Village Adaa Woreda. PP. 211-216. *Proceeding Annual Conference of 10th Ethiopian Society of Animal Production*, August 21-23/2003.
- Nnadi, P.S. and George, S.O. (2010): A Cross-sectional survey on parasites of chickens in selected villages in the sub humid zones of south-eastern Nigeria. *Journal of Parasitology Research*, 1 – 19.
- Nyoni, N. M. B. and Masika, P.J. (2012). Village chicken production practices in the Amatola Basin of the Eastern Cape Province, South Africa. *Afr. J. Agric. Res.*, 7: 2647-2652.
- Ogbogu, D. A. (1988). Coping with UPS and DOWNS in Poultry production. *Nigeria livestock farmer (Journal of NVRI)* 8:16.
- OIE, (2004). IBD (Gumboro). OIE manual for diagnostic techniques of livestock disease. Paris, France. Pp 496-506.
- Okeke, E. N. and Lamorde, A. G. (1988). Newcastle disease and its control in Nigeria. In: *viral disease of Animal in Africa* (A. O. Williams, and W. M. Masinge Edited) CTA of ACP/EEC (lome convention). Pp. 283-300.
- Pedersen, C. V., Kristensen, A. R. and Madsen, J. (2002). On farm research leading to a dynamic model of traditional chicken production system. In: *Proceedings of the joint 17th Scientific Conference of the Tanzania Society for Animal Production and the 20th Scientific Conference of the Tanzania Veterinary Association held in Arusha, Tanzania on 3rd to 5th December, 2002*. Pp. 237-247.
- Pettit, J. R., Gough A. W. Gagnon, A. N. (1975). Infectious bursal disease of chickens (Gumboro disease). <http://www.medarchives/site1/en/GumboroDisease.htm>
- Rosenberger, K. J. and Gelb, J. (1978). Response to several avian respiratory viruses as affected by infectious bursal disease virus. *Avian disease* 22: 95-105.
- Saif, Y. M. (1984). Infectious bursal disease virus types. In: *Proceedings of the 19th National meeting of poultry health condemnation*, Ocean city. Pp. 105 – 107
- Saif, Y. M. (1998). Food animal research programs, the Ohio Agricultural research and development centre. *Poultry science* 77: 1186 –1189.
- Sonaiya, E. B. (2007). Review article: Family poultry, food security and the impact of HPAI. *World's Poult. Sci. J.*, 63: 132-138.
- Spradbrow, P. B. (1987). The isolation of Newcastle disease an overview. In: *Newcastle disease in poultry. A new food pellet vaccine*: Edited by Joyn W. Copland, Australian centre, for international Agricultural Research. Ramsay ware printing Melbourne. Pp. 255-262.
- Spradbrow, P. B. and Samuel, J. C. (1991). Oral Newcastle disease vaccination with V4 virus in chicken comparison with other routes. *Australian veterinary journal* 68 (3): 55-64.
- Sule, A. G., Umoh, J. U., Abdu, P. A., Ajogi, J., Jibrin, U. M., Tijjani, A. O. Atsanda, N. N. and Gidado, A. S. (2013). A serological survey for infectious bursal disease virus antibodies among village chickens in Yobe State, Nigeria. *Int. J. Agric. Sci.*, 3: 596-598.
- Tantaswasdi, U. Dan Vivatanaoim, J. Siriwan, P. Chaisingh, A. and

- Spradbrow, P. B. (1992). Evaluation of an Oral Newcastle disease vaccine in Thailand. *Preventive veterinary medicine* 12:89 – 94.
- Tesfaheywet Z, Hair-Bejo M, Rasedee A (2012). Hemorrhagic and Clotting Abnormalities in infectious bursal disease in specificpathogen- free chicks. *World Appl. Sci. J.* 16(8):1123-1130.
- Thrusfield, M. (2005). *Veterinary Epidemiology* 3rd ed. Black well science Ltd, London. Pp 178 -236.
- Winterfield, T. W., Hitchner, S. B. and Cosgrove, A. S. (1962). Avian necrosis, nephritis and Gumboro disease L and M news views 3: 103.
- Woldemariam S. and Wossene A. 2007. Infectious bursal disease (Gumboro Disease): Case report at Andasa poultry farm, Amhara region. *Ethiopian Veterinary Journal*, 11:141-150.
- Wyeth, P. J. and Cullen, G. A. (1979). The use of an activated infectious bursal disease oil emulsion vaccine in commercial broiler parent chicken *Vet. Rec.* 104: 188-193.
- Yakubu, A. (2010): Indigenous chicken flocks of Nassarawa State, Nigeria: Their characteristics, husbandry and productivity. *Tropical and Subtropical Agroecosystems*, 12 (1): 69 – 76.