Determination of the seroprevalence of Newcastle disease virus (avian paramyxovirus type 1) in Zambian backyard chicken flocks

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Dates:
Received: 15 July 2012
Accepted: 10 Oct. 2012
Published: 07 Dec. 2012

How to cite this article:
Musako, C. & Abolnik, C., 2012, 'Determination of the seroprevalence of Newcastle disease virus (avian paramyxovirus type 1) in Zambian backyard chicken flocks', *Onderstepoort Journal of Veterinary Research* 79(1), Art. #502, 4 pages. http://dx.doi.org/10.4102/ojvr.v79i1.502

A cross-sectional study was conducted in five provinces and 11 districts of Zambia to determine the seroprevalence of Newcastle disease in Zambian backyard chicken flocks. Of the chickens sampled, 73.9% tested positive for avian paramyxovirus type 1 antibodies by means of an enzyme-linked immunosorbent assay. Seroprevalence varied amongst the five provinces sampled, ranging from 82.6% in the Eastern Province to 48.3% in Luapula Province. Seroprevalence also varied amongst the 11 districts sampled, ranging from 91.3% in Monze district of Southern Province to 22.8% in Mufulira district of the Copperbelt province. Overall, the seroprevalence of Newcastle disease in Zambian backyard chicken flocks has increased since the previous study conducted in 1994.

Introduction

Newcastle disease (ND), notifiable to the World Organisation of Animal Health (OIE 2010), is caused by virulent avian paramyxovirus type 1 (APMV-1) strains. It is a contagious disease of birds that is widely distributed throughout the world, affecting many domestic and wild avian species and causes severe economic losses in the poultry sector (Cattoli *et al*. 2009). Although two classes (I and II) and multiple genotypes are described, only a single serotype of APMV-1 exists (Czegledi *et al*. 2006).

The poultry industry in Zambia is based on two distinct systems. The first is the commercial system, where broilers or layers are obtained from hatcheries and reared on commercial feed and in properly designed chicken houses. Strict vaccination schedules for important avian diseases such as ND are followed, and the lentogenic LaSota/46 vaccine strain is widely used. Most commercial systems are situated along the railway line in close proximity to major towns. The second system is the village or backyard production system, where chickens scavenge for food and subsist with little input from their owners. Vaccination schedules are not followed for these chickens (Songolo & Katongo 2000).

In a previous study (Alders, Inoue & Katongo 1994) the seroprevalence of ND in chickens was determined to be 36.9%, based on the haemagglutination inhibition (HI) titres of 2000 blood samples. Seroprevalence varied between provinces, ranging from 29.2% in the Northern Province to 51.3% in the Copperbelt Province. Recognising that small-scale farming has the potential to make an important developmental contribution to the national economy, the government is now considering improving support to the agricultural sector, thus spurring sectoral growth and productivity. It is anticipated that the right agricultural policy environment will improve sectoral income and savings, and contribute to poverty reduction. The purpose of the current study was to determine updated information on the seroprevalence of ND virus in Zambian village chickens, as a starting point to justify an official policy for ND control.

Materials and methods

Sampling

Serum samples were collected from various local chicken breeds in various districts of Zambia between June and December in both 2009 and 2010. Border areas, busy market places and sites near water bodies where wild birds congregate were selected. Only chickens that had not been vaccinated against ND and were apparently healthy at the time of sample collection were included in the survey. The formula of Cannon and Roe (1982) was used to determine the sample size, based on the assumption that the prevalence of ND in the country was 25%, with a 95% probability of detecting at least one infected chicken. Eleven districts from five provinces were sampled and 29 households from each district were included in the study. The resulting sample size was 1595. However, owing to logistical problems, only 1012 samples were obtained. A blood sample

of 2 mL was collected from the wing vein of each bird. The blood was allowed to clot at room temperature, after which the serum was separated and stored at –20 °C until testing.

Enzyme-linked immunosorbent assay

Samples were tested using a commercial enzyme-linked immunosorbent assay (ELISA) kit for ND (FlockChek®, IDEXX Laboratories, Maine) according to the recommended procedure. The laboratory work was performed at the Central Veterinary Research Institute in Lusaka, Zambia. Results were read using a microplate reader (Multiskan,Labsystems). Absorbance values were measured at 650 nm. Sample/positive ratios (S/P) were calculated according to the recommended procedure. Serum samples with S/P ≤ 0.2 were considered to be negative, whereas those with S/P ≥ 0.2 were considered to be positive.

Statistical analysis

The prevalence of ND was calculated for each province and district using SPSS software (IBM Corporation, New York). The prevalence within the provinces and between the districts was compared using Fisher's exact test. Analysis of variance was used to determine whether the log-transformed titres from the various provinces were significantly different. Values were considered significant at $p \leq 0.05$.

Ethical considerations

A blood sample of 2 mL was collected from the wing vein of each bird sampled. The procedure was done as humanely as possible. The birds did not show any undesirable reaction after sample collection.

Results

The results regarding ND seroprevalence in each of the five provinces are shown in descending order in Table 1. The highest seroprevalence was recorded for the Eastern Province (82.6%; CI = 74.9% – 90.4%), followed by the Southern Province (80.0%; CI = 76.7% – 83.3%), the Northern Province (77.3%; CI = 70.4% – 84.2%), the Copperbelt Province (51.6%; CI = 44.4% – 58.8%) and finally Luapula Province (48.3%; CI = 30.1% – 66.5%). The seroprevalence of ND was significantly different ($p < 0.001$) amongst the provinces.

The seroprevalence of ND was also determined at district level. In Southern Province, the highest seroprevalence was recorded in Monze district (91.3%; CI = 85.5% – 97.1%), whilst the lowest was recorded for Kazungula district (71.7%; CI = 65.2% – 78.2%). Of the two districts that were sampled in the Northern Province, seroprevalence was highest in Mpulungu (87.8%; CI= 78.6% – 97.0%) and lowest in Nakonde (71.7%; CI = 62.5% – 80.9%). For the two districts that were sampled in the Copperbelt Province, the highest seroprevalence was recorded in Chililabombwe (80.4%; CI = 72.3% – 88.5%) and the lowest in Mufulira (22.8%; CI = 14.2% – 31.4%). Only one district in both the Eastern and Luapula provinces was sampled and therefore seroprevalences could not be compared. The seroprevalence of ND was significantly different ($p < 0.001$) amongst the districts (see Figure 1 for the location of the districts).

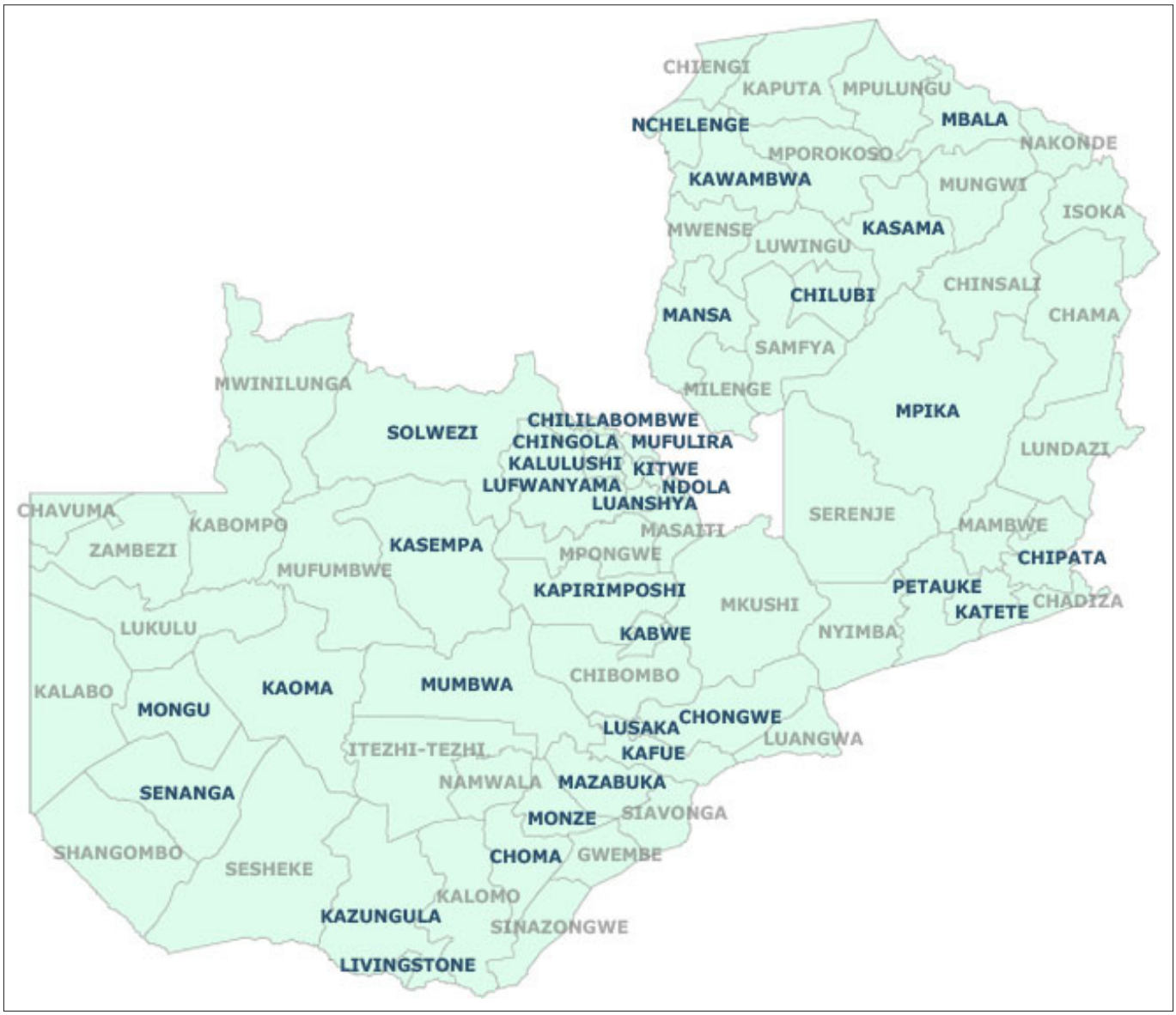
Discussion

When ND was first reported in Zambia in 1952, outbreaks were concentrated along the railway line, where the largest population of poultry occurred (Songolo & Katongo 2000). In a study conducted by Alders *et al.* (1994), seroprevalence levels varied amongst the provinces, ranging from 29.2% in the Northern Province to 51.3% in the Copperbelt Province. In the current study, we demonstrated not only that ND seroprevalence in flocks in the informal sector had increased to 77.3% in the Northern Province (Copperbelt Province remained roughly the same at 51.6%) but also that seroprevalence was 82.6% in the Eastern Province, 80% in the Southern Province and 48.3% in Luapula Province. It is likely that the increasing population of chickens provided a sustainable reservoir for the maintenance of ND strains, which could have allowed the infection to persist or facilitated the introduction of viruses more frequently. Therefore, although the Eastern and Northern Provinces are not situated along the railway line, the increase in chicken numbers could have contributed to the infection shifting to these areas.

The samples were collected from apparently healthy, unvaccinated birds, suggesting that the infections were due

TABLE 1: Seroprevalence of Newcastle disease in each province and district sampled, in descending order.

Province	District	Number of birds sampled	Number of positive birds	Prevalence	95% confidence interval
Eastern	Chipata	92	76	82.6	74.9–90.4
Southern	Monze	92	84	91.3	85.5–97.1
	Namwala	92	77	87.4	81.8–93.0
	Siavonga	92	71	77.2	68.6–85.8
	Itezhitezhi	63	48	76.2	65.9–86.7
	Kazungula	184	133	71.7	65.2–78.2
	Subtotal	**523**	**413**	**80.0**	**76.7–83.3**
Northern	Mpulungu	92	84	87.8	78.6–97.0
	Nakonde	92	66	71.7	62.5–80.9
	Subtotal	**184**	**150**	**77.3**	**70.4–84.2**
Copperbelt	Chililabombwe	92	74	80.4	72.3–88.5
	Mufulira	92	21	22.8	14.2–31.4
	Subtotal	**184**	**95**	**51.6**	**44.4–58.8**
Luapula	Samfya	29	14	48.3	30.1–66.5



Source: National Malaria Control Centre, 2009, *Enumeration reports*, viewed 20 November 2012, from http://www.nmcc.org.zm/report-map.htm

FIGURE 1: Map of Zambian districts.

to circulating avirulent strains. Whether these originated from vaccine strains or another source, for example Class I or Class II lineage 1 ND viruses, which are common in wild birds (Czegledi *et al*. 2006), is unknown. Since only a single serotype of APMV-1 exists, we were unable to distinguish between vaccine-related and field-strain antibodies. The live lentogenic LaSota/46 vaccine strain is widely used in the commercial sector and it is possible that some spillover of vaccine into backyard chickens occurred, especially where spent layers end up in villages. It has been shown that vaccines alter the epidemiology of ND to some extent, since they prevent disease but not infection. Vaccinated birds exposed to virulent virus strains develop no clinical signs; however, some replication of the infecting virus occurs and birds excrete virulent ND virus. This would probably not be excreted in similar quantities as by susceptible birds, but there would be sufficient virus to infect other birds (Mavale 2001; Miller *et al*. 2007). In the Zambian situation, active vaccination of village and backyard chickens against ND is rarely practiced owing to cost and problems associated with maintaining the cold chain for the heat-labile live vaccine.

There was a slight peak in ND seroprevalence between January and March, and from September to November. A similar trend was reported in Mozambique (Harun & Massango 2001; Songolo & Katongo 2000). This may be attributed to either a seasonal or a social reason. The period from January to March is cool and humid, with heavy rains, whereas the period from September to November is hot, windy and dry. Another important factor associated with the transmission of ND is the keeping of flocks of various ages. This is common in village chicken husbandry and although mortality is higher in young chickens, 100% mortality may occur in adult flocks, depending on the strain (Alexander 1998; Songolo & Katongo 2000; Spradbrow 2001). Social aspects that affect transmission of ND in Zambia include the transport of live chickens during extensive travelling (both locally and across borders) to visit friends and family

over the festive season, and increased trading at markets in January to generate income for school fees.

Conclusion

Although unidentified ND strains are apparently widespread in Zambia, little official attention has been given to the control of the disease in chickens since the government ceased subsidised ND vaccinations in the 1980s. The increasing seroprevalence of ND, albeit due to unknown strains, highlights the ease with which the infection can spread. Incursion of a highly virulent ND strain to which the poultry population has insufficient immunity could have devastating consequences. However, these could be mitigated by a good vaccination strategy. Improved small-scale poultry farming would make an important contribution to the national economy and this could motivate the re-establishment of an official, subsidised ND vaccination programme in Zambia. Similarly, a vaccination programme amongst village and backyard chickens would limit mortalities, improve flock numbers and ultimately improve food security and generate much-needed income for many poor households in the country. Since the last study to isolate and characterise ND strains responsible for losses in the Zambian poultry sector was published almost 30 years ago (Hussein *et al*. 1984), there is a need to isolate and genetically characterise currently circulating strains affecting poultry in Zambia to allow appropriate control decisions to be taken.

Acknowledgements

We wish to acknowledge the following people who contributed to the study: Martin Simuunza (University of Zambia) performed the statistical analysis, Oliver Chibomba (Central Veterinary Research Institute) collected some of the samples and performed some of the experiments and Cheelo Mweene, Gregory Bwalya, Tingiya Sikombe, Linford Moya (Central Veterinary Research Institute) collected some of the samples. The district veterinary officers (Ministry of Livestock and Fisheries) in the various districts from where the samples were collected provided additional information. This work was financially supported by the Institute of Tropical Medicine, Belgium.

Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this paper.

Authors' contributions

C.M. (Ministry Livestock and Fisheries) was responsible for conceptual design, data analysis and contributed to writing the manuscript. C.A. (University of Pretoria) was the study leader, responsible for experimental design, and contributed to writing the manuscript.

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