Seroprevalence of infectious bronchitis virus and avian reovirus in free backyard chickens

Infectious bronchitis virus (IBV) and avian reovirus (ARV) cause significant losses in the poultry industry throughout the world. A cross-sectional study was conducted in four villages in Manjacaze district, Southern Mozambique, to determine the seroprevalence of IBV and ARV. A total of 467 serum samples from adult unvaccinated backyard chickens were screened using commercial and competitive enzyme-linked immunoabsorbent assay kits. Our results showed anti-IBV and anti-ARV antibodies in all surveyed households and villages. The overall seroprevalence was 89.5% (95% confidence interval [CI]: 77.2–97.4) and 95.7% (95% CI: 88.0–99.2) for IBV and ARV, respectively. The risk of becoming exposed to IBV was lower in Chidenguele village compared with the other three villages (p > 0.05). However, no statistically significant differences were observed for becoming exposed to ARV between villages (p < 0.05). The backyard chickens tested in this study had no previous history of vaccination, outbreaks or typical clinical signs of IB and AR diseases. Therefore, the presence of antibodies to IBV and ARV was considered clear evidence that the birds have been naturally exposed to those two infectious agents, and the infection was of subclinical type. It is concluded that IBV and ARV are widespread in backyard chickens in the studied area. These obtained data are essential for design and implementation of chicken health development programmes.

Introduction

Infectious bronchitis (IB) is a highly contagious disease with severe economic consequences in the chicken industry worldwide (Cavanagh 2007). The etiologic agent, infectious bronchitis virus (IBV), belongs to the family Coronaviridae and subfamily Coronavirus within the genera Gammacoronaviridae. Infectious bronchitis virus is an enveloped virus with single-stranded positive-sense linear ribonucleic acid (RNA) with the genome of about 27 kB (Jackwood 2012). Birds infected with IBV may show depression, watery eyes, mucus in the nares and trachea, gasping, coughing and tracheal rales (Jackwood 2012). In laying birds, IB disease can also cause a decrease in egg production and egg quality (Cavanagh & Naqi 2003; Jackwood, Hall & Handel 2012). Morbidity is normally 100%, while mortality is low but can be higher than 50%, depending on the age of the birds, strain of the virus and presence of secondary infections (Jackwood 2012; Jackwood et al. 2012). At necropsy, gross lesions observed on respiratory organs of chickens naturally infected with IBV include the presence of mucous secretion, congestion and hyperaemia in the trachea and mild focal areas of lung consolidation (Khataby et al. 2016). In backyard chickens, IB has been reported in a few countries worldwide, including Zimbabwe (Kelly et al. 1994), South Africa (Thekiso et al. 2003), Botswana (Mushi et al. 2006), Nigeria (Owoade, Ducatez & Muller 2006), Ethiopia (Chaka et al. 2012), Ivory Coast (Kouakou et al. 2015), Mexico (Gutierrez-Ruiz et al. 2000), Switzerland (Keller-Berger & Hoop 1993), Bangladesh (Biswas et al. 2009) and Iran (Hadipour et al. 2011). There are no published reports of this disease in chickens in Mozambique.

Avian reovirus (ARV) is a pathogenic agent in chickens (Gallus gallus) and a member of the genus Orthoreovirus in the Reoviridae family. The ARV genome contains 10 segments of double-stranded RNA that encode at least eight structural proteins (IA, IB, IC, mA, mB, sA, sB and sC) and four nonstructural proteins (mNS, P10, P17 and sNS) (Bodelon et al. 2001; Saif et al. 2003; Wundervald
Reovirus infections are prevalent worldwide in chickens, turkeys and other avian species (Bodelon et al. 2001), where they are associated with several diseases such as viral arthritis or malabsorption syndrome, causing considerable economic losses in the chicken industry worldwide (Jackwood 2012; Jackwood et al. 2012; Khataby et al. 2016). Economic losses related to reoviral infections are frequently associated with increased mortality, viral arthritis or tenosynovitis and general lack of performance (Jackwood 2012; Jackwood et al. 2012; Khataby et al. 2016). Literature on the epidemiology and significance of ARV infections in backyard chickens is limited to only one report from Zimbabwe (Kelly et al. 1994). To our knowledge, there are no reports of ARV presence in either backyard, broiler or layer chickens in Mozambique.

Several government and nongovernmental backyard chicken development and social projects are being implemented in Southern Mozambique. The aim of these initiatives is to improve availability of protein of animal origin and income generation for villagers. Data on disease prevalence are needed for design and implementation of chicken disease control strategies.

Unlike other viral and bacterial diseases (Messa et al. 2017; Taunde et al. 2017a, 2017b), little is known about the IB and AR status of backyard chickens in Mozambique. This study aimed to determine the seroprevalence of IBV and ARV in backyard chicken health in Southern Mozambique.

Research methods and design

Study area and sampling

From June 2020 to July 2020, a cross-sectional study was conducted in four villages of Manjakazi district, Southern Mozambique (Figure 1). The study villages were selected because public and private chicken development projects are being implemented. The farmer households were selected based on owners’ willingness to participate in the study. The required minimum sample size was calculated using the formula:

$$n = \left( \frac{Z_{\alpha/2} \times p \times q}{L^2} \right)$$  \[Eqn 1\]

where $n$ = sample size required; $Z_{\alpha/2} = 1.96$ is the value required for confidence of 95%; $p = a$ priori estimate of the prevalence; $q = 1 - p$ the complementary of prior estimate; and $L = 5\%$, the precision of estimate (Emikpe et al. 2003). A previous estimate of the prevalence of 50% was used, as there were no previous studies regarding IB and ARV in Mozambique. A total of 467 serum samples were screened to determine the seroprevalence of IBV and ARV. Blood samples were collected aseptically from the wing vein of each chicken. About 2 mL – 4 mL of blood samples were collected using a sterile disposable syringe with 22 gauge 1 and a quarter needle size. Serum was acquired after 5 min centrifugation at 1500 rotation per minute (rpm) of the coagulated blood samples and stored at −20 °C before testing.

Serology

Serum samples were analysed using commercial enzyme-linked immunosorbent assay (ELISA) kits for the presence of anti-IBV antibodies (ProFLOK® Infectious Bronchitis Virus Antibody Test Kit, Synbiotics Corp., San Diego, CA, United States) and anti-ARV antibodies (ProFLOK® Avian Reovirus Virus Antibody Test Kit, Synbiotics Corp., San Diego, CA, United States) according to the manufacturer’s instructions. Optical reading was performed at 450 nm, and the cut-off values used were according to the manufacturer’s instructions.

Statistical analysis

Data were entered into a Microsoft Excel® spreadsheet (Microsoft Corporation, Redmond, Washington, United States) and exported to Stata® version 12.1 (Stata IC 12.1 for Windows) software (StataCorp LLC, College Station, Texas, United States) for analysis. Prevalence data were analysed using the chi-square test ($\chi^2$-test). Logistic regression models were used to compute odds ratios (OR) to identify the risk for being infected as dichotomous dependent variable and independent variable (location). In all chi-square tests, $p < 0.05$ was considered statistically significant.

Ethical considerations

Ethical review and approval were granted by the Scientific Board of the Faculty of Veterinary Medicine, Eduardo Mondlane University (ethical clearance number 280/FAVET).

Results

Antibodies to IBV and ARV were detected in chickens from all villages included in the study. Serological results are presented in Table 1. The seroprevalences for IBV showed...
wide variation between villages. Chidenguele and Macuacua villages showed the highest and lowest seroprevalence, respectively. The risk of becoming exposed to IBV was significantly higher in Macuacua and Chizavane villages than Chidenguele village \( (p < 0.05) \). Narrowed seroprevalence variations between villages were seen for ARV. Seroprevalences of ARV did not exhibit significant differences between villages \( (p = 0.05) \).

**Discussion**

This study is the first cross-sectional survey conducted to determine the seroprevalence of IBV and ARV among backyard chickens at the village level in Southern Mozambique. In unvaccinated flocks, positive serological results are clear evidence that the birds have been exposed to the infectious agent under consideration, although without identifying the infectious strains \( (\text{Chaka et al. 2012}) \). In the present study area and elsewhere in Mozambique, backyard chickens are not vaccinated against IBV or ARV. Therefore, the presence of antibodies to IBV and ARV was considered evidence of exposure to natural infection.

This study’s findings indicated that IBV is widespread among backyard chickens in the studied villages, with a seroprevalence of 93.3\%. This high prevalence agrees with reports from Zimbabwe (86\%) \( (\text{Kelly et al. 1994}) \), Switzerland (\( > 75\% \)) \( (\text{Keller-Berger & Hoop 1993}) \), Bangladesh (74\%) \( (\text{Biswa et al. 2009}) \), central Ethiopia (91\% and 97.46\%) \( (\text{Habte et al. 2022}) \), and Nigeria (82.95\%) \( (\text{Ijoma et al. 2022}) \). Relatively lower IBV seroprevalences had been recorded in Mexico (56.5\%) \( (\text{Gutierrez-Ruiz et al. 2000}) \), South Africa (43\%) \( (\text{Thekiseoe et al. 2003}) \), Botswana (65.22\%) \( (\text{Mushi et al. 2006}) \), and north-west Ethiopia (23.96\%) \( (\text{Birhan et al. 2021}) \). The high proportion of serologically positive samples with the absence of any clinical signs or significant mortality may imply that village chickens are resistant or had been infected by less virulent strains, as has been described in Kenya \( (\text{Mbuthia et al. 2008}) \).

To date, very few studies on seroprevalence of ARV in backyard chickens have been conducted worldwide. The present study’s findings showed that the seroprevalence of ARV is very high (94.4\%), which agrees with reports from western Turkey (86.53\%) \( (\text{Erol & Sengil 2012}) \) and Korea (100\%) \( (\text{Jae-Kyo et al. 2019}) \). Lower ARV seroprevalences were reported from Zimbabwe (3\%) \( (\text{Kelly et al. 1994}) \). Although a high seroprevalence (94.4\%) of ARV was found in the present study, the importance of ARV in backyard chickens remains still to be determined.

In backyard flocks from the present study sites, biosecurity measures are not employed, flocks are often composed of a mixture of ages and species and new chickens are continually added. The authors speculate that these husbandry practices and the backyard chickens–wild birds contact may explain the high seroprevalence reported in this study for both IBV and ARV \( (\text{Wille & Holmes 2020}) \).

In summary, it is concluded that IBV and ARV are circulating among indigenous chickens in the investigated area. Therefore, the infected chickens may represent a threat in the transmission of those viruses to wild birds, broiler or layer chickens in that region. However, additional research is warranted to identify the circulating strains and the epidemiology of these diseases.

**Acknowledgements**

We would like to acknowledge the farmers for their helpful cooperation.

**Competing interests**

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

**Authors’ contributions**

S.C.P., J.A., K.C., A.G.D. and C.G.B. contributed for the conceptualisation, validation, writing, review and editing of the article. A.G.C. contributed to the methodology design and statistical analysis. C.G.B. contributed to project administration, resources, funding and supervision. All authors have read and agreed to the submitted version of the manuscript.

**Funding information**

The research was funded by the Fundo Nacional de Investigação, Maputo – Mozambique.

**Data availability**

The authors confirm that the data supporting the findings of this study are available within the article.

**Disclaimer**

Any opinion, finding and conclusion or recommendation expressed in this material are those of the authors and do not necessarily reflect the views of the organisation that provided support for the project and the publisher. The funders had no role in the study design or the decision to submit the work for publication.
References

Birhan, M., Temeqene, M., Shite, A., Berhan, N., Bitew, M., Gelaye, E. et al., 2021, 'Seroprevalence and associated risk factors of infectious bronchitis virus in chicken in Northwest Ethiopia', Scientific World Journal 2021, 4553890. https://doi.org/10.1155/2021/4553890

Biswas, P.K., Barua, H., Uddin, G.M., Biswas, D., Ahad, A. & Debnath, N.C., 2009, 'Serosurvey of five viruses in chickens on smallholdings in Bangladesh', Preventive Veterinary Medicine 88(1), 67–71. https://doi.org/10.1016/j.prevetmed.2008.06.018

Bodelon, G., Labrada, L., Martinez-Costas, I. & Benavente, J., 2001, 'The avian reovirus genome segment S1 is a functionally tricistronic gene that expresses one structural and two nonstructural proteins in infected cells', Virology 290(2), 181–191. https://doi.org/10.1006/viro.2001.1159

Cavanaugh, D. & Gelb, Jr., G., 2008, 'Infectious bronchitis', in Y.M. Saif (ed.), Diseases of poultry, 12th edn., pp. 117–135, Blackwell Publishing, Ames, IA.

Cavanaugh, D. & Naqi, S.A., 2003, Diseases of poultry, 11th edn., Iowa State University Press, Ames, IA.

Chaka, H., Goutard, F., Bisschop, S.P.R. & Thompson, P.N., 2012, 'Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa zone, Ethiopia', Poultry Science 91(4), 862–869. https://doi.org/10.3382/ps.2011-01906

Emikoe, B.O., Ohore, O.G., Oluwauelu, D.O., Oladele, O.A., Ockiiya, M.A. & Enioka, S.O., 2003, 'Serorevelance of antibodies to infectious bronchitis virus in Nigerian indigenous chickens in Ibadan, Nigeria', Nigerian Veterinary Journal 24(3), 9–12.

Erol, N. & Sengül, S.S., 2012, 'Seroprevalence of avian reovirus infections in chickens in Western provinces of Turkey', Kathis Universitesi Veteriner Fakultesi Dergisi 18(4), 653–656. https://doi.org/10.9775/kvfvd.2012.6171

Gutierrez-Ruiz, E.J., Ramirez-Cruz, G.T., Camara Gamboa, E.I., Alexander, D.J. & Gough, R.E., 2000, 'A serological survey for avian infectious bronchitis virus and Newcastle disease virus antibodies in backyard (free-range) village chickens in Mexico', Tropical Animal Health and Production 32(6), 381–390. https://doi.org/10.1023/A:1005281619260

Habte, T., Gerber, P.F., Ibrahim, F., Groves, P.J. & Walkden-Brown, S.W., 2022, 'Seroprevalence of major respiratory diseases of chickens in the Qwa-Qwa district of the northeastern Free State province of South Africa', Journal of the South African Veterinary Association 83(8), 102, 83–88. https://doi.org/10.4314/sokjvs.v18i4.6

Hadijopur, M.M., Azad, F., Vosoughi, A., Fakhrabadipour, M. & Olyaei, A., 2011, 'Measurement of antibodies to infectious bronchitis virus in indigenous chicken farms around Maharloo lake in Iran', International Journal of Animal Veterinary Advances 3(3), 182–185.

Ijoma, S., Shittu, I., Chinyere, C., Olawuyi, K.A., Gado, D.A., Nwagbo, I.O. et al., 2020, 'Seroprevalence and associated risk factors of infectious bronchitis virus in backyard chickens in Southern Mozambique', Journal of Veterinary Medicine A 2020, 1715. https://doi.org/10.1155/2020/743187

Ijoma, S., Shittu, I., Chinyere, C., Olawuyi, K.A., Gado, D.A., Nwagbo, I.O. et al., 2020, 'Seroprevalence of major respiratory diseases of chickens in the Qwa-Qwa district of the northeastern Free State province of South Africa', Journal of the South African Veterinary Association 83(8), 102, 83–88. https://doi.org/10.4314/sokjvs.v18i4.6

Kelly, P.J., Chitauro, D., Rohde, C., Rukwawa, J., Majok, A., Davelaar, F. et al., 1994, 'Diseases and management of backyard chicken flocks in Chitungwaza, Zimbabwe', Avian Diseases 38(1), 626–629. https://doi.org/10.2307/1592089

Khataby, K., Fellahi, S., Mustapha, E.M., Loutfi, C. & Khataby, M., 2015, 'Avian infectious bronchitis virus in Africa: A review', Veterinary Quarterly 36(2), 71–75. https://doi.org/10.1007/s11250-015-0130-4

Kouakou, A.V., Kouakou, V., Kouakou, C., Godji, P., Kouassi, A.L., Krou, H.A. et al., 2015, 'Prevalence of Newcastle disease virus and infectious bronchitis virus in avian influenza negative birds from live bird markets and backyard and commercial farms in Ivory-Cost', Research in Veterinary Science 102, 83–88. https://doi.org/10.1016/j.rvsc.2015.07.015

Mbithua, P.G., Ngaji, W.L., Nyaga, P.N., Bebora, L.C., Minga, U., Kamundia, J. et al., 2008, 'Pasteurella multocida in scavenging family chickens and ducks: Carrier status, age susceptibility and transmission between species', Avian Pathology 37(1), 51–57. https://doi.org/10.1080/03079450501784891

Messa, J.A., Taunde, P., Zandamela, A.F., Junior, A.P., Chilundo, A., Costa, R. et al., 2017, 'Serological screening suggests extensive presence of Mycoplasma gallisepticum and Mycoplasma synoviae in backyard chickens in Southern Mozambique', Journal of Veterinary Medicine A 2017, 2743187. https://doi.org/10.1155/2017/2743187

Mushio, T., Binta, M.G., Chabo, R.G. & Itebegu, K., 2006, 'Diseases of indigenous chickens in Bokaa village, Kpateng district, Botswana', Journal of the South African Veterinary Association 77(3), 131–133. https://doi.org/10.4102/jsava.v77i3.360

Owoade, A.A., Ducatze, M.F. & Muller, C.P., 2006, 'Serorevelance of avian influenza virus, infectious bronchitis virus, revovirus, avian pneumovirus, infectious laryngotracheitis virus, and avian leukosis virus in Nigerian poultry', Avian Diseases 50(2), 222–227. https://doi.org/10.1637/142-0715005.1

Saif, Y.M., Barnes, H.J., Giissson, J.R., Fadly, A.M., McDougald, L.R. & Swayne, D.E., 2017, Diseases of poultry, 11th edn., Blackwell Publishing Professional, New Jersey.

Shiferaw, J., Degu, T., Tefera, M. & Tamiru, Y., 2012, 'Seroprevalence of infectious bronchitis virus in live and layer farms of Central Ethiopia', Biomed Research International 2012, 8915400. https://doi.org/10.1155/2012/8915400

Taunde, P., Lucas, A.F., Chilundo, A., Costa, R. & Bila, C.G., 2017a, 'Serological survey of avian influenza virus infection of unvaccinated backyard chickens in Mandilhakazi, Southern Mozambique', Avian Pacific Journal of Tropical Biomedicine 7(4), 686–688. https://doi.org/10.4102/apjtb.2017.07.008

Taunde, P., Timbe, P., Lucas, A.F., Tcham, C., Chilundo, A., Dos Anjos, F. et al., 2017b, 'Serological evidence of avian encephalomyelitis virus and Pasteurella multocida infection in free-range indigenous chickens in Southern Mozambique', Tropical Animal Health and Production 49, 1047–1050. https://doi.org/10.1007/s11250-017-1304-x

Thekiso, M.M., Mbatia, P.A. & Bisschop, S.P., 2003, 'Diseases of free-ranging chickens in the Qwa-Qwa district of the northeastern Free State province of South Africa', Journal of the South African Veterinary Association 74(1), 14–16. https://doi.org/10.4102/jsava.v74i1.490

Wille, M. & Holmes, E.C., 2020, 'Wild birds as reservoirs for diverse and abundant gamma- and deltacoronaviruses', FEBS Microbiology Reviews 44(5), 631–646. https://doi.org/10.1093/femsre/fuu026

Wundervald, W. & Hoop, R.K., 2002, 'Serological monitoring of 40 Swiss fancy breed poultry flocks', Avian Pathology 31(2), 157–162. https://doi.org/10.1080/03079450120118649