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Molecular detection of infectious bronchitis and avian metapneumoviruses in Oman backyard poultry

Thunai Al-Shekaili, Matthew Baylis [1], Kannan Ganapathy *

Institute of Infection and Global Health, University of Liverpool, Leahurst Campus, Neston, Cheshire, CH64 7TE, UK

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ABSTRACT

Infectious bronchitis virus (IBV) and avian metapneumovirus (aMPV) are economically important viral pathogens infecting chickens globally. Identification of endemic IBV and aMPV strains promotes better control of both diseases and prevents production losses. Orophrayngeal swab samples were taken from 2317 birds within 243 different backyard flocks in Oman. Swabs from each flock were examined by RT-PCR using part-S1 and G gene primers for IBV and aMPV respectively. Thirty-nine chicken flocks were positive for IBV. Thirty two of these were genotyped and they were closely related to 793/B, M41, D274, IS/1494/06 and IS/885/00. 793/B-like IBV was also found in one turkey and one duck flock. Five flocks were positive for aMPV subtype B. Though no disease was witnessed at the time of sampling, identified viruses including variant IBV strains, may still pose a threat for both backyard and commercial poultry in Oman.

1. Introduction

Infectious bronchitis virus (IBV) is a highly contagious viral pathogen of chickens. It is a type 3 coronavirus and part of the family *Coronaviridae* (Cavanagh, 2001). Most IBVs infect the respiratory, urinary and reproductive tracts causing considerable production losses (Dolz et al., 2008; Jones, 2010; Roussan et al., 2008; Villarreal et al., 2007; Worthington et al., 2008). IBV infections can also be further aggravated by the presence of bacterial infections such as *Escherichia coli*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Ornithobacterium rhinotracheale* (Landman and Feberwee, 2004; Matthijs et al., 2003; Naqi et al., 2001; van Empel et al., 1996).

Since the first description of IBV in 1931 (Schalk and Hawn, 1931), a number of different IBV genotypes have been detected worldwide (Jackwood, 2012). Virulent IBV genotypes (eg. 793/B, QX, IS/1494/06, IS/885/00, Q1) that have a severe impact on chicken health and production have been reported in recent decades (Gough et al, 1992; Kahya et al., 2013; Meir et al., 2004b; Yu et al., 2001). Infections from different IBV genotypes present a challenge for poultry producers worldwide (Dolz et al., 2008; Jones, 2010; Worthington et al., 2008), and also for owners of backyard chicken flocks. The spike subunit 1 (S1) is highly variable in IBV and analysis of S1 using reverse transcription–polymerase chain reaction (RT-PCR) and sequencing has allowed for genotyping of IBV strains (Kingham et al., 2000) .

Avian metapneumovirus (aMPV) is an avian virus belonging to the *Paramyxoviridae* family (Lee et al., 2007b). It is capable of infecting the respiratory tract of birds, causing avian rhinotracheitis in turkeys (Jones, 2010) and swollen head syndrome in chickens (Georgiades et al., 2001). Furthermore, it also causes a drop in egg production and/or egg quality in both turkeys and chickens (Banet-Noach et al., 2005; Hess et al., 2004). The virus was first reported in South Africa in the 1970s (Buys et al., 1989) and has since spread to other continents (Jones, 2010). There are four distinct aMPV subtypes; A, B, C and D (Cook and Cavanagh, 2002). Subtypes A and B are widespread throughout Asia, Europe, Africa and South America (Jones, 2010; Kwon et al., 2010; Owoade et al., 2008). Reports of infections by subtypes C and D are infrequent and to date, subtype C has been reported in France, Korea and the US (Alvarez et al., 2003; Bayon-Auboyer et al., 2000; Lee et al., 2007a), with D so far only detected in France (Bayon-Auboyer et al., 2000).

There is a particular paucity of information from Oman, with almost no published studies of avian respiratory viruses for any species. This is despite Oman's geographic location (between the horn of Africa and southern Asia), its importance as a site for migrating wild birds and the presence of large commercial poultry production farms. These farms produce the majority of the Omani poultry requirements; however census data in 2004 reports there were around 25,000 backyard flocks bred for household consumption (Anon, 2004). Due to the avian influenza contingency plan implemented between 2004 and 2012, this number has been reduced to nearly 10,000 flocks (Rural Women Development Department, personal communication, 2012). Maintaining a good health

* Corresponding author. Institute of Infection and Global Health, University of Liverpool, Leahurst Campus, Neston, Cheshire, CH64 7TE, UK. Tel.: +44 151 7946019; fax: +44 151 7946005.
E-mail address: gana@liv.ac.uk (K. Ganapathy).
[1] Health Protection Research Unit in Emerging Infections and Zoonoses.

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status of backyard flocks is crucial for both the flock owners and the owners of nearby commercial flocks (McBride et al., 1991). Backyard poultry in Oman are not vaccinated against IBV or aMPV.

This paper reports the first study on the prevalence of IBV genotypes and aMPV subtypes within backyard poultry flocks in Oman.

2. Materials and methods

2.1. Sampling method

Oropharyngeal swabs were collected from a total of 243 backyard flocks (2317 birds) from 237 backyard farms within all regions and governorates of Oman (Fig. 1), from June to September 2012. More than one flock was sampled if the farm had more than one species of bird. The samples were collected during a study on the prevalence of respiratory viruses, such as avian influenza (AI), Newcastle disease (ND), IB and aMPV. The number and location of sampled farms was determined based on the estimated prevalence of Avian Influenza (AI) and Newcastle Disease (ND) in Oman backyard poultry. Sampling criteria were calculated based on an estimated prevalence of AI of 30% and between-cluster variance of 0.7. The number of flocks to be sampled was stratified by region according to the number of poultry farms, total number of poultry, number of people and number of backyard poultry present in each region. A confidence level of 95% was utilised along with a two-stage cluster sampling method (Thrusfield, 1986).

The total backyard poultry population in Oman was estimated by the Ministry of Agriculture and Fisheries, Department of Rural Women Development to be approximately 10,000 poultry flocks (Table 1) with a median size of 50 birds per flock. The vast majority of the sampled farms raised local village chickens; however turkeys, guinea fowl, duck and geese were also present.

Local veterinarians and animal health engineers from the Ministry of Agriculture and Fisheries in each state aided with the selection of farms at different locations. Inclusion criteria involved a minimum distance between two farms (>1 km) and <3 farms from each village. If only one flock was present at a farm, 10 healthy adult (>3 months) birds were selected randomly and sampled. If more than one species of bird was present in the farm, then two flocks would be randomly chosen and 10 birds sampled from each flock. If there were fewer than 10 birds within a chosen flock, then all were sampled.

For detection of respiratory viruses, oropharyngeal swabs were collected from each flock and pooled into 1.5 ml distilled water in a sterile 5 ml plastic bijou container. All samples were kept cool in crushed ice within a thermal-box and brought to the nearby Veterinary Research Centre. The bijou was vortexed and 100 µl was inoculated into the centre of a Flinders Technology Associates (FTA) card (Sigma Aldrich, Dorset, UK) using a sterile pipette and tips. Cards were left to dry for 1 hour at room temperature (22 °C), away from direct light sources, then stored at 4 °C in air-tight plastic bags. Samples were transported to the University of Liverpool, UK, for processing and analysis.

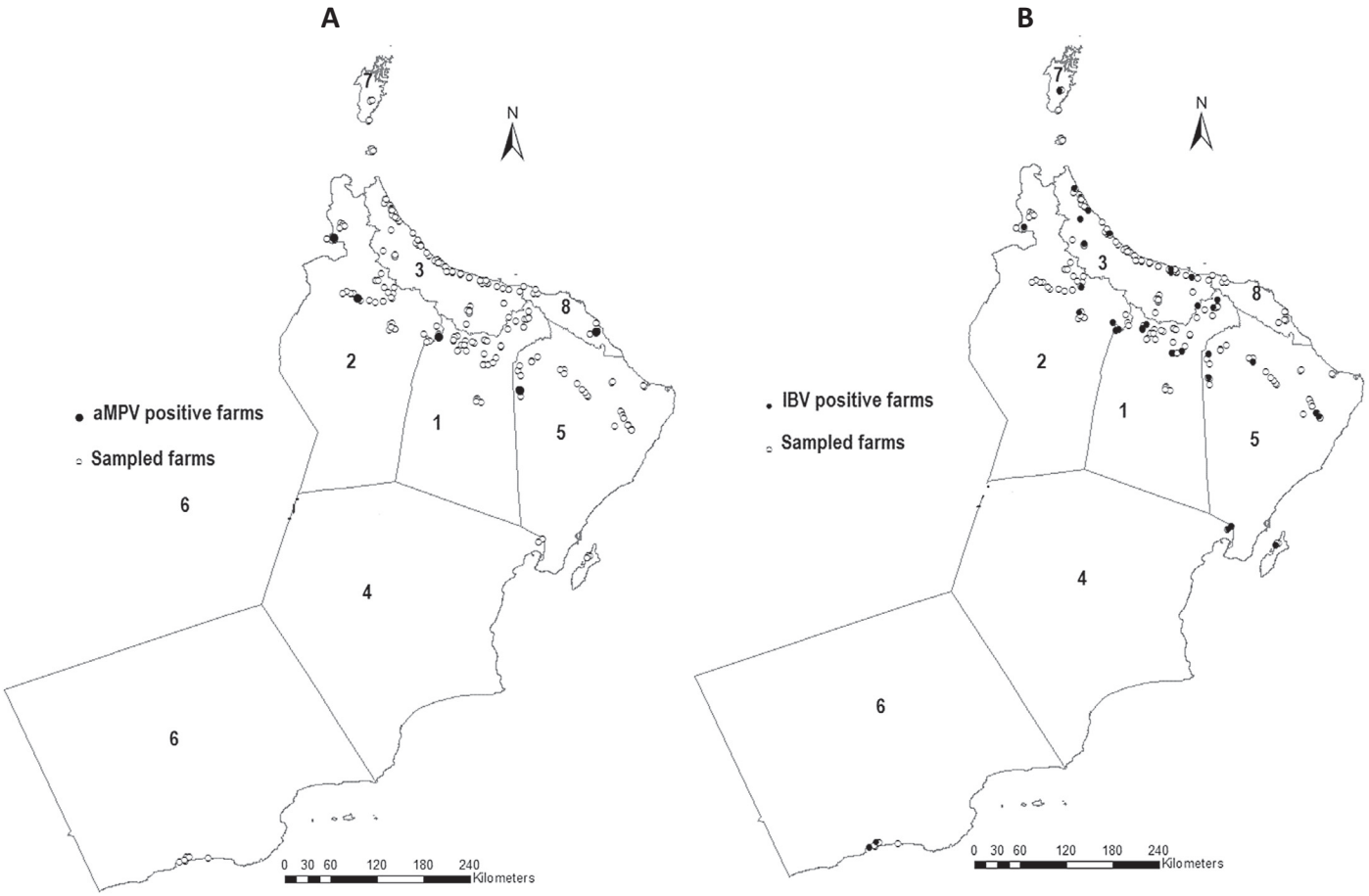

Fig. 1. Locations of sampled farms ($n = 237$) and (A) aMPV positive flocks ($n = 5$) and (B) Locations of IBV positive flocks ($n = 39$). 1 = Ad Dakhliyah, 2 = Adh Dhahirah, 3 = Al Batinah, 4 = Al Wusta, 5 = Ash Sharqiyah, 6 = Dhofar Governorate, 7 = Musandam Governorate, 8 = Muscat Governorate.