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SHORT COMMUNICATIONS

Investigation of infectious laryngotracheitis outbreaks in Namibia in 2018

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Abstract

Between July and August 2018, two outbreaks of infectious laryngotracheitis caused the death of over 116,000 commercial poultry (layers and broilers) near the city of Windhoek, Namibia. A third outbreak occurred in September 2018 in the north of the country approximately 800 km from the original outbreaks. Sample collection and molecular epidemiological analyses revealed that the outbreaks were most likely caused by poor vaccination practices leading to the reversion to virulence of an ILT vaccine strain. The analyses also indicate that inaccurate declarations were made by one of the farms involved and that illegal movement of animals most likely occurred.

Keywords Poultry · Infectious laryngotracheitis · Gallid alphaherpesvirus-1 · Namibia · Phylogenetic analysis · ICP4 · Vaccination

Introduction

Infectious laryngotracheitis (ILT) is an acute and highly contagious respiratory disease of chickens, pheasants, peafowl and turkeys that has a significant economic impact on the poultry industry globally (García et al. 2013). The disease is caused by the Gallid alphaherpesvirus-1 (GaHV-1) which is a member of the genus *Iltovirus*, family *Herpesviridae*, subfamily *Alphaherpesvirinae*. The virus has a dsDNA genome of approximately 150 kb that encodes 80 viral proteins (Menendez et al. 2014).

ILT is primarily associated with morbidity (50–100%), increased mortality (up to 70%) and decreases in egg production in flocks. Transmission between farms can occur by airborne particles or fomites with movement and mixing of flocks being recognised as important factors in the spread of disease. A number of different types of vaccines are available for the control of the disease (García 2017). These include killed, live attenuated and recombinant vaccines. The live attenuated vaccines are produced by sequential passaging in either tissue cultures or embryonated eggs but have been known to revert to virulence and cause outbreaks following bird-to-bird passage or poor administration procedures (Chacón and Ferreira 2009; Lee et al. 2012; Shehata et al. 2013). Molecular epidemiological tools (e.g. PCR-RFLP and PCR-sequencing of specific genes) can be used to differentiate between field isolates of ILT and vaccine strains (Menendez et al. 2014)

Evidence for the presence of ILT in Africa is limited to a small number of publications from Morocco, Egypt and Nigeria (El Houadfi et al. 2005; Shehata et al. 2013; Shittu et al. 2016). The present study describes three outbreaks that occurred in poultry in Namibia in 2018 and, to the best of our knowledge, is the first such report for southern Africa (Menendez et al. 2014).

Materials and methods

Sample processing

Dead chickens ($n = 80$) were collected from three farms [A ($n = 30$), B ($n = 45$), and C ($n = 5$)]. Tracheal samples from

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each bird were homogenised using a TissueLyzer II (Qiagen GmbH, Düsseldorf, Germany). DNA was then extracted using a Maxwell®16 Tissue DNA Purification Kit (Promega, Madison, WI, USA) and eluted in a volume of 300 μl. Amplification of a segment of the infected-cell polypeptide 4 (ICP4) gene was performed on the DNA samples using primers 1F: 5′ GGGTCTTGTTCTGCAGGATTCT 3′ and R1: 5′ CATCGGGACATTCTCCAGGTAGCA 3′ according to Chacón and Ferreira (2009). The following thermal profile was used: 94 °C for 3 min as initial denaturation followed by 35 cycles of denaturation at 94 °C for 60 s, annealing at 62 °C for 60 s and elongation at 72 °C for 90 s, followed by a final elongation at 72 °C for 10 min. Samples were also tested for avian influenza (AI), Newcastle disease virus (NDV), infectious bursal disease virus (IBDV), *Pasteurella multocida* and *Mycoplasma* spp. using standard techniques.

Sequence and phylogenetic analysis

Amplicons were purified using the Wizard® SV gel and PCR Clean-up system (Promega) and sent for sequencing at LGC genomics (Berlin, Germany). The Staden Package (http://staden.sourceforge.net/) was used to assemble the generated sequences, and multiple sequence alignment was performed using MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/) with default settings, incorporating all the sequences generated here combined with a selection of representative sequences available in GenBank. Phylogenetic trees were estimated using the maximum likelihood (ML) method available in MEGA 6 (Tamura et al. 2013) employing the Kimura 2-parameter model of nucleotide substitution with uniform rates among sites and 500 bootstrap replications.

Results and discussion

Between August 21 and September 10, 2018, diseased chicken carcases from three farms (Farm A, Farm B and Farm C) were sent by state veterinarians to the Central Veterinary Laboratory (CVL), Windhoek, Namibia, for further disease investigation (Table 1). Farms A and B are located 8 km from each other and approximately 20 km from Windhoek while Farm C is approximately 800 km from Windhoek (Fig. 1). Both Farm A (layers) and Farm B (broilers) reported 70% mortality among their flocks while Farm C (broilers) reported 23% mortality. Prior to death, all of the diseased birds showed respiratory difficulties which included gasping, extension of the neck, sinusitis and coughing. In Farm A, an 8% drop in egg production was also recorded. Upon arrival at the CVL, a full necroscopy was performed on the dead birds which revealed severe laryngotracheitis and the presence of blood and yellow exudates in the trachea.

All of the samples investigated were positive for ILT by PCR. None of the samples were positive for AI, NDV, IBDV, *P. multocida* or *Mycoplasma* spp. Four representative amplicons were purified and sequenced as described. All sequences generated in this study have been submitted to GenBank under accession numbers (MK089452 to MK089455). Phylogenetic analysis (Fig. 2) revealed that the samples analysed were identical to the Nobilis® ILT vaccine strain. This vaccine is from a chicken embryo propagated culture of a modified Serva strain of ILT. Although this vaccine is registered for use in Namibia, it is not currently available for purchase in the country (March 2019).

Up until December 2017, Farm A declared that it vaccinated all birds at 4 weeks of age with Nobilis® ILT vaccine strain with a follow-up vaccination at 10 weeks of age. However, between January and May 2018, Farm A ceased vaccination with Nobilis® ILT as the vaccine was not available for purchase in Namibia during that period. Farm A then claimed to have recommenced vaccination in June 2018 with LT-IVAX® (containing a live virus of chicken tissue culture origin). However, they administered the vaccine by aerosol spray and not by eye drop as recommended by the manufacturer. From the phylogenetic analysis (Fig. 2) of the samples collected from Farm A (4476 and 4960), it is clear that the ILT virus identified was more similar to the Nobilis® ILT vaccine strain than the LT-IVAX ® strain. This data would therefore suggest one of two possibilities: (1) Farm A inaccurately reported using LT-IVAX ® in June 2018 and instead used Nobilis® ILT vaccine which was most probably purchased outside of Namibia (e.g. in South Africa); (2) the Nobilis® ILT vaccine strain detected in the diseased birds in August 2018 originated from the Nobilis® ILT vaccine used up until May 2018 (i.e. from older birds in the flock). In either case, it is believed that the incorrect administration of the Nobilis®

Table 1 Description of samples analysed in this study

Farm	Date collected	ID	Bird	Age (days)	Mortality	Vaccination declared	GenBank no.
A	21/08/2018 21/09/2018	4476 4960	Layer	35	70%	Nobilis MSD and then LT-IvaX MSD	MK089452 MK089455
B	22/08/2018	4501	Broiler	26	70%	LT-IvaX MSD (breeders only)	MK089453
C	10/09/2018	4840	Broiler	29	23%	No vaccination	MK089454

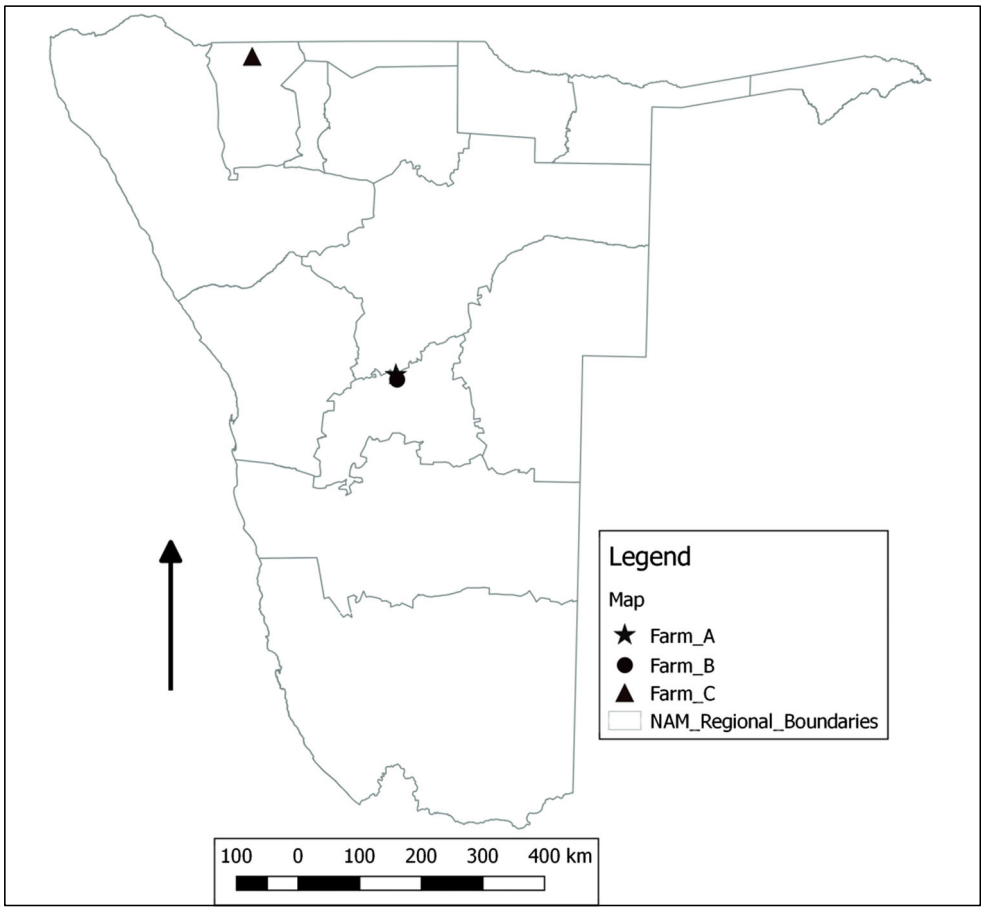

Fig. 1 Map of Namibia showing the locations of Farms A, B and C

ILT vaccine (i.e. through aerosol spray rather than eye drop) led to the incomplete coverage of the flock and the reversion to virulence of the Nobilis® ILT vaccine strain.

Farm B consists of breeders vaccinated with LT-IVAX® and un-vaccinated broilers. It was only the broilers that succumbed to ILT. They were most probably infected by the revertant virus originating from Farm A via airborne transmission or contaminated fomites due to the proximity of the farms (Johnson et al. 2005: Dufour-Zavala 2008). July and August are windy months in Namibia, particularly in the area where

Fig. 2 Maximum-likelihood analysis of a partial segments (between 587 and 602 bp) of the ICP4 gene of ILTVs using the MEGA6 software. The samples from this study are shown with black circles. The numbers indicate the bootstrap values calculated from 500 replicates

Farms A and B are located. In addition, workers from Farm A and Farm B live in the same settlement located between the two farms and this may also have facilitated viral transmission. Farm C claims to have never vaccinated its small flock of chickens ($n = 90$) for ILT. Namibian authorities suspect that this farm may have purchased birds from Farm A resulting in ILTV transmission. This theory is supported by the fact that in September 2018, a truck containing 5000 birds from Farm A was stopped by authorities while travelling to northern Namibia despite the ban on animal movement that had been placed on this farm.

As a result of these ILT outbreaks, over 200,000 chickens were lost and a mortality of 10% continues in Farms A and B as of December 2018. This outcome highlights the impact that ILT can have on poultry populations when incorrect vaccination procedures are used. This study has also confirmed the utility of molecular epidemiological analysis as a valuable tool in outbreak investigations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval This article does not contain studies on live animals.

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