IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 13, Issue 12 Ser. III (December 2020), PP 55-61
www.iosrjournals.org

Effect of Thermo Stable Newcastle Disease Vaccine on Productivity of Free Ranging Indigenous Chicken

Mali B[1], Nakavuma J L[1], State A[1] and Byarugaba D K[1]
College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University Kampala, Uganda
Corresponding Author: Mali B

Abstract: *The purpose of this study was to establish the effect of vaccination against Newcastle disease using I-2 thermo stable vaccine, on the productivity of free range chicken. It was carried out in Bulyansiime village, Iganga District, Uganda. A baseline survey was conducted to determine the serological status and flock dynamics of the free range chicken. This was followed by blood sample collection and vaccination of adult birds after every 3 months for a period of one year. Haemagglutination Inhibition tests were performed on serum samples derived from blood collected, to determine the level of antibody titers against New Castle Disease Virus. Flock production data per household was recorded alongside the vaccination and blood sample collection exercise. Flock production data derived was compared with those obtained from an unvaccinated group of birds in a neighboring village (Kikunu) at the end of the study. Analysis of variance was used to determine significant differences in geometric mean antibody titers and flock size of vaccinated birds. Independent sample t test was used to determine significant differences in flock size between the vaccinated and unvaccinated flocks. IBM SPSS statistics, version 24 was used for statistical analysis. Results were presented graphically and in tables. There was a significant difference ($p<0.05$) in geometric mean antibody titers of the experimental flock, which increased from log_2 2.0±1.22 to log_2 5.57±0.82. The level of protection of the flock derived from the baseline data was 32% and increased to 83% and 87% after administration of the vaccine in the first and second quarter of the study respectively. A 100% level of protection was attained after the third and fourth vaccinations. An increase in flock size from 10.83±0.64 to 23.44±0.89 birds per household was observed after one year. Significant differences ($p<0.05$) were observed between the mean flock sizes of vaccinated group (23.44±0.89) and unvaccinated group (10.88±0.46) at the end of the study. Vaccination of free ranging chicken against New castle Disease using I-2 thermo stable vaccine led to an increase in their antibody titers to levels that offered them protection against NCD. Use of the vaccine increased flock productivity by enhancing survivability of the flock.*

Date of Submission: 15-12-2020 Date of Acceptance: 29-12-2020

I. Introduction

Free range poultry play a significant role in the lives of poor rural communities in developing countries. Besides their use in cultural and religious ceremonies, they provide animal protein in form of meat or eggs and can be sold to generate income that enables rural households cater for necessities like tuition for children, medical care and clothes. Free range poultry, mainly owned by the rural poor, are a stepping stone towards asset accumulation because earnings from their sale enable households invest in crop and other livestock enterprises like goats and cattle (Dolberg 2003). In addition, the authority and responsibility of looking after poultry is left to the women so income generated from the birds acts as additional income that they use in provisioning for the household (Alders and Pym, 2009).

Village based projects have exhibited the potential of free range poultry to further transform the lives of rural poor through improved methods of management that offer protection to chicks for the first two months and vaccination against New Castle disease (NCD), a highly contagious disease (Alexander 1997). Vaccination against New Castle disease leads to a reduction in poultry mortality (Alexander et al 2004). In order to ensure success of New Castle disease control programs, village based projects have included supply of NCD vaccines with adequate shelf life and active involvement of farmers in the vaccination programs (Alders et al 2010). Monitoring of these vaccination programs and diagnosis of New Castle disease is enhanced by the use of serological tests adapted to the conditions where vaccination is carried out (Tabidi et al 2004).

II. Materials and Methods

Study area: The study was carried out in Bulyansiime village, Igombe Sub County, Iganga District, Uganda.

Research design: A longitudinal study was carried out. Only birds reared under free ranging system were included in the study.

Sample size determination

The sample size of birds for the study was calculated (Ariola 2006) as:

$n = N/ (1+Ne^2)$

Where n= Number of samples

N=Total population of birds

e = margin of error

Using a 95% confidence interval (0.05 margin of error) the sample size was;

$n = 2000/ (1+2000*0.05^2) = 333$ birds

Using simple random selection, blood was drawn from every 6^{th} bird picked from the population. A sample size of 333 birds was maintained throughout the study.

Baseline survey, serum collection and vaccination

A baseline survey to establish the flock demographics was carried out with the aid of a questionnaire. A respondent from each of the 110 households, was interviewed to determine flock numbers per household, starting from one end of the village to the other. Farmers were asked a day before to restrain their birds for the blood collection and vaccination exercise. Majority kept them locked up in the houses while others tethered them in gardens. In order to establish baseline serological status of birds, door to door blood sample collection was carried out. Birds then received I-2 thermostable NCD vaccine. A maximum of 2mls of blood was collected aseptically from the wing vein of adult birds, using a 2.5ml non-auto lock syringe with a 23-gauge needle and placed in 4ml plain vaccutainer. The samples were placed on a bench for 12 hours to allow clotting of blood. The clear sera was collected and stored at -20°C and used to carry out Haemagglutination inhibition tests. I-2 Thermo stable NCD vaccine was administered through eye droplet method, using 5ml syringes with 21 gauge needles. All birds received the vaccine. Subsequent vaccinations were done concurrently with sera collection after every 3 months for a period of one year.

Haemagglutination inhibition tests

The antigen used for the test was reconstituted I-2 Thermo stable vaccine. Initially, 0.025 ml of Phosphate buffered Saline (PBS) was dispensed into each well of a plastic V-bottomed micro titer plate up to the 8^{th} well. This was followed by 0.025 ml of the sera sample which was placed in the first well and two fold dilutions of 0.025 ml volumes of the sera were made up to the 7^{th} well. 0.025ml of 4HAUs of antigen (I-2 vaccine) was dispensed to each well across the plate. The solution was mixed gently and allowed to settle for 30 minutes at room temperature. 0.025 ml of 1% (v/v) chicken red blood cells was dispensed to each well. The solution was mixed by tapping the plate gently and allowed to settle for 40 minutes at a room temperature of 25°C. The wells for each sera sample in which red blood cells settled as pinpoint buttons at the bottom of the well were recorded as positive. The HI titer for each serum sample was expressed as a reciprocal and transformed to $\log_2$. Titers $\geq \log_2 3$ were considered protective (Grimes, 2002).

Flock production data

Quarterly flock production records were taken during the vaccination and sera collection exercise for the households participating in the study, using a simple form that was tailored to this study. Flock deaths, number of cocks, hens and chicks were recorded. After one year, flock production data of vaccinated birds was compared with data derived from households with unvaccinated flock from a neighbouring village.

Statistical analysis

Using SPSS, analysis of Variance (ANOVA) was carried out to determine significant differences in mean antibody titers, flock size and deaths obtained at the different points in time during the study. Independent sample t test was used to determine significant difference in mean flock size between vaccinated and unvaccinated birds. Post Hoc analyses using Least significant Difference (LSD) was carried out. IBM SPSS statistics, version 24 was used for statistical analysis. The results were presented graphically and by means of tables.

III. Results

Serological status of the flock

The level of flock protection against New Castle Disease was established through determination of antibody titer levels of the flock through haemagglutination inhibition tests carried out on sera samples obtained from the birds. The protection levels obtained pre vaccination (baseline) was 32%. Levels of protection achieved after the first and second vaccination were 83% and 87% respectively. After the third and fourth vaccination, the level of protection was 100%. The Geometric mean titer value (GMT) increased from 2.0± 1.22 to 5.57±0.82 as shown in figure 1. Post hoc analysis revealed significant differences ($P<0.05$) in geometric mean titers throughout the study.

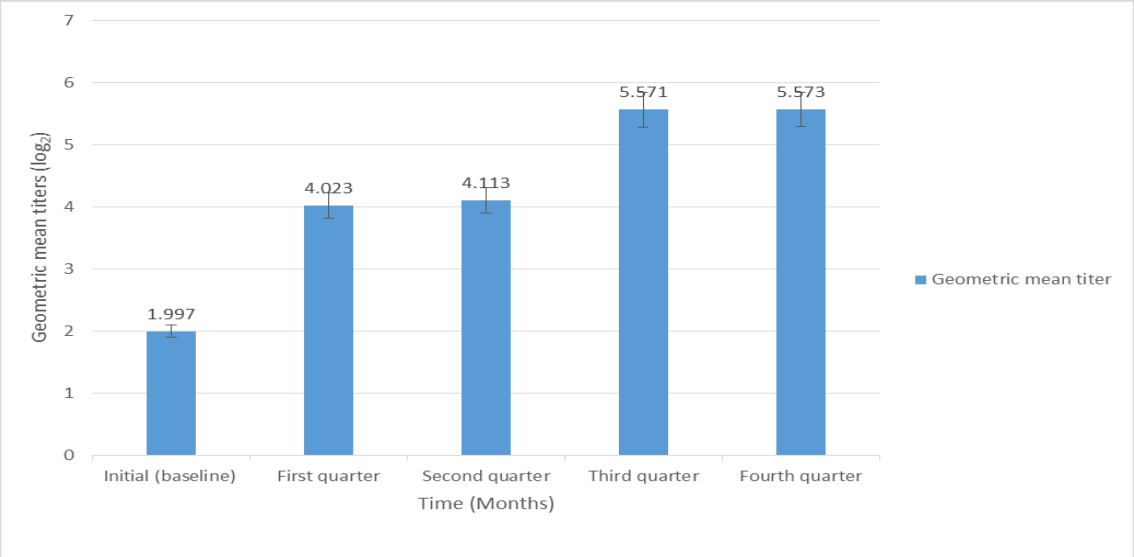

Figure 1: Geometric mean titers against time

Flock production

Flock production data was taken concurrently with the vaccination and sera collection exercise. Generally, the flock size increased from 10.83±0.64 to 23.44±0.89 birds as shown in table 1.

Table 1: Average flock sizes and deaths per household

Period	Initial (Baseline)	1st quarter	2nd Quarter	3rd Quarter	4th Quarter	P value
Flock size	10.83±0.644[a]	17.11±0.776[b]	15.93±0.78[b]	20.11±0.882[c]	23.44±0.886[d]	0.000
Cocks	0.90±0.067[a]	0.99± 0.071[a]	1.36±0.103[b]	1.42±0.095[b]	2.03±0.116[c]	0.000
Hens	3.81±0.266[a]	5.26±0.341[b]	6.56±0.471[b]	10.09±0.669[c]	12.47±0.649[d]	0.000
Chicks	6.12±0.483[a]	10.86±0.562[b]	8.02±0.521[c]	8.60±0.499[c]	9.05±0.522[c]	0.000
Flock deaths	4.31±0.285[a]	2.08±0.189[b]	3.91±0.289[c]	3.44±0.236[c]	3.31±0.236[c]	0.000

Mean and SEM values

[a, b, c, d] values on the same row with different superscript are significantly different at p=0.05.

Post Hoc analysis revealed significant differences in reported flock deaths and chick numbers in the first and second quarter of the year. Significant differences ($p<0.05$) in flock size were observed in first, third and fourth quarter. Significant differences in number of cocks ($p<0.05$) were observed in second and fourth quarter while significant differences ($p<0.05$) in hen numbers were observed in first, second, third and fourth quarter of the year. Significant differences ($p<0.05$) were observed in the household flock sizes and deaths among vaccinated and unvaccinated groups by the last quarter of the study as shown in table 2.

Table 2: Average household flock sizes and deaths for unvaccinated and vaccinated groups by the last quarter of the study

	Unvaccinated	Vaccinated	P value
Flock size	10.88±0.455	23.44±0.886	0.000
Cocks	1.05±0.071	2.03±0.116	0.000
Hens	4.65±0.273	12.47±0.649	0.000
Chicks	5.17±0.318	9.05±0.522	0.000
Flock deaths	8.08±0.437	3.31±0.236	0.000

Mean ± SEM values

IV. Discussion

The study showed that birds in the village were sporadically or never vaccinated against New castle Disease. The low protective titer of 32% and Mean HI titer of $\log_2$ 2.0±0.12 obtained at baseline showed that antibodies against NCD existed within the flock and was an indication that the birds could have earlier on been vaccinated or exposed to a wild virus. However due to prolonged absence of vaccine administration or exposure to wild virus strains in the village, the antibodies in birds against Newcastle decreased to levels that could not offer birds protection against the disease. A similar scenario was observed in Malawi where a single dose of I-2 thermostable vaccine administered to birds in three villages led to an increase in antibody titers against NCD and protection levels in all villages in the first month but gradually decreased in one village from $\log_2$5.39±0.26 to 2.99 in a period of four months (Mgomezulu et al 2009).

Within the same period, the other two villages registered higher titer values of 4.00±0.22 and 5.95±0.34 respectively. The titer values obtained at the end of the study were attributed to exposure of the two villages to wild virus strain that increased their antibodies to above protection levels. Because birds in the third village were not exposed to the wild virus strain, the antibodies created in response to vaccination gradually reduced hence a decline in mean antibody titer and protection levels (Mgomezulu et al 2009). The low Geometric Mean antibody Titer (GMT) and flock protection of 32% obtained at baseline in this study was an indication that no recent NCD outbreak had occurred and depicted that no vaccination exercise was recently carried out in the area, which is in agreement with Alder's observation of the inability of village farmers rearing chicken on free range to routinely vaccinate their flock hence rendering birds susceptible to NCD (Alders et al 2009).

The eye drop method was selected as the route for vaccine administration for this study because in comparison to other methods, a higher immune response is produced in birds. Studies done in Uganda and Tanzania demonstrated that the antibodies generated and level of protection against NCD that follows administration of the vaccine by eye drop method were superior to what is achieved when the vaccine was administered to birds orally (Illango et al 2008; Wambura et al, 2000).

Following the initial vaccination, GMT values rose to $\log_2$4.02±0.11 demonstrating I-2 vaccines ability to invoke an immune response that leads to an increment in antibody titers against NDV in birds. A study done in 2008 demonstrated a rise in mean HI titers from $\log_2$ 2.58 to $\log_2$ 4.05 observed among birds vaccinated with a single dose of I-2 thermostable vaccine (Illango et al 2008). Consequently due to an increase in GMT, the level of protection against NDV increased to 83%. A study in Mozambique showed 82% survival of adult birds five months later following administration of I-2 vaccine via the eye drop method (Dias et al 2001).When HI tests were done using blood obtained from adult birds in a study done by Albano in Tanzania, 81.2% had antibody titers above protection levels after initial I-2 vaccine administration (Albano et al 2012).

Attainment of a 100% level of protection after the third vaccination and fourth vaccination exercise could be attributed not only to administration of the vaccine, but also exposure of birds to a wild virus strain. In controlled environments such as laboratories, administration of I-2 vaccine alone by eye drop method can generate protection levels up to 100%. This was demonstrated by Illango where housed birds attained a 100% level of protection after administration of the third treatment of I-2 themostable vaccine in comparison to 89.5% in un housed birds (Illango et al 2008). In Tanzania and Nigeria, 100% of housed birds in the study that received I-2 vaccine via eye drop survived the challenge with a virulent field isolate (Wambura et al 2000; Musa et al 2010). However, a study in Malawi revealed a 100% level of protection in free ranging chicken that resulted due to exposure of the birds to a wild strain of New Castle Disease (Mgomezulu et al 2009). An encounter with wild strains of NDV led to infection of the flock with the virus; the infected birds however did not succumb to the disease. This encounter led to a rise in the mean antibody titer of the exposed flock (Mgomezulu et al 2009; Musa et al 2010).

The 116% increase in flock size from 10.83±0.64 to 23.44±0.89 at the end of the study period could be attributed to protection against New castle disease as a result of vaccination. A similar study by Dias et al 2001

demonstrated the ability of thermo stable vaccines to single handedly increase chicken flock sizes by 50% in five months and 144% within one year.

The significant differences observed between flock sizes in the vaccinated and unvaccinated groups in this study could probably be due to the differences in flock deaths. A six month study carried out in Eastern Uganda by Nahamya et al 2006 registered a 56% and 50% difference in flock size and flock deaths respectively between vaccinated and unvaccinated groups of chicken. The percentage of chicken in the unvaccinated area with antibody titers above protection levels was 96%. These levels were an indication of exposure of the unvaccinated flock to a wild virus strain of NDV that could have culminated in death of birds. Even though a 74.5% level of protection was achieved in the vaccinated area, flock deaths were lower implying birds were protected from the NDV wild strain (Nahamya et al 2006). The vaccinated flock in this study might have been protected from the New Castle disease field virus strain that all birds in and around the neighboring villages were exposed to. Unvaccinated birds however succumbed to the disease.

Despite the intervention of NCD vaccination, flock deaths still occurred in the study area. Cases of death could have arisen due to encounters with predators, rain and other emerging diseases such as fowl pox which contribute to the death toll among free ranging chicken.

Increase in flock size is dependent on chick survivability since they act as replacement stock. Increase in the number of chicks in the first quarter depicted an increase in their survivability which could be attributed to the vaccine. As demonstrated by Henning 2004, I-2 thermo stable vaccine reduced the proportions of mortalities in growers and chicks that were attributed to New castle disease (Henning et al 2009). However, in the free range system, vulnerable chicks are exposed to predators, diseases and extreme weather conditions as they forage for food. Exposure of chicks to cold during heavy rain could have led to death and a decrease in the number of chicks observed during the rainy period encountered in the third quarter of the study. Studies have attempted to improve on housing for chicks for the first two months of their life in order to protect them from adverse weather conditions such as sunshine and rain (Lwesya et al 2004). Mgomezulu in Malawi noted that flock losses were majorly due to chick mortalities (Mgomezulu et al 2009). At the time of the baseline survey of this study, no outbreak of NCD had occurred for six months implying cause of death of birds at the time could have been other diseases, predation or harsh weather.

V. Conclusion

Vaccination of free ranging chicken against New castle Disease using I-2 thermostable vaccine led to an increase their antibody titers to levels that offered them protection against NCD. Use of the vaccine increased flock productivity by enhancing survivability of the flock.

Acknowledgement

This work is supported by RUFORUM under Grant # RU2010 GRG 02 . The project also received thermo stable vaccines from BRENTEC Ltd

References

[1]. Albano O M, Komba E V G and Rutashobya C T M 2012 Adoption of I2 Vaccine in Immunization of Village Chickens against Newcastle Disease Virus in Southern Tanzania: Immune Status of Farmer Vaccinated Birds, Journal of Agricultural Science, 4 (4): 23, Tanzania, http://dx.doi.org/10.5539/jas.v4n4p23

[2]. Alders R G 2002 Strategies for vaccination of family Poultry against New Castle disease in Africa; Characteristics and parameters of family poultry production in Africa, Results of a FAO/IAEA coordinated Research Programme on Assessment of the Effectiveness of Vaccination Strategies against New Castle disease and Gumboro disease using Immunoassay based Technologies for increasing Farmyard Poultry Production in Africa, Rome.

[3]. Alders R G 2000 Strategies for vaccination of family Poultry against New Castle disease in Africa, Second IAEA/FAO Research Coordination Meeting on Improvement of Health and Management of Family Poultry Production in Africa, Morogoro.

[4]. Alders R G, Bagnol B and Young M P 2010 Technically sound and sustainable Newcastle disease control in village chickens: lessons learnt over fifteen years, World's Poultry Science Journal, 66, doi:10.1017/s0043933910000516.

[5]. Alders R G and Bagnol B 2007 Effective communication: the key to efficient HPAI prevention and control. World's Poultry Science Journal, 63 (1): 143-151.

[6]. Alders R G and Pym R A E 2009 Village poultry: still important to millions, eight thousand years after domestication. World's Poultry Science Journal, 65 (2): 181-190. doi:10.1017/S0043933909000117.

[7]. Alders R and Spadbrow P 2001 Controlling New Castle Disease in Village chicken, A Field Manual, Version 14.03, ACIAR, Sydney.

[8]. Alders R G, Spradbrow P B and Young M P 2009 Thermo stable vaccines in the control of Newcastle disease in village chickens: a history, Village chickens, poverty alleviation and the sustainable control of Newcastle disease. Proceedings of an international conference held in Dar es Salaam, Tanzania, 5–7 October 2005, ACIAR Proceedings No. 131, 235 pp. ISBN 978 1 921531 75 0 (print), ISBN 978 1 921531 76 7 (online), ACIAR, Canberra.

[9]. Alexander D J 1997 Newcastle disease and other avian Paramyxoviridae infections, Diseases of Poultry, 10th Edition, Pg 541-570, Iowa State University Press, Iowa, USA.

[10]. Alexander D J, Bell J G and Alders R G 2004 Technology Review: Newcastle disease with special emphasis on its effect on village chickens. FAO Animal Production and Health Paper No. 161. Rome, FAO. 63pp, ISSN 0254-6019, ISBN 92-5- 105080-5.

[11]. Ariola M M 2006 Principles and methods of research, Pg 78, 1st Edition, Rex Book store Inc, Manila, Phillipines, ISBN 978-971-23-4548-7.
[12]. Bagnol B 2009 Improving village chicken by employing gender sensitive methodologies, Village chickens, poverty alleviation and sustainable contol of new castle disease, Proceedings of an international conference in Dar es Salaam, Tanzania, 2005, edited by Alders R.G and Spadbrow P.B and Young M.P, Canberra, ACIAR proceedings, No 131:235, ISBN 978 1 921531 76 7.
[13]. Bell J G 2001 A comparison for different vaccines available for the control of New Castle disease in Village Chicken; Proceedings of an international Workshop on New Castle Disease Control in Village Chickens, Maputo,2000, edited by Alders R.G and Spadbrow, Canberra, ACIAR proceedings, No103: 56-60.
[14]. Bell J G, El Hakim E l, Alaoui M and Jaouzi T 1991 An enzyme-linked immunosorbent assay for antibodies against Newcastle disease virus suitable for use in kit form in tropical countries, World Animal Review 69, 59-63.
[15]. Carrilho J 2001 Opening speech by the Mozambican Vice-Minister for Agriculture and Rural Development, in: Proceedings of an international workshop on Newcastle Disease Control in Village chickens, Maputo, 2000, edited by Alders R.G and Spadbrow Canberra, ACIAR Proceedings No. 103: ix-xi.
[16]. Chen J and Wang C 2001 Clinical Epidemiology and Experimental Evidence for the transmission of New Castle Disease Virus through Eggs, Journal of Avian Diseases,46:461-465.
[17]. 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.
[18]. Dias P T, Alders R G, Fringe R and Mata B V 2001 Laboratory and field trials with thermo stable live Newcastle Disease vaccines in Mozambique, In: R. G. Alders and P. B. Spradbrow, ACIAR proceedings: SADC Planning workshop on Newcastle Disease control in village chickens. SADC Planning Workshop on Newcastle Disease Control in Village Chickens, Maputo, Mozambique, (91-96). 6-9 March, 2000.
[19]. Denzin N K and Yvonna S L 2000 Collecting and Interpreting Qualitative Materials, 3rd Edition, Sage Publications, California.
[20]. Dolberg F 2003 Review of household poultry production as a tool in poverty reduction with focus on Bangladesh and India, FAO Pro-Poor Livestock Policy Initiative Working Paper No. 6.
[21]. FAO 2002a Organic Agriculture, environment and food security, Environment and Natural Resource Series No 4, 258pp, ISBN 92-5-104819-3, ISSN 1684-8241, Adapted from http://www.fao.org/DOCREP/005/Y4137E/y4137e07b.htm#P865_120559.
[22]. FAO 2002b Laboratory Manual for the Small-Scale Production and Testing of I-2 Newcastle Disease Vaccine, RAP publication, 22; ISBN 974-7946-26-2.
[23]. FAO 2008 Poultry Sector Country Review Report for Uganda, ftp://ftp.fao.org/docrep/fao/011/ai378e/ai378e00.pdf
[24]. FAO 2009 Poultry Genetic Resources and Small Poultry Production Systems in Uganda, Prepared by Busuulwa S.Henry, AHBL - Promoting strategies for prevention and control of HPAI. Rome.
[25]. Gillespie J R 2002 Modern Livestock and Poultry Production, 6th Edition, Delmar, New York, ISBN 0-7668-1607-9.
[26]. Grimes S E 2002 A basic laboratory manual for the small scale production and testing of I-2 New Castle Disease Vaccine, FAO-APHCA, FAO regional Office for Asia and Pacific (RAP), ISBN-974-7946.26-2.
[27]. Henning J, Morton J Pym R, Hla T and Meers J 2009 Evaluation of strategies to improve village chicken production: controlled field trials to assess effects of Newcastle disease vaccination and altered chick rearing in Myanmar, Journal of Preventive Veterinary Medicine 1;90(1-2):17-30. Epub 2009 May 13, PubMed, USA.
[28]. Illango J, Mukiibi-Muka G, Abila P P, Musisi G and Etoori A 2008. The value of the Newcastle disease I-2 thermo stable vaccine use in the rural free-range poultry management system in Uganda. Journal of livestock research for rural development, Volume 20, Article #145. Retrieved February 1, 2013, from http://www.lrrd.org/lrrd20/9/illa20145.htm.
[29]. Jordan F T W, Pattison M, Alexander D J and Faragher T T 2001 Poultry Diseases, 5th Edition, 257-267, W.B Saunders, London.
[30]. Khalafall A I, Hassan W, Elnur M and ALI A S 2006 Observations on production, laboratory testing and field application of I-2 thermo stable Newcastle disease vaccine in the Sudan, Improving farmyard poultry production in Africa: Interventions and their economic assessment, PP232, FAO/IAEA, Vienna, ISBN 92–0–101206–3.
[31]. Kirunda H, Mukiibi-Muka G 2003 Study of causes of chick mortality in free-range poultry in Busede Sub-county, Jinja District. Proceedings of LSRP Annual Scientific Workshop 2003, Hotel Africana. pp: 133-141.
[32]. Lwesya H, Phoya R K D, Safalaoh A C L and Gondwe T N P 2004 Rearing chicks in enclosures under village conditions: effect on chick growth and reproductive performance of mother hens. Livestock Research for Rural Development. Vol. 16, Art. #89. Retrieved September 12, 2012, from http://www.lrrd.org/lrrd16/11/wesr16089.htm.
[33]. Mgomezulu R A, Alders R G, Chikungwa P B, Young M P, Lipita W G, and Wanda G W 2009 Trials with a thermo tolerant I-2 Newcastle disease vaccine in confined Australorp chickens and scavenging village chickens in Malawi, Poverty alleviation and the sustainable control of Newcastle disease, ACIAR Proceedings No. 131. Australian Centre for International Agricultural Research: Canberra. ISBN: 978 1 921531 75 0 (print) 978 1 921531 76 7 (online) Canberra, Australia, pp232 Editted by Alders R.G, Spradbrow P.B. and Young M.P.
[34]. Musa U, Abdu P A, Mera U M, Emmenna P E and Ahmed M S 2010 Vaccination with Newcastle disease vaccines strain I2 and Lasota in commercial and local chickens in plateau state Nigeria, Nigerian Veterinary Journal Vol 31(1):46-55.
[35]. Mutayoba S K, Katule A K, Minga U, Mtambo M M and Olsen. J E 2012 The effect of supplementation on the performance of free range local chickens in Tanzania, Livestock research for rural Development, 24(5), http://www.lrrd.org/lrrd24/5/muta24093.htm, Colombia.
[36]. Nahamya F H, Mukiibi-Muka G, Nasinyama G W and Kabasa J D 2006 Assessment of the cost effectiveness of vaccinating free range poultry against Newcastle disease in Busedde sub-county, Jinja district, Uganda. Livestock Research for Rural Development. Volume 18, Article No. 158. Retrieved December 18, 2012, from http://www.lrrd.org/lrrd18/11/naha18158.htm, ISSN 0121-3784.
[37]. Njue S W, Kasiiti J L, Macharia J M, Gacheru S G, and Mbugua H C W 2001 A Survey of the disease status of Village chicken in Kenya. In: Proceedings of Association of the Institutes of Tropical Veterinary Medicine (AIMVT) conference proceedings, 20-23 August 2001, Copenhagen, Denmark, 36.
[38]. OIE 2008 Manual of Diagnostic Tests and Vaccines for Terrestrial animals, 6th Edition, Volumes 1 and 2, France, ISBN 978-92-9044-718-4.
[39]. Saif Y M, Barnes H J, Glisson J R, Fadly A M, Mc Dougald L R and Swayne D E 2003 Diseases of Poultry, 11th Edition, 63-69, Ames, Iowa State Press, Iowa, USA.
[40]. Spadbrow PB 2001 Thermo stable New Castle Disease Vaccine, Proceedings of an international Workshop on New Castle Disease Control in Village Chickens, Maputo, 2000, edited by Alders R.G and Spadbrow, ACIAR proceedings No 103:61-66, Canberra.

[41]. Tabidi M H, Makkawi A, Mahasin E and Ali A S 2004 Comparative Evaluation of Haemagglutination Inhibition Test and Enzyme-linked Immunosorbent Assay for Detection of Antibodies Against Newcastle Disease Vaccine in Broiler Chicks, International Journal of Poultry Science 3 (10): 668-670., 2, Khartoum.
[42]. UBOS 2010 Statistical Abstract, chapter 3: 40-46, Kampala
[43]. Wambura P N, Kapaga A M and Hyera J M 2000 Experimental trials with a thermo stable Newcastle disease virus (strain I2) in commercial and village chickens in Tanzania, Journal of Preventive Vet Medicine, Jan 20;43(2):75-83, PubMed, USA.
[44]. Wells K F 1948, Newcastle Disease, Canadian Journal of comparative Medicine, volume XII, No 4, Canada.
[45]. Williams R, Boshoff C H, Verwoerd D, Shoeman M, Wyk A V, Gerdes T H, and Roos K 1997. Detection of Antibodies to New Castle Disease Virus in Ostriches (Struthio camelus) by an indirect ELISA, Journal of Avian Diseases, 41:864-869 (Oct-Dec), American Association of Avian Pathologists, Onderstepo

Mali B. "Effect of Thermo Stable Newcastle Disease Vaccine on Productivity of Free Ranging Indigenous Chicken." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 13(12), 2020, pp. 55-61.