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Research Article

Four Serotypes of Infectious Bronchitis Virus are Widespread in Unvaccinated Backyard Chicken and Commercial Farms in Ethiopia

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Abstract

Avian infectious bronchitis is highly contagious disease of respiratory, urogenital and reproductive tissues of chicken causing considerable losses due to death, egg drop and reduced production. Little information is available on infectious bronchitis in chicken in Ethiopia. The objectives of this study were to estimate seroprevalence of infectious bronchitis and identify the serotypes of infectious bronchitis virus infecting chicken in the country. A total of 711 serum samples were collected from chicken reared under ten commercial and six backyard farms in Oromia and Southern Nations Nationalities and People's Regional States. The presence of antibody against infectious bronchitis virus was detected using standard quantitative indirect ELISA while the serotypes of the virus was identified by hemagglutination inhibition test. Out of the 711-chicken tested 502 (70.60%; CI: 67.11, 73.93) of them were found positive for anti-infectious bronchitis virus antibody. The prevalence was 68.75% (341/496; CI: 64.47, 72.81) and 74.88% (161/215; CI: 68.53, 80.53) in chicken raised under commercial settings and backyard systems, respectively. Four serotypes of infectious bronchitis virus namely M41, D-274, 793B and Qx were identified from unvaccinated backyard and commercial farms. In conclusion this study revealed that the four serotypes of IBV tested are widespread in many backyard and commercial farms in Ethiopia. The seroprevalence observed is high in both commercial and backyard chicken farms.

Keywords: Infectious bronchitis; IBV; Indirect ELISA; Hemagglutination inhibition assay; Backyard; Chicken; Ethiopia

Introduction

Avian Infectious Bronchitis (IB) caused by Infectious Bronchitis Virus (IBV) is acute and highly contagious disease of respiratory, urogenital and reproductive tissues of chicken causing considerable losses due to death, egg drop and reduced production [1-3]. Since its first recognition in USA in 1941 IB has been reported in chicken worldwide. Mortality due to IB is suggested to range from 44% to 100% [4-6]. The variable mortality is associated with the diversified serotypes, pathotypes and genotypes of IBV, which arise as a result of recombination and mutation (insertion or deletion) [7-10]. Vaccination is the most appropriate control option but vaccination failure is frequent event due to little or no cross-protection among the serotypes of the virus and emergence of new serotypes [11].

Chicken production is well entrenched business in Ethiopia. It is an integral part of agricultural activity in all production systems. Despite reports of respiratory diseases in chicken from all production systems, information available on IB and IBV is very scarce. To the best of the knowledge of the authors only one study was carried out thus far revealing the circulation of IBV serotype designated 793B in commercial chicken farms in the country [12]. However, there is empirical evidence in the field suggesting the widespread occurrence of IB in the field. Characterization of IBV is important to understand the epidemiology of the disease and natural history of the virus, which will be helpful devising effective control measures. Therefore, the objectives of this study were to estimate seroprevalence of IB and identify the serotypes of IBV in unvaccinated backyard and commercial chicken farms in Ethiopia.

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Materials and Methods

Study areas

The study was conducted in chicken reared under commercial and backyard systems from two Regional States: Oromia Regional State and Southern Nations, Nationalities and People's Regional Sate between December 2017 and November 2018 (Figure 1). Oromia Regional State is the largest State in Ethiopia hosting nearly half of the country's population and livestock including chicken. The majority of the commercial poultry farms and breeding centers are found in this region. Southern Nations, Nationalities and People's Regional Sate is the second largest Sate of the country where small-scale poultry farms are growing with few commercial farms. It is densely populated region with diversified livestock production.

Study population and design

The chicken used in this study include exotic breeds (Cobb broiler, Rose broiler, Bovans brown, Issa brown and Kokokey) and indigenous chicken ecotypes comprising all age groups. Cross-sectional approach was employed to collect samples from chicken reared on ten commercial farms, eight of which were located in Oromia Regional

State. They include Alema, ELFORA, Genesis, Maranata, Hage and Elere (located in Bishoftu) and AYMA and SW (situated in Awash). In addition, two commercial farms namely Hawassa and Bonga were selected from Southern Nations, Nationalities and People's Regional State. The commercial farms selected vaccinated their chicken with H120 (Masatustes type) and 793B (variant type) except Hawassa and Bonga. Indigenous chicken reared on six backyard farms (three from each Regional States) were also sampled. The indigenous chickens have not been vaccinated and were sampled from live bird markets and villages surrounding the commercial farms investigated. Overall a total of 711 chickens (496 from commercial farms and 215 from backyard settings) were sampled.

Blood sample collection and laboratory analysis

Blood samples were collected from wing vein of each chicken using syringe and 21 Gauge needle. The blood samples were kept at room temperature overnight to allow separation of serum from the clot. Then serum samples were transferred to cryovial tubes, labelled individually and transported in ice box to National Animal Health Diagnostic and Investigation Center (NAHDIC) and kept at -20°C until laboratory analysis was performed.

Indirect ELISA for the detection of antibodies against IBV in chicken sera (ID Screen®, IDvet, 310, rue Louis Pasteur - Grabels, France) was used for estimation of seroprevalence according to the manufacturer's instructions. Test kit was validated and verified before analyzing the samples. The antibody titer was estimated from sample to positive ratio (S/P) using Equation 1 as described by the manufacturer. Those serum samples with S/P value of greater than 0.2 or antibody titer greater than 853 were considered positive. Positive samples were subjected to Hemagglutination Inhibition (HI) test for identification of the serotype of IBV.

$$S/P \text{ ratio} = OD_{sample} - OD_{NC} / OD_{PC} - OD_{NC}$$

$$Log10^{(titer)} = 1.00 + log10^{(S/P)} + 3.520 \quad \text{Equation 1}$$

$$Titer = 10^{log10\,(titer)}$$

Antigens prepared from four serotypes of IBV designated M41, 793B, Qx and D274 and positive control (GD laboratory, The Netherlands) were used for serotyping of positive samples. These serotypes were selected purposively based on their worldwide distribution and their dominance in countries that provide parent chicken stock to Ethiopian poultry enterprises. Hemagglutination inhibition assay was carried out after two-fold serial dilution of the test sera in 96-well microtitre plates. The antigens were standardized to 8 IBV hemagglutinating units (HA-units) prior to use. Heat-inactivation of serum samples for 30 minutes at 56°C was done to reduce non-specific inhibition. Serum sample was regarded as positive when it had a titer of 2^4 or more [13].

Results

Seroprevalence

Out of the 711 serum samples tested 502 (70.60%; CI: 67.11, 73.93) of them were found positive for anti-infectious bronchitis virus antibody. The prevalence was 68.75% (341/496; CI: 64.47, 72.81) and 74.88% (161/215; CI: 68.53, 80.53) in chicken raised under commercial settings and backyard systems, respectively. The seroprevalence ranges from 10% in chicken from Bonga to 100% in chicken from central Oromia (Table 1). Overall higher antibody titer was recorded in chicken reared under commercial firms ranging from 288.5 (in Bonga) to 22993.3 (in AYMA) than chicken from backyard systems, which ranges from 1011.7 to 9109.3 (Figure 1).

Figure 1: Map of Ethiopia depicting the regional states where samples were collected.

Table 1: Seroprevalence of anti-infectious bronchitis virus antibody in chicken reared under commercial backyard production systems in two regions of Ethiopia.

Farm	Region	No. Sampled	No. Positive (%)	Mean Ab. Titer
Commercial Farms				
Alema	Oromia	134	115(85.82)	10121
ELFORA	Oromia	43	39(90.69)	10599.2
Maranata	Oromia	47	45(95.74)	15474.2
Genesis	Oromia	25	25(100.00)	16232.8
Elere	Oromia	25	24 (96.00)	17267.5
Hage	Oromia	25	25 (100.00)	6409.9
SW	Oromia	20	20 (100.00)	5172.1
Ayema	Oromia	26	26 (100.00)	22993.3
Bonga*	SNNP	50	5 (10.00)	288.5
Hawassa*	SNNP	101	17 (16.83)	562.61
Sub-total		**496**	**341 (68.75)**	
Backyard farms				
Farm 1	Oromia	37	35(94.54)	9109.3
Farm 2	Oromia	35	27(77.14)	5659
Farm 3	Oromia	39	33(84.62	8366.3
Farm 1	SNNP	42	23(54.76)	1597.7
Farm 2	SNNP	41	33(80.49)	3667.7
Farm 3	SNNP	21	10 (47.62)	1011.7
Sub-total		**215**	**161(74.88)**	

Note*: Unvaccinated flock

Serotyping

The 502 serum samples that were positive for anti-infectious bronchitis virus antibody were tested using HI test to identify the serotypes of IBV. Four serotypes of IBV designated M41, D274, 793B (4/91) and Qx were identified. These serotypes were detected in all commercial farms and interestingly in 100% of unvaccinated chicken from backyard production system. To the best knowledge the authors the serotypes designated M41, D274 and Qx are reported for the first time in Ethiopia in this study. The results of serotyping are given in (Table 2).

Discussion

Identification of the serotypes of IBV is important to establish the catalogue of the virus, which is important for understanding of the epidemiology of the disease and devise effective control measures [14]. Little is known about epidemiology of IB in Ethiopia and horn of Africa in general. The main purpose of this study was to identify the serotypes of IBV circulating among major chicken farming regions of Ethiopia. The results revealed the occurrence of four serotypes of IBV in both commercial and unvaccinated backyard chicken farms. Except the European 793B serotype, which was reported in Ethiopia previously [12], the remaining three (M41, D274 and Qx) are reported for the first time. Since live attenuated vaccines are imported from European countries where IB is endemic, the virus is suggested to

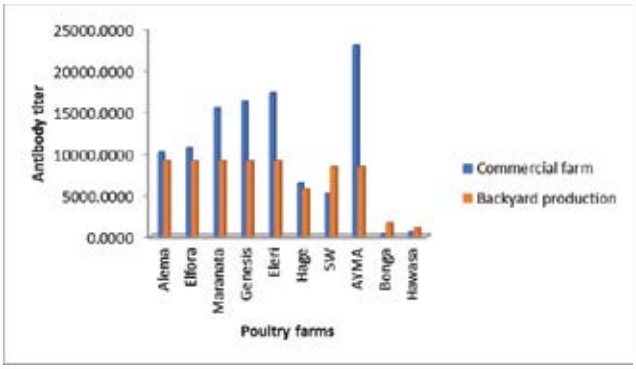

Figure 2: Results of comparison of anti-infectious bronchitis virus antibody titer in chickens under commercial and backyard production systems.

Table 2: Serotypes of infectious bronchitis virus identified in unvaccinated backyard and commercial chicken in Ethiopia.

Serotypes/Antibody Titer				
Study farms/areas	M41	D274	793B (4/91)	Qx
Commercial farms		**Titer**		
Alema	1:256	1:256	1.256	1.256
ELFORA	1:256	1:256	1:256	1:128
Maranata	1:256	1:256	1:256	1:256
Genesis	1:256	1:256	1:256	1:256
Elere	1:256	1:64	1:64	1:32
Hage	1:256	1:256	1:256	1:256
Bonga	1:256	1:256	1:256	1:256
Hawassa	1:256	1:256	1:256	1:256
Backyard farms				
Farm1 (Oromia)	1:256	1:256	1:256	1:256
Farm 2 (Oromia)	1:256	1:256	1:256	1:256
Farm 3 (Oromia)	1:256	1:256	1:256	1:256
Farm 1 (SNNP)	1:256	1:256	1:256	1:256
Farm 2 (SNNP)	1:256	1:256	1:256	1:256
Farm 3 (SNNP)	1:256	1:256	1:256	1:256

be introduced from those countries although this issue needs to be clarified using genetic studies in the future. The absence of bio security measures might have favored its widespread occurrence among various commercial farms and several unvaccinated backyard chicken farms found in the vicinity of commercial farms. The results of this study provide useful information on the serotypes of IBV circulating in Ethiopia. The veterinary and livestock authorities should take this finding into account.

The serotype M41 was shown to cause infection of oviduct in Korea [3]. Identified this serotype in 92% of samples collected from chicken flocks in Jordan, also reported it in poultry breeder farms in Pakistan [15]. That is, it is widespread and may cause reproductive problems in chicken [16]. The Qx serotype has been reported from various parts of the world such as Europe [17], Asia causing up to 40% mortality. It infects respiratory system, spleen, kidney, bursa and proventriculus [3]. It is considered to be one of the predominant serotypes of IBV in Russia [18]. Have reported this serotype in backyard chicken from Pakistan [19], reported the serotype designated D274 to be among the predominant IBV serotypes in Russia revealed that 40% of chicken sera were positive for D274 in Pakistan [18,20] and also revealed the existence of D274 in chicken breeder farms in Pakistan [16]. Since chicken production is an important activity, which is prioritized by the Ministry of Agriculture, the widespread occurrence of these serotypes of IBV shows the huge potential of IB to constrain the sector and hamper the attempts made in achieving food security.

The seroprevalence observed in backyard chicken is quite high. The high antibody titer observed in the absence of vaccination in backyard chicken suggests the exposure of chickens to virulent strains of IBV. Antibody against all of the four serotypes of IBV tested was detected in all farms investigated showing uncontrolled spread of the virus among different farms and areas. Uncontrolled movement and exchange of chicken and farm equipment is evident not only among backyard chicken farmers but also it is frequent among backyard and near commercial farmers. The seroprevalence observed in commercial chicken is also high and anti-IBV antibody was detected in all farms investigated at varying proportions. This high prevalence and widespread occurrence of IBV have significant implication to the poultry farmers and the veterinary authorities should consider control of infectious bronchitis. The overall prevalence observed in this study is in agreement to the reports of [15,16,21]. This showed that IB occurs in many chicken rearing areas of the world. However, our observation is higher than the prevalence reported by in Pakistan [22]. This is difference could be due to the fact that Hussain and colleague investigated small number of commercial farms and used different tests, which has different sensitivity and specificity than the test used during this study.

The high prevalence in backyard chicken is specifically alarming. There is no use of IB vaccine in backyard chicken. Besides, the density of chicken is low in this farming system. That is, the virus has spread widely without recognition of the veterinary authorities. This has important implication to developing countries like Ethiopia where backyard chicken production accounts for 96.9% of the nation's poultry population [23]. In consent to this, in countries where there are weak or no bio security measures high prevalence and widespread occurrence of IB has been reported [1,19,24-31]. This implies that IB is an important impediment to chicken production. Infection of chicken with IBV leads to considerable economic losses as a result of reduced growth in broilers and dropped egg production in layers. Death of infected birds as high as 30% has also been reported elsewhere in the world [10].

In conclusion this study revealed that the four serotypes of IBV tested are widespread in many commercial and backyard chickens in Ethiopia. The seroprevalence observed is high in both commercial and backyard chicken farms. Vaccines incorporating all serotypes are needed to control infectious bronchitis and the veterinary authorities should take this into account.

Ethical approval and consent to participate

This study was submitted to the ethics committee named "Animal Research Ethics Review Committee 'and approved. Ethical clearance was obtained from the Ethics Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University. The clearance identification number is VM/ERC/24/05/2018. Handling of the study animals throughout the study period was done according to this guideline.

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References

1. Sabarinath A, Sabarinath GP, Tiwari KP, Kumthekar SM, Thomas D, Sharma RN. Seroprevalence of infectious bronchitis virus in birds of Grenada. Int J Poult Sci. 2011;10(4):266-8.
2. Jinling F, Yanxin H, Zhijun M, Qi Y, Jixun Z, Xiaodong L, et al. Virulent avian infectious bronchitis virus, people's Republic of China. Emerg Infect Dis. 2012;18(12):1-8.
3. Lin SY, Chen HW. Infectious bronchitis virus variants: analysis pathogenicity investigation. Int J Mol Sci. 2017;18(10):E2030.
4. Delaplane JP, Stuart HO. The modification of infectious bronchitis virus of chickens as the result of propagation in embryonated chicken eggs. Rhode Island Agricultural Experimental Statistical Bulletin. 1941.
5. Cavanagh D. Corona viruses in poultry other birds. Avian Pathol. 2005;34(6):439-48.
6. Yu L, Jiang Y, Low S, Wang Z, Nam SJ, Liu W, et al. Characterization of three infectious bronchitis virus isolates from China associated with proventriculus in vaccinated chickens. Avian Dis. 2001;45(2):416-24.
7. Gelb J, Weisman Y, Ladman BS, Meir R. S1 gene characteristics efficacy of vaccination against infectious bronchitis virus field isolates from the United States and Israel (1996 to 2000). Avian Pathol. 2005;34(3):194-203.
8. Lee CW, Jackwood MW. Evidence of genetic diversity generated by recombination among avian coronavirus IBV. Arch Virol. 2000;145(10):213-48.
9. Alvarado IR, Villegas P, Mossos N, Jackwood MW. Molecular characterization of avian infectious bronchitis virus strains isolated in Colombia during 2003. Avian Dis. 2005;49(4):494-9.
10. Chen Y, Jiang L, Zhao W, Liu L, Zhao Y, Shao Y, et al. Identification molecular characterization of a novel serotype infectious bronchitis virus (GI-28) in China. Vet Microbiol. 2017;198:108-15.
11. De Wit JJ. Detection of infectious bronchitis virus. Avian Pathol. 2000;29(2):71-93.
12. Hutton S, Bettridge J, Christley R, Habte T, Ganapathy K. Detection of infectious bronchitis virus 793B, avian metapneumovirus, Mycoplasma gallisepticum Mycoplasma synoviae in poultry in Ethiopia. Trop Anim Health Prod. 2017;49(2):317-22.
13. OIE (World Organization for Animal Health). Avian Infectious Bronchitis. Terrestrial Manual 2018; Chapter 2.3.2.
14. Shettima YM, El-Yuguda AD, Zanna MY, Abubakar MB, Hamisu TM, Maina MM, et al. Serological evidence of infectious bronchitis virus among some poultry species in Maiduguri, Nigeria. Alexandria J Vet Sci. 2016;51(1):135-9.
15. Dergham AR, Ghassan YK, Ibrahem AS. Infectious bronchitis virus in Jordanian chickens: Seroprevalence detected from Iranian unvaccinated backyard chickens. Iran J Microbiol. 2009;10(1):65-71.
16. Barberis A, Alloui N, Boudaoud A, Ammar OB. Seroprevalence of Infectious Bronchitis Virus in Broiler Farms in Batna, East Algeria. Int J Poult Sci. 2018;17(9):418-22.
17. Bande F, Arshad SS, Omar AR, Hair-Bejo M, Mahmuda AV. Global distributions strain diversity of avian infectious bronchitis virus: a review. Anim Health Res Rev. 2017;18(1):70-83.
18. Evgeniya VO, Yury AB, Lidiya OS, Zoya BN, Nikolay GZ, Nikolay PE, et al. Molecular characterization of infectious bronchitis virus isolates from Russia neighboring countries: identification of intertypic recombination in the S1 gene. Avian Pathol. 2011;40(5):507-14.
19. Shima S, Vahid K, Arash G, Langeroudi M, Vasfi M, Masoud H, et al. Seroprevalence genotyping of avian infectious bronchitis virus detected from Iranian unvaccinated backyard chickens. Iran J Microbiol. 2018;10(1):65-71.
20. Ahmad Z, Hameed A. Detection seroprevalence of infectious bronchitis virus strains in commercial poultry in Pakistan. Poult Sci. 2007;86(7):1329-35.
21. Emikpe BO, Ohore OG, Olujonwo M, Akpavie SO. Prevalence of antibodies to infectious bronchitis virus (IBV) in chickens in Southwestern Nigeria. Afr J Microbiol Res. 2010;4(1):92-5.
22. Hussain A, Khan AS, Khalid M, Hamid T. Seroprevalence polypeptide analysis of infectious bronchitis virus in broilers. Pak Vet J. 2005;25(4):194-6.
23. CSA. Central Statistical Authority of Ethiopia. Statistical analysis report, Addis Ababa. Ethiopia. 2013.
24. Gutierrez-Ruiz EJ, Ramirez-Cruz GT, Gamboa EI, Alexander DJ, Gough RE. A serological survey for avian infectious bronchitis virus Newcastle disease virus antibodies in backyard (free-range) village chickens in Mexico. Trop Anim Health Prod. 2000;32(6):381-90.
25. Hadipour MM, Azad F, Vosoughi A, Fakhrabadipour MA. Measurement of Antibodies to Infectious Bronchitis Virus in Indigenous Chicken Flocks Around Maharlou Lake in Iran. Int J Anim Vet Adv. 2011;3(3):182-5.
26. Selma OA, Ballal A. Seroprevalence of Selected Avian Pathogens of Backyard Poultry in Sinar, Sudan. Bulletin of Animal Health Production in Africa. 2013;61(2):209-14.
27. Abraham S S, Geetha S, Viswanathan H. Prevalence of infectious bronchitis in the organized poultry farms of Kerala. J Ind Vet Assoc. 2014;12(1):88-90.
28. Waleed S, Arash GL, Vahid K, Omid M, Mehdi VM, Masoud H. Prevalence of avian infectious bronchitis virus in broiler chicken farms in south of Iraq, 2014 - 2015. Vet Res Forum. 2016;7(4):317-21.
29. Adebiyi AF. Infectious bronchitis virus in Captured free-living, free-range intensively Reared birds in southwest Nigeria. Folia Veterinaria. 2017;61(1):23-6.
30. Matilda A, Doreen DO, Hilda EO, Agyekum OA, Patricia H, Michael A, et al. Serological Molecular Surveillance of Infectious Bronchitis Virus Infection in Free-Range Chickens Guinea Fowls in the Ga-East District of Ghana. J Vet Med. 2018;1-6.
31. Zafar AB, Giasuddin MD, Zahed U, Mahmood K. Seroprevalence of infectious bronchitis virus in different types of chicken in Bangladesh. Asian J Med. 2018;4(1):132-6.