

DETECTION OF ANTIBODIES AGAINST NEWCASTLE AND INFECTIOUS BURSAL DISEASE ON CHICKEN IN NORTH GONDAR ZONE, ETHIOPIA

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✉Supporting Information

ABSTRACT: Infectious Bursal (IBD) and Newcastle diseases (NCD) are an acute highly contagious and destructive illness of chickens that occur almost any time of the year. In this study, we aimed to detect antibodies against NCD and IBD. The study was compared a compressive investigation on the Ab detection local and exotic breed with different serological technique of Indirect-ELISA and Haemagglutination inhibition (HIT) test. In this study, a cross-sectional study design was used and applied to collect 384 serum samples from chickens which were selected by simple random sampling. Serum samples were collected from three different districts which were Dembiya, Dabark and Metema. The serum sample collections were also considered different parameters like sex, age, breed, infection, and vaccination during the data collection time. The results showed that the overall Ab detection of IBD and NCD Virus were 74.4% and 79.6% respectively. Epidemiological status of both IBD and NCD Virus of each districts were indicted Metema (72.2%, 86.0%), Dembiya (89.7%, 92.4%) and Dabark (67.2%, 69.1%) respectively. In conclusion, these research findings extend the presence of detectable antibodies to NCD and IBD in chickens, in the three districts of North Gondar Zone. As it is known Chickens' might have been high detectable antibodies if they are either susceptible to the pathogenic or are vaccinated.

Keywords: Newcastle, infectious bursal, Antibody, Chickens, Indirect-ELISA test and Haemagglutination inhibition.

INTRODUCTION

Chicken production has a major role in the economy of developing countries and backyard production is particularly important to women (Wilson, 2010). Several programmes, in Ethiopia and elsewhere, have attempted to improve chicken production as a means to reduce poverty (Sambo et al., 2015).

Like away in the developing world, backyard poultry rearing is a common practice in rural Ethiopia. Village backyard poultry, characterized by traditional production methods and local breeds, represents 98% of the total Ethiopian poultry population of 38 million (Chaka et al., 2012).

Small and large-scale chicken farms are rapidly growing in Ethiopia. The chicken strains imported are temperate breeds that are less adapted to the heat stress and disease challenges in the country. Accompanying intensification of poultry farming, there is occurrence of epidemics of newly introduced diseases and/or epidemics of endemic diseases (Anebo et al., 2014). One of the diseases that are of growing concern in poultry is Infectious Bursal disease (Gumboro disease). As in this report a large scale occurrence of Infectious Bursal disease in the central part of Ethiopia with intensive and high-density juvenile farms (Zelege et al., 2005).

Infectious bursal disease (IBD) is a highly contagious, immunosuppressive infection of immature chickens with a worldwide distribution (Negash et al., 2012). Two serotypes of IBD virus strains are described: 1 and 2. Serotype 2 strains are classified as a pathogenic, and serotype 1 strain, pathogenic to chickens, is classified into several pathotypes, from mild to hyper virulent, according to their virulence (Chaka et al., 2012).

IBD infection results in lymphoid tissue depletion and the final destruction of the bursa, which is the predominant feature of its pathogenicity (Okwor et al., 2012). This virus may exacerbate infection with other etiologic agents and reduce the chicken's ability to respond to vaccination. Susceptibility and breed of flock, types of virus strains, inter-current primary and secondary pathogens, and environmental and management factors influence the economic impact of IBD (Jenbreie et al., 2012)

The other disease of poultry is Newcastle disease (NCD) also known as Ranikhet disease (RD), locally known as Fungal, is caused by avian paramyxovirus serotype 1 (APMV-1) belonging to the family Paramyxoviridae, genus *Avulavirus* (Ezema et al., 2009). Newcastle disease virus (NCD) can be categorized into highly pathogenic (velogenic), intermediate (mesogenic), and less pathogenic (lentogenic) strains based on pathogenicity in chickens and are divided in two clades (class I and class II) (Ali et al., 2004).

The epidemiology and control of NCD and IBD has been extensively studied and documented in commercial poultry systems, but has been poorly documented in village poultry (Awan et al., 1994). The large differences in management between commercial and village poultry prohibit the transfer of epidemiological data and control programmes of NCD and IBD from the commercial sector to the village environment (Yongolo et al., 2002). In developing countries these vaccines are available most often as live vaccines. The use of live vaccines can result in vaccination reactions especially if the birds are stressed (Cserep, 2008).

With the ever expanding flock sizes and the increasing farm size it is possible for individual bird inoculation, which ensures even distribution of the vaccine but involves the chasing and catching of the birds for hours, to constitute a major stress. It also increases labour and cost. To save labour, and possibly minimize stress due to chasing and catching of the birds, it may be possible to administer the two vaccines simultaneously. However, this procedure may have some effects on immune responses to the vaccines, feed consumption and weight gain (Okwor et al., 2013). Although the diseases are the major health constraints responsible for marked economic losses in a country, the dynamicity and the status of the disease in chickens in the study areas have not been yet studied to a full extent and not well documented.

MATERIALS AND METHODS

Study setting

Cross-sectional study type was conducted from October 2015 to April 2016 in selected districts of North Gondar zone, Amhara National Regional State, located in the northwestern part of Ethiopia. The study districts included were Dabark, Dembiya, and Metema, located between 700 and 778kms northwest of the capital, Addis Ababa. The study zone is located between geographically coordinates 12.3° to 13.38° north latitudes and 35.5° to 38.3° east longitudes and the altitude ranges from 550 to 4620 meters above sea level (masl) in western lowland and in north Semen Mountain, respectively. The average annual rain fall vary from 880mm to 1772 mm, which is characterized by a monomodal type of distribution. The mean annual minimum and maximum temperature is 10°C in the highland and 44.5°C in the lowland (Eshete, 2002).

The indigenous chicken population in the study zone is estimated at 3.75million. Most of the poultry is found in the highland and mid highland areas of the region, which is associated with the ecology and human demography. Indigenous chickens are major family poultry types in the area managed under backyard free-range system (Fentie et al., 2013).

Sampling procedure and study population

In this study, a cross-sectional study design was used and applied to collect 384 serum samples from chickens which were selected by simple random sampling method (lottery drawing methods). Serum sample were collected from three different districts which were Dembiya, Dabark and Metema. The serum sample collections were also considered different parameters like sex, age, breed, infection, and vaccination during the data collection time. The serum sample was collected from October 2015 to April 2016. The laboratory tests were conducted in Deberzit National Veterinary Institute, Ethiopia from April to May 2016 using the laboratory techniques of Haemagglutination inhibition test and Indirect-ELISA test for NCD and IBD respectively. The sample size was determined using the formula described by Thrusfield (1995). The study was considered an expected prevalence of 50% and an absolute precision of 5% (5% significance level) with 95% CI. For both IBD and NCD, the total serum sample in Metema, Dembiya and Dabark were collected 114, 92 and 178 respectively.

Data collection instrument and procedures

Totals of 384 chicken serums were collected both from local and exotic (Faso T44) breeds of chickens in the three districts and 2-3 ml of blood were collected from the humeral region of the wing vein with a syringe and needle of 5ml size. The syringe with blood, then kept horizontally until the blood clots. After clotting, the syringe was returned to a vertical, but invert position and left on a bench overnight to permit the serum to ooze out. The separated serum was transferred into Eppendorf tubes, labeled and stored at -20 Cuntil. The Haemagglutination-inhibition test (HIT) and indirect ELISA tests were carried out. HIT and Indirect ELISA tests were performed to detect antibodies against NCD and IBD respectively.

Protocol HIT to detect specific antibodies

i) 0.025 ml of PBS is dispensed into each well of a plastic V-bottomed microtitre plate, ii) 0.025ml of serum is placed into the first well of the plate ,iii) Two fold dilutions of 0.025 ml volumes of the serum are made an exotic the plate, iv) 4 HAU virus/antigen in 0.025 ml is add to each well and the plate is left for a minimum of 30 minutes at room temperature, i.e. about 20°C, or 60 minutes at 4°C, v) 0.025 ml of 1% (v/v) chicken RBCs is added to each well and, after gentle mixing, the RBCs are allowed to settle for about 40 minutes at room temperature, i.e. about 20°C, or for about 60 minutes at 4°C if ambient temperatures are high, when control RBCs should be settled to a distinct button, vi) The HI titer is the highest dilution of serum causing complete inhibition of 4 HAU of antigen (Zelege et al., 2005).

The agglutination is assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (positive serum, virus/antigen and PBS controls) should be considered to show inhibition, vii) The validity of results shall be assessed against a negative control serum, which should not give a titer $>1/4$ (>22 or $>\log_2 22$ when expressed as the reciprocal), and a positive control serum for which the titer should be within one dilution of the known titer. The value of serology in diagnosis is clearly related to the expected immune status of the affected poultry. HI titers may be regarded as being positive if there is inhibition at a serum dilution of $1/16$ (24 or $\log_2 24$ when expressed as the reciprocal) or more against 4 HAU of antigen. Some laboratories prefer to use 8 HAU in HI tests. While this is permissible, it affects the interpretation of results so that a positive titer is $1/8$ (23 or $\log_2 3$) or more. Back titration of antigen should be included in all tests to verify the number of HAU used. In vaccinate flocks that are being monitored serologically, it may be possible to identify anamnestic responses as the result of a challenge infection with field virus (Alexander & Allan, 1974), but great care should be exercised as variations may occur from other causes. For example, it has been demonstrated that APMV-3 virus infections of ND-virus-vaccinated turkeys will result in substantially increased titers to NCD (Vui, Lohr et al., 2002).

Protocol Indirect ELISA to detect specific antibodies

Coat plate with antigen using a multichannel pipet and tips; dispense 50 μ l antigen solutions into each well of micro titer plate. Tap or shake the plate to ensure that the antigen solution is evenly distributed over the bottom of each well. 2. Wrap coated plates in plastic wrap to seal and incubate overnight at room temperature or 2 hr at 37°C. 3. Rinse coated plate over a sink by filling wells with deionized or distilled water Flick the water into the sink and rinse with water two more times. Block residual binding capacity of plate. 4. Fill each well with blocking buffer dispensed as a stream from a squirt bottle and incubate 30 min at room temperature. 5. Rinse plate three times in water as in step 5 Add Ab to plate. 6. Add 50 μ l Ab samples diluted in blocking buffer to each of the coated wells, wrap plate in plastic wrap, and incubate ≥ 2 hr at room temperature. 8. Fill each well with blocking buffer, vortex, and incubate 10 min at room temperature. 9. Rinse three times in water as in step 5 Wash the plate. 7. Rinse plates three times in water as in step 5 and 8. Fill each well with blocking buffer, vortex, and incubate 10 min at room temperature. 9. Rinse three times in water as in step 5 Wash the plate. 7. Rinse plate three times in water as in step 5 Add developing reagent to plate. 10. Add 50 μ l developing reagent in blocking buffer to each well, wrap in plastic wrap, and incubate ≥ 2 hr at room temperature. 11. Wash plates as in steps 9 to 11 Add substrate and measure hydrolysis. 12. Add 75 μ l MUP or NPP substrate solution to each well and incubate 1 hr at room temperature. 13. Monitor hydrolysis qualitatively by visual inspection or quantitatively with a microtitre plate reader. Hydrolysis can be stopped by adding 25 μ l of 0.5 M NaOH. Read out visually, hydrolysis of NPP can be detected by the appearance of a yellow color. If using a micro titer plate reader to measure NPP hydrolysis, use a 405-nm filter (Williams et al., 1997).

Washing of RBCs: A total of 5 ml of chicken blood was collected aseptically in a disposable syringe containing 1 mL of sodium citrate (4% solution) as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 min. The plasma and Buffy coat was pipetted off. After washing thrice with phosphate buffer saline (PBS), 1% suspension in PBS was made to be used in HI test (Miers et al., 1983).

Data analysis

Data were managed using both in hard and soft copies. Data entry was done using Microsoft office excels and processed using SPSS version 21 statistical software. Descriptive statistics, Chi-Square and ANOVA tests were computed for all the parameters and to analyze the differences in the Sero-prevalence between sexes, ages, vaccine, infection, breeds and among districts. $P < 0.05$ was considered to best statistical significant variation.

RESULTS

This study is aimed to make a comprehensive study on the detection of Ab against IBD and NCD of Chicken by considering multi factors analysis namely: age, district, vaccination, infection, breed, and Sex. As it is stated in the methodology section, the study considered 384 serum samples in all the factors.

Ab Detection of IBD

The results revealed that out of the total 156 male chickens Sero-positive were 81.6%, whereas detection of Ab of females from the total 228 serum samples was found to be 69.4%. The highest Ab detection was found in males (81.6%) as compared to females (69.4%). The findings of this study indicating that 0% Ab in local and 83.3% in exotics do not agree with the findings of (Halima et al., 2007). 314 young chickens a total of 285 serum samples were found Ab positive (90.9%). Likewise, out of the 70 adult chickens a total 0 serum samples were found Ab positive (0.0%).

The results showed that out of the total 48 infected chickens Ab detection were 80%, whereas out of the total 336 non-infected Ab detection 73.6% were found to be Ab positive. The highest Ab detection was found on vaccinated Chickens 83.3% were serum antibody, as compared to non-vaccinated Chickens with 0% Ab detection which is in line with the findings of (Jenbreie et al., 2012).

This indicates there are Sero-positive rate of 72.2%, 89.7% and 67.9% in Metema, Dembiya and Debark district respectively. It shows that compared to the others, Dembiya has highest Ab detection rate among the three districts. These findings agree with the findings of Halima et al. (2007) and Tadesse et al. (2005). The possible explanation for this could be there are few chicken farms in the highland area of the study sites and chicken population number is a factor for the transmission of the disease.

The two-sided *asymptotic significance* of the chi-square statistic ($\chi^2 = 135.76$) was less than 0.05 ($P=0.00$). Therefore it is safe to say that the differences between vaccinated and non-vaccinated chickens with respect to IBD was not due to chance variation, which implies that vaccinated chickens have a higher level of prevalence on IBD than non-vaccinated chickens. Except sex and disease all the parameters such as district, age, breed shows similar result. However, the findings shows that IBD was independent of sex and infection with $P= 0.09$ and 0.48 respectively.

Detection of Antibody to Infection Bursal Disease Virus by Sex, Age and Breed

In this section, detection serological antibodies of IBDV of chicken are investigated by sex, age and breed. As it is shown in Table 2, the findings of sex, age and breed are listed.

Table 1 - Detection of antibodies against infectious bursal disease by sex, age and breed

Variable	No examined Chickens	No. antibody Positive	Antibody detection	Total antibody detection	$\chi^2(P\text{-value})$	P-value*
Male	156	127	81.6%	285(74.4%)	71.1(0.09)	0.07
Female	228	158	69.9%			
Adult	70	0	0.0%	285(74.4%)	246.44(0.00)	0.00
Young	314	285	90.0%			
Local	42	0	0.0%	285(74.4%)	135.16(0.00)	0.00
Exotics	342	342	83.3%			

χ^2 = the chi-square value, *=the annova p-value

Table 2 - Detection of antibodies against infectious bursal disease by vaccinated, infection and districts

Variable	No. Examined Chickens	No. antibody positive	Antibody Detection Rate	Total Antibody rate	$\chi^2 (P\text{-value})$	P-value
Vaccinated	342	285	83.3%	285(74.4%)	135.76(0.00)	0.00
Non-vaccinated	42	0	0.0%			
Infected	48	38	80.0%	285(74.4%)	0.7(0.48)	0.345
Non- infected	336	247	73.6%			
Dembiya	92	82	89.7%	285(74.4%)	14.63(0.01)	0.00
Dabark	114	82	72.2%			
Metema	178	120	67.9%			

Ab Detection of NCD

The 156 male chickens Serological Ab positive were account 79.57% and the 228 female Chickens also account 79.56%. This shows that both male and female Chickens have equal serum Ab with respect to NCD, which indicates being male or female is not a decisive factor in the resistance of NCD. And from the total 32 local chickens Ab positive were 76.19%, whereas, exotics or Faso T44 chickens from the total 274 serum samples were found to be

80.12% Ab positive. The highest Ab detection was found in exotics (8.12%) as compared to local (76.19%) I agree with (Chaka et al., 2013).

In addition to this, further comparisons between local and exotics (Faso T44) Chickens were made. The result of the findings is similar to Moges (2010) reports. It shows that there no statistical significance difference (P-Value =0.113) between local and exotics or Faso T44 Chickens with respect to NCD. It shows that, out of the total of 286 young chickens Sero-positive were 91.1%, whereas the serological prevalence of adult from the total 70 serum samples was found to be 28.6%. The highest prevalence was found in young (91.1%) as compared to adult (28.6%). The results of the study indicating 91.1% Ab in young and 28.6% in adult agree with those of who reported highest prevalence in young and the lowest in the adult.

The results revealed that the Serological Ab of Metema was found to be 86.0%, that of Dembiya was 92.4% and that of Dabark was 69.1 %. Among them, the highest Ab detection was found in Dembiya (92.4%) as compared to Metema (86.0%) and Dabark (69.1%). The results of the study indicating 86.0% Ab in Metema, 92.4% in Dembiya chickens and 69.1% in Dabark was however, observed that susceptibility was the highest to Debark as compared to Metema and Dembiya which was in line with previous studies I agree with (Ashenafi & Eshetu, 2004).

The results revealed that out of the total 342 vaccinated and 42 non-vaccinated chickens 89.5% and 0%, serological Ab detection is respectively found. When it was represented proportionally the total of 38 infected chickens Serological Ab were account 79.2%, whereas the serological Ab of non-infected from the total of 286 serum samples was found to be 79.8%, which shows that there is no serological Ab difference between infected and non-infected Chickens with respect to NCD.

The two-sided asymptotic significance of the chi-square statistic ($\chi^2 = 135.76$) is less than 0.05 (P= 0.00). Therefore it is safe to say that the differences between vaccinated and non-vaccinated chickens with respect to NCD is not due to chance variation, which implies that vaccinated chickens have higher level of prevalence on NCD than non-vaccinated chickens. Similarly the report in the same table shows that except sex and infection all the parameters such as district, age, breed show similar result. However, the finding show that NCD was independent of sex and breed with P= 0.036 and 0.551 respectively.

Table 3 - Detection of antibodies against Newcastle disease by sex, age and breed

Variable	No. examined chickens	No. Positive	Antibody Detection rate	Total Antibody detection rate	χ^2 (p-value)	p-value
Male	156	124	79.59%	306(79.9%)	0.007(0.936)	0.999
Female	228	182	79.56%			
Adult	70	20	28.6%	306(79.9%)	138.19(0.00)	0.0001
Young	314	286	91.19%			
Local	42	32	76.19%	306(79.9%)	0.36(0.551)	0.0001
Exotics	342	274	80.12%			

Table 4 - Detection of antibodies against Newcastle disease by vaccine, infection and districts.

Variable	No. examined chickens	NO. antibody positive	Antibody detection Rate	Total antibody Rate	χ^2 (p-value)	p-value
Vaccinated	342	306	89.5%	306 (79.6)	18.1 (0.00)	0.0001
Non-vaccinated	42	0	0.00%			
Infected	42	38	79.2%	306 (79.6)	73.73 (0.00)	0.834
Non- infected	48	268	79.8%			
Dembiya	92	85	92.4%	306 (79.6)	24.27 (0.00)	0.0001
Metema	114	98	86.6%			
Dabark	178	123	69.0%			

DISCUSSION

In vaccinated flocks, positive serological results are clear evidence that the birds have been exposed to the infectious agent under investigation, although without identifying the infecting strains. In the present study, we confirmed from the sellers during purchase that all of them had vaccinated their chickens for any poultry diseases especially cross breeds. Hence, the presence of antibodies to NCD and IBD was considered evidence of not the exposure to natural infection.

The study revealed that the prevalence of NCD antibodies in backyard chickens was generally high, around 76.19%. This is considerably higher than previous reports by Geresu et al. (2016) who reported prevalences of 19.8%

in the southern and Rift Valley districts and 32.2% in central Ethiopia, respectively, but our results were not close to those reported by (Regasa, Fufa, Berihanu, & Hunduma, 2007) in southern Ethiopia (11%). Our results are also consistent with seroprevalence in backyard poultry of 76.19% in Mauritania. When chickens are affected by a velogenic NCD virus that results in very high mortality, one is likely to find few or no survivors with antibodies. Up to 30% of market sellers claimed to have observed poultry disease signs (sudden death, diarrhea, and nervous signs) resembling Newcastle disease during previous months. There was no observed seasonal or geographic variation in seroprevalence, in the present study, suggesting that the disease is widespread and occurs throughout the year in the study area.

The survey also indicated that IBD is widespread among village chickens in the study area, with a seroprevalence of 74.4%. This agrees closely with reports by Degefu et al. (2010) from Ethiopia, and Chaka et al. (2012) from India, who reported seroprevalence of 76.6%, 100, 74, and 73.7% respectively. However, relatively lower IBD seroprevalence were recorded in Mauritania (15.8%; Chrysostome et al., 1995), Zimbabwe (55%; Mazengia et al., 2010). The higher seroprevalence of the disease in the study area, in the apparent absence of mortality, could be due to an IBD virus of lower pathogenicity, unlike the case reported from the Amhara region of Ethiopia (Mazengia et al., 2009) or the outbreak in a commercial broiler farm with evident mortalities (Jenberie et al., 2014). It is also possible that the birds were infected with IBD virus as adults, at which stage they simply seroconvert without any apparent clinical disease. With such a high seroprevalence and low mortality of infected birds, there is the possibility of genetic resistance among indigenous breeds of chickens in Ethiopia, as reported from Egypt (Hassan, 2004). This is difficult to demonstrate using serological studies, but further studies could be undertaken to investigate this.

CONCLUSION

In conclusion, these research findings extend the presence of detectable antibodies to NCD and IBD in chickens, in the three districts of North Gondar Zone. As it is known Chickens' which might found in different districts have been high variable detectable antibodies if they are either susceptible to the pathogenic or vaccinated and either female or male.

DECLARATIONS

Acknowledgment

First of all, the authors would like to express their sincere gratitude to the study participants for their willingness to take part in the study. The authors' heartfelt thanks will also go to the University of Gondar, Vice President of research and community service office for the financially supporting the study.

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Authors' contributions

MB1 conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript. ST participated in drafting and reviewing the manuscript. MB2 conceived the study, coordinated the overall activity, and reviewed the manuscript. ST participated in drafting and reviewing the manuscript. AT participated in the design of the study, and reviewed the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

Data will be made available upon request of the primary author

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by the University of Gondar. The views presented in the article are of the authors and do not necessarily express the views of the funding organization. The University of Gondar was not involved in the design of the study, data collection, analysis, and interpretation.

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