A STUDY OF RURAL CHICKEN FARMERS, DISEASES AND REMEDIES IN THE EASTERN CAPE PROVINCE OF SOUTH AFRICA

V. Simbizi [a, b, e,*], R. Moerane [b], G. Ramsay [c], C. Mubamba [d], C. Abolnik [b] and B. Gummow [e, b]

[a] *State Veterinary Services, Department of Rural Development and Agrarian Reform, Lady Frere 5410, South Africa*

[b] *Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa*

[c] *School of Animal & Veterinary Sciences and Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga 2650, Australia*

[d] *Department of Veterinary Services, Ministry of Fisheries and Livestock, Lusaka 50060, Zambia*

[e] *Discipline of Veterinary Sciences, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville 4811, Australia*

*Correspondence**:** Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, RSA; Tel: +27 12 529 8013; Fax: +27 12 529 8315; Email: vsimbizi@gmail.com

ABSTRACT

The source of emerging diseases and antimicrobial resistance is of increasing interest to epidemiologists. This paper looks at village chickens as such a source. In addition, infectious diseases constitute a major challenge to the growth and profitability of the rural poultry sector in Sub-Saharan Africa. A serological survey was conducted to estimate the apparent seroprevalence of selected chicken diseases in the Eastern Cape Province of South Africa alongside a sociological survey of poultry farmers and the remedies most commonly used to prevent diseases in their flocks. Sera collected from village chickens (n=1007) in the province

were screened for specific antibodies against Newcastle disease (ND), avian influenza (AI), avian infectious bronchitis (IB) and *Mycoplasma gallisepticum* (MG).

The overall seroprevalence of ND, AI, IB and MG in the province was found to be 69.2% (95% CI 51.9-86.5%); 1.8% (95% CI 0.2-3.4%); 78.5% (95 % CI 74.9-82%) and 55.8% (95% CI 41.3-70.3%) respectively with clustering found at the District level. Cross hemagglutination inhibition (HI) tests indicated that the chickens were exposed to the ND vaccine. AI ELISA-positive samples were tested using HIs against the H5, H6 and H7-subtypes, but only H6-specific antibodies were detected. Avian influenza strains shared the common ancestor responsible for the 2002 chicken outbreak in KwaZulu-Natal Province.

The majority of chicken farmers were females and pensioners (69% and 66.1% respectively) and had a primary school education (47.1%). Traditional remedies were commonly used by farmers (47.15%) and among the remedies, *Aloe* plant (*Aloe ferox* Mill.) or ikhala (Xhosa) was the most commonly used product (28.23%) for preventing and reducing mortalities among village chickens.

The findings stress the importance of village chickens as a substitute for social welfare and highlight the exposure of village chickens to important chicken pathogens. The economic impact of these pathogens on the development of this sub-sector needs further investigation. Village chickens are a potential source of virulent Newcastle disease virus (NDV) because of the lack of vaccination and biosecurity. They may serve as amplification hosts which increases the probability that virulent NDV could spill over into commercial poultry flocks due to large amounts of circulating virus. The zoonotic threat of circulating H6N2 viruses raise concern due to their mutation and reassortment among chickens and a potential movement of infected birds within the province. Finally, the use of antibiotics by untrained chicken farmers constitute another major concern as it could serve as a source of antimicrobial resistance (AMR).

Keywords: Chicken diseases, traditional remedies, antibiotic use, village farmers, emerging diseases

1. Introduction

In Southern Africa, village chickens are reared under an extensive or scavenging system and to a lesser extent in a semi-intensive system under subsistence farming, with few or no inputs for housing, feeding and health care (Mtileni et al., 2009). They play a vital role in many poor rural households by providing scarce animal protein in the form of meat and eggs and can be sold or bartered to meet essential family needs such as medicine, clothes and school fees (Alders and Pym, 2009). They are mostly owned and managed by women and children and are often essential elements of female-headed households (Gueye, 2000).

The Eastern Cape Province (ECP) is the second largest province in South Africa (Fig 1) and village chickens are reported to be the second most populous domesticated animal species in the province (STATS, 2016). The productivity of these chickens is however hampered by several factors, including a wide range of infectious diseases such as Newcastle disease (ND), avian influenza (AI), *Mycoplasma gallisepticum* (MG) Gumboro disease or infectious bursal disease (IBD), fowl cholera and avian infectious bronchitis (IB) (DAFF, 2020; Simbizi, 2020). In addition, village chickens could be a potential reservoir of these pathogens that could jeopardise the development of local semi-commercial poultry production (Chaka et al., 2012). The reverse is also true when spent hens from commercial farms are introduced into village settings (Musako and Abolnik, 2012).

Data on the prevalence of poultry diseases in the rural sector of Southern Africa is limited. Similarly, only a few studies on the demographics of rural chicken farmers and their remedies used to treat infectious diseases have been published.

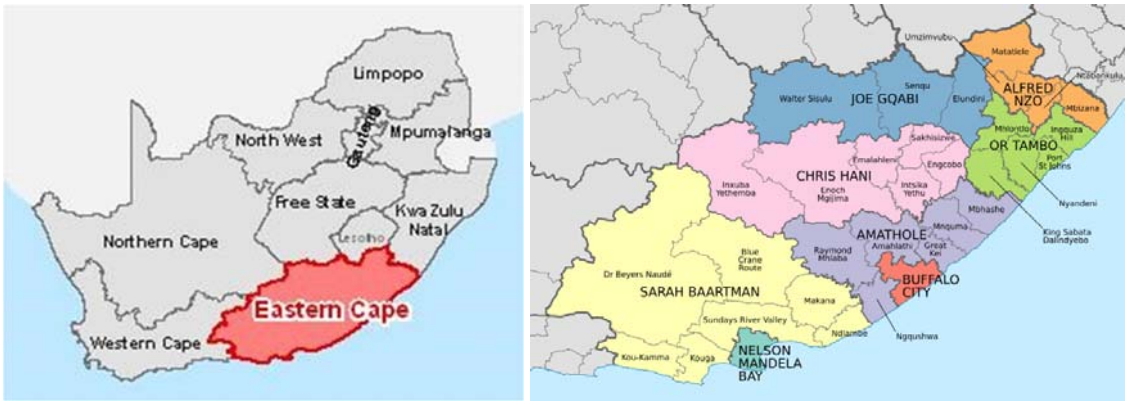

Fig 1: Position of Eastern Cape Province and its District municipalities (Source: Wikipedia)

The objectives of this study were therefore to describe the demographics of village chicken farmers in the ECP, to describe the remedies used by farmers to treat and prevent chicken diseases and to determine the apparent seroprevalence of Newcastle disease (ND), avian influenza (AI), avian infectious bronchitis (IB) and *Mycoplasma gallisepticum* (MG), the important diseases affecting chickens in Southern Africa.

2. Materials and Methods

2.1. Study design

The Eastern Cape Province is divided into two metropolitan municipalities, Buffalo City and Nelson Mandela Bay, and six district municipalities (Fig 1). The district municipalities are in turn divided into thirty-one local municipalities. All thirty-one local municipalities plus the two metropolitan municipalities were included in the study. A two-stage sampling strategy was used to calculate the required number of villages and households to be used in the study (Thrusfield, 2005). Three villages per municipality were randomly selected, giving a total number of 99 villages for the whole province. Since the study design included a pig survey (data to be published elsewhere), a list of farmers with at least four chickens and four pigs was generated with the help of the extension officers and a sample of five households per selected

village was randomly selected giving a total number of 15 households (or 15 farmers) per local municipality (approximately 500 households in total which could be divided into 250 chicken farmers and 250 pig farmers).

An interview-based questionnaire of households with village chickens was carried out by the research team with the assistance of veterinary and extension services from the Department of Rural Development and Agrarian Reform, Eastern Cape Province. A section on farm owner demographics (age of the farmers, sex etc.), farm husbandry (number of poultry kept, breed, farm raising system etc.) and poultry diseases and their treatment was included in the questionnaire.

2.2 Blood collection

The serological survey was conducted from August 2019 to March 2020 and targeted 500 households based upon the two-stage sampling strategy described. Two chickens from each household were sampled to give a total of approximately 1000 samples (Thrusfield, 2005).

Only non-vaccinated chickens were sampled. Blood samples were collected from the brachial vein in 3-mL disposable syringes, and transferred into 10 ml blood collection tubes to allow the serum to separate before they were sent to the Queenstown Veterinary Provincial laboratory. Each tube was labelled with a unique number describing each chicken bled (sex, breed, age, owner's name and village name). At the laboratory, serum was collected in 2-mL cryovial tubes with a unique corresponding code and stored at −20°C until testing.

2.2.1 Serological tests

Sera were shipped to NOSA (Pty) Ltd in Centurion, Pretoria, a national accredited veterinary laboratory for serological testing. Sera were analyzed using commercial ELISA kits for the presence of antibodies to NDV (Newcastle Disease Virus Antibody Test Kit: BioChek, United

Kingdom), AI (IDEXX Influenza A virus Antibody test; Montpellier SAS, France) and MG (IDEXX Mycoplasma Gallisepticum Test Kit; Montpellier SAS, France) according to the manufacturers' recommended procedures. For IB, the ELISA method to detected antibodies to IB was developed in-house. The NDV assay worked on the principle of indirect ELISA and was developed to detect specific antibodies against PMV-1 in serum. Microtitre plates were pre-coated with purified NDV antigens. Chicken serum samples were diluted and added to the microtitre wells where any anti-NDV antibodies present would bind and form antigen-antibody complex. Non-specific antibodies and other proteins were then washed away. Anti-chicken IgG labelled with the alkaline phosphatase were added to the wells to bind to any chicken anti-NDV antibodies bound to the antigen. After another wash to remove the unreacted conjugate, substrate was added in the form of *para*-Nitrophenylphosphate (pNPP) chromogen. A yellow colour was developed when anti-NDV antibody was present. The intensity was related to the amount of the anti-NDV antibody present in the sample. The sample and control OD values were read using an ELISA reader at 405 nm. For each sample, the sample-to-positive (S/P) ratios were calculated from OD values by the formula:

S/P ratio = (OD_{sample} – negative control mean OD)/ (positive control mean OD-negative control mean OD). ND positive samples had an S/P > 0.2 whereas samples with an S/P ≤ 0.2 were regarded as negative.

The Influenza A assay was performed in a microtitre well coated with Influenza A viral antigen. During the first incubation, at room temperature, Influenza A antibodies present in the sample reacted with immobilized antigens. After a wash step, an Anti-Influenza A monoclonal antibody enzyme conjugate was added to the micro well. In the absence of any Anti-Influenza A antibodies in the sample, the enzyme-conjugated monoclonal antibodies were blocked from reacting with the antigen. Following this incubation period, the excess conjugate was removed by washing and a substrate/chromogen solution was added. In the presence of enzyme, the

substrate was converted to a product which reacted with the chromophore to generate a blue colour. The absorbance was read at 620 nm using a spectrophotometer.

Results were calculated by dividing the OD value of the sample by the mean OD of the negative control, resulting in a sample to negative (S/N) value (S/N ratio=Sample OD/negative control OD). The quantity of antibodies to Influenza A was inversely proportional to the OD value, and thus, to the S/N value. The same principle applied to all IDEXX kit test for MG.

For the AI assay to be valid, the negative control optical density had to be ≥ 0.50 and the positive control S/N (sample to negative) had to be <0.5. Samples with S/N ratios ≥ 0.50 were therefore considered as negative whereas samples with S/N ratios <0.5 were considered as positive.

For MG, positive samples had an S/P ≥ 0.5 whereas samples with an S/P ≤ 0.49 were regarded as negative.

All ELISA AI positive samples were tested using the HI tests for H5/ H6/ H7 subtyping according to the OIE-recommended protocol, with a cut-off of 2^2 or $>\log_2 2$ for a positive sample (OIE, 2018a).

A sub-set of ELISA-positive ND samples (n=38) with titre $>2^2$ (or $>\log_2 2$ when expressed as the reciprocal) were tested with the cross haemagglutination inhibition (HI) tests (OIE, 2018b) using antigens that distinguish virulent genotype VII and avirulent genotype II. Cross-HI tests for NDV-specific antibodies were performed at the accredited Serology laboratory of the Department of Veterinary Tropical Diseases, University of Pretoria.

2.3 Data analysis

All data from the questionnaire were entered into the software programmes Epi Info® 7, NCSS and Microsoft Excel for statistical analysis. Data from the questionnaire were analysed using descriptive statistics.

Apparent seroprevalence was computed by dividing the number of seropositive chickens by the total number of chickens sampled. Published values for specificity and sensitivity of the ELISA test (Table 1) were used to calculate the true prevalence and the 95% confidence interval (CI) of each disease using the Epi Tools Epidemiological calculators (http://epitools.ausvet.com.au).

Table 1: Characteristics of ELISA test used to calculate the true prevalence

Disease	Sensitivity	Specificity	Reference
ND	98.9	98.4	(Phan et al., 2013)
AI	98	98	(Shriner et al., 2016)
IB	98	97.2	(Chen et al., 2011)
MG	97.2	100	(Ewing et al., 1996)

Spatial analysis was done using ArcGIS Desktop 10.7® software by comparing the districts with the highest seroprevalence of ND, AI, IB and MG.

The overall seroprevalence and 95% confidence interval of selected disease in the province was calculated taking into account clustering within the data using Equation 1 and Equation 2 (Thrusfield, 2005).

$$\hat{P}\text{-}1.96\left\{\frac{C}{T}\sqrt{\frac{V}{C(C-1)}}\right\}, \hat{P}\text{+}1.96\left\{\frac{C}{T}\sqrt{\frac{V}{C(C-1)}}\right\}, \qquad \text{(Equation 1)}$$

Where:

C=number of clusters in the sample

T=total number of animals in the sample

and:

$$V= \hat{P}^2(\sum n^2) - 2\hat{P}(\sum nm)+(\sum m^2), \qquad \text{(Equation 2)}$$

Where:

n=number of animals sampled in each cluster

m=number of diseased animals sampled in each cluster

3. Results

3.1 Demographics of village chicken farmers

Among farmers interviewed, females were more represented (69%) than males (31%). For the purpose of analysis, farmers interviewed were grouped into three categories according to their age: youth (from 0-35 years); adults (36-55 years) and pensioners (56-89). The survey showed that pensioners were more represented (66.1%; 95% CI 64.6-67.5) followed by the adults (46.4%; 95% CI 44.9-47.9) and youth (30.2%; 95% CI 27.9-32.6). The survey found that 47.1 % of farmers had primary education (from grade 1-9) followed by farmers with secondary education (grade 10-12) (37.1%); 7.1% of farmers had tertiary education and 8.6% of farmers had no education.

3.2 Farming system and remedies used to treat infectious diseases in village chickens

The chicken production systems in this study were classified using the FAO family poultry production system classification guidelines (FAO, 2014). The study found that 40% of rural farmers were using a small extensive scavenging system, i.e. chickens that scavenge for food around the yard or village during the day with almost no supplementation and kept in poultry houses at night whereas 37.62% of farmers used an extensive scavenging system where poultry are allowed to wander around the village looking for food with occasional supplementation. A semi-intensive system, where chickens were always kept in a confined area with regular supplementation was used by 22.38% of rural farmers.

Farmers were using remedies for the prevention and treatment of chicken diseases which could be grouped into one of four groups: Sulpha products; Tetracyclines, traditional remedies and chicken vaccines (Appendix 4).

Traditional remedies were most commonly used by farmers (47.15%). Among this group, *Aloe* (*Aloe ferox* Mill.) was the most predominant product used (28.23%). The second group of medicines used by farmers was tetracyclines (17.42%) followed by the Sulpha products (12.01%). Farmers had access to these antibiotics as over-the-counter products through the local licensed selling companies. Chicken vaccines was the last group of remedies frequently used by farmers which comprised ND vaccine (6.91%); Gumboro (4.8%) and avian infectious bronchitis vaccine (0.9%) (Appendix 4). The study also found that Stresspac (Phenix ® Stresspac for Poultry and Ostriches: Virbac) was commonly used by chicken farmers as a supplement (10.33%) (Appendix 4).

Seventy-eight farmers (37.1%) were using a combination of one or more of the above-mentioned remedies whereas 110 farmers (52.4%) were using only one of these products. Twenty-two farmers (10.4%) were not using any remedies for the prevention of chicken diseases.

3.3 Seroprevalence of chicken diseases

A total of 1007 village chickens from 71 villages in the ECP were sampled (Appendix 1). The ages of these chickens were ranged from 1 months to 6 years. Among these chickens, 120 were layers, 666 were Xhosa or local breed and 221 were broilers.

The apparent prevalence of ND, AI, IB and MG was calculated at the district level with 95% CI (Table 2).

Table 2: Apparent prevalence of Newcastle disease (ND), avian influenza (AI), avian infectious bronchitis (IB) and *M. gallisepticum* (MG) in districts of the Eastern Cape Province (From August 2019 to February 2020).

Disease	District	Total no. collected	No. positives	Prevalence	95% CI*
ND	Chris Hani	411	231	56.2%	51.4-60.9%
	Alfred Nzo	88	83	94.3%	87.4-97.6%
	Joe Gqabi	66	60	90.9%	81.6-95.8%
	Buffalo City	34	33	97.1%	85.1-99.5%
	OR Tambo	96	93	96.9%	91.2-98.9%
	Sarah Baartman	84	82	97.6%	91.7-99.3%
	Amathole	228	115	50.4%	44-56.9%
AI	Chris Hani	411	6	1.5%	0.7-3.2%
	Alfred Nzo	88	7	8%	3.9-15.5%
	Joe Gqabi	66	0	0%	0-6%
	Buffalo City	34	0	0%	0-10.2%
	OR Tambo	96	4	4.2%	1.6-10.2%
	Sarah Baartman	84	1	1.2%	0.2-6.4%
	Amathole	228	0	0%	0-1.7%
IB	Chris Hani	411	325	79.1%	74.9-82.7%
	Alfred Nzo	88	73	83%	73.8-89.4%
	Joe Gqabi	66	50	75.8%	64.2-84.5%
	Buffalo City	34	29	85.3%	69.9-93.6%
	OR Tambo	96	63	65.6%	55.7-74.4%
	Sarah Baartman	84	62	73.8%	63.5-82%
	Amathole	228	188	82.5%	77-86.8%
MG	Chris Hani	411	197	47.9%	43.1-52.8%
	Alfred Nzo	88	61	69.3%	59-78%
	Joe Gqabi	66	39	59.1%	47-70.1%
	Buffalo City	34	31	91.2%	77-97%
	OR Tambo	96	74	77.1%	67.7-84.4%
	Sarah Baartman	84	78	92.9%	85.3-96.7%
	Amathole	228	82	36%	30-42.4%

*95% CI: Confidence interval calculated based on the sensitivity and specificity of the test (Table 1)

The overall seroprevalence of ND, AI, IB and MG in the province was found to be 69.2% (95% CI 51.9-86.5%); 1.8% (95% CI 0.2-3.4%); 78.5% (95 % CI 74.9-82%) and 55.8% (95% CI 41.3-70.3%) respectively.

The true prevalence of each selected disease at provincial level was calculated considering the clustering effect during the sampling. A cluster was considered as a batch of chickens originating from one household (Table 3).

Table 3: True prevalence of chicken diseases in the Eastern Cape Province (From August 2019 to February 2020) at provincial level

Disease	Number of positive samples	Apparent prevalence	True prevalence
Newcastle	697/1007	69.2%	51.9-86.5%
Avian influenza	18/1007	1.8%	0.2-3.4%
Avian infectious bronchitis	790/1007	78.5%	74.9-82%
Mycoplasma gallisepticum	562/1007	55.8%	41.3-70.3%

3.4 The cross-HI test results for ND positive samples

The results from the cross-HI test showed that 31 samples out of 38 from chickens exposed to the vaccine strains were identified by the Genotype II (avirulent vaccine) antigen giving a higher Log_2 HI titre in every instance, by 1 to 2 logs (Appendix 2).

3.5 The cross-HI test results for AI positive samples

Fourteen AI ELISA-positives samples were tested using HIs against the H5, H6 and H7-subtypes. Nine samples (ADA1; CAA1; HAA5; HCA1; ACA1; PAA2; PAA4, PAA9 and PAA10) presented high titres to H6. Four samples (AFB 18; AFC11; AFD 11 and AFE6) were negative to all AI subtypes. All samples were negative to H5 but some presented non-specific

reactions (ADA1; CAA1; HAA5; HCA1; ICA1; ICB2; PAA2; PAA4; PAA9 and PAA10 (Appendix 3).

4. Discussion

Village chickens were owned mainly by females (69%) compared to men (31%). The main reason for keeping chickens was for selling (income generation) and human consumption (meat and eggs). This was consistent with other findings published on village chickens (Mushi et al., 2000; Alders and Pym, 2009; Mtileni et al., 2012; Mtileni et al., 2013) stating that females dominate most of the activities around chicken production; feeding, watering, cleaning, selling of chickens and eggs. It also emphasizes the importance of poultry farming as an income source for women.

Among village chicken farmers' pensioners were the most represented compared to youth and adults and village chickens can be regarded as an important source of income for most pensioners, which is highly significant considering the virtual lack of welfare system in many African countries.

Farmers with only a primary school level of education were predominantly involved in chicken farming (47.1 %) compared to those with secondary and tertiary education level. This is similar to what was reported previously in two studies in the Eastern Cape Province (Nyoni and Masika, 2012; Idowu et al., 2018) and chickens are therefore an important source of income for a sector of the population that may find other employment opportunities difficult due to their low level of education.

A small extensive scavenging system was the most commonly used by village chicken farmers in the Eastern Cape Province (40%), compared to those using an extensive scavenging (37.62%) and a semi-intensive system (22.38%). This agrees with what was found in previous

studies (Idowu et al., 2018; Mubamba et al., 2018) where it was shown that this system of farming is the most cost effective in that environment.

Traditional remedies were commonly used by farmers (47.15%) and among these, *Aloe* was the most predominant product used (28.23%). *Aloe* plants *(Asphodelaceae*) have been widely known and used for centuries due to their health, beauty, medicinal, and skin care properties (Boudreau and Beland, 2006). *Aloe arborescens, Aloe barbadensis, Aloe ferox*, and *Aloe vera* are among the well-investigated *Aloe* species and are among the most economically important medicinal plants commonly used in primary health treatment (Salehi et al., 2018). *Aloe ferox* Mill. or ikhala in Xhosa which was predominantly used by farmers in this study has been reported to be effective in the prevention of chicken diseases including ND (Waihenya et al., 2002a; Mwale et al., 2005) and *Salmonella gallinarum* (Waihenya et al., 2002b). Leaves are generally used and are prepared by crushing a leaf and mixing it with a litre of water (Masimba et al., 2011). The solution is then given to the chickens until they show signs of good health (Mwale et al., 2005).

Seventy-eight percent (78%) of farmers interviewed reported “ikhala” prevented and reduced mortalities among village chickens. Tetracyclines and Sulpha products were the second group of remedies used by chicken farmers which could be explained by their low cost compared to other chicken remedies as well as their availability on the market. Their availability and use by untrained farmers is concerning as this could be contributing to anti-microbial resistance (AMR). These findings highlight the need for more detailed look at antibiotic use in these communities.

Chicken vaccines were only used by a small number of farmers and included ND vaccine (6.91%), Gumboro (4.8%) and avian infectious bronchitis disease (0.9%). The study demonstrated that chicken vaccines were not widely used by village chicken farmers probably due to lack of knowledge, availability of vaccines and inaccessibility of veterinary and

extension services. This was consistent with the findings from similar studies in South Africa (Mtileni et al., 2009; Mtileni et al., 2012; Mtileni et al., 2013), Botswana (Mushi et al., 2000) and Zimbabwe (Kelley et al., 1994).

The overall seroprevalence of ND in the province was found to be 69.2% (95% CI 51.9-86.5%) (Table 3 and Figure 2) but varied from 50.4% to 97.6% at the District level. Estimates of prevalence of ND across many SADC countries were reported somewhere else (Alders and Spradbrow, 2001).

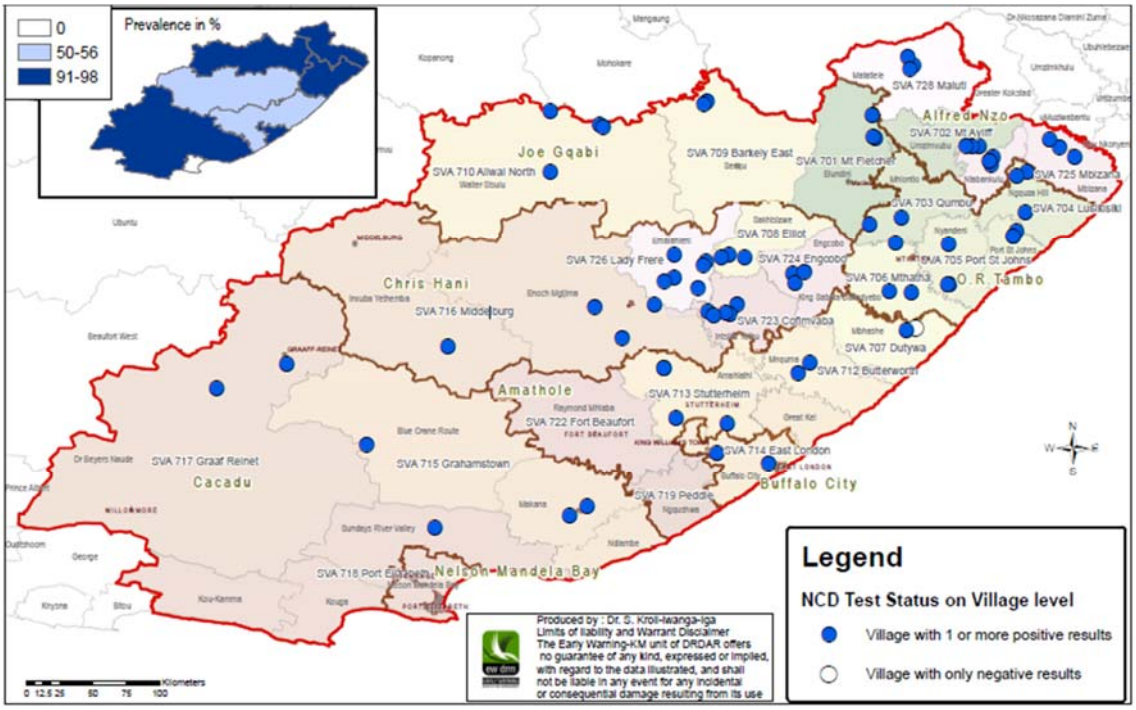

Figure 2: Apparent prevalence of ND at district level, ECP, from August 2019 to February 2020

In South Africa, this prevalence was higher than that reported in the North West Province (Thekisoe et al., 2003). The samples were collected from apparently healthy, unvaccinated birds, suggesting that the infections were probably due to circulating avirulent strains and this was shown through cross-HI tests. The cross-HI assay for ND positive samples showed that antibodies identified by the LaSota antigen (II) had high titres compared to the ones produced

by the N2057 antigen (VII). Different studies on the cross-HI tests have demonstrated antigenic differences between different NDV genotypes (Miller et al., 2007; Li et al., 2010). The live lentogenic LaSota vaccine strain is widely used in the commercial sector and it is possible that some spillover of vaccine into village chickens occurred, especially where spent layers end up in the village (Musako and Abolnik, 2012). 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 (Musako and Abolnik, 2012). The extent to which the propagation of these vaccine strains may have occurred still needs to be determined given the high and widespread seroprevalence found in this study. In the rural Eastern Cape, active vaccination of village chickens against ND is rarely practiced mainly due to the lack of knowledge from farmers, inaccessibility of veterinary and extension services and unavailability of the vaccines in remote rural area. Furthermore, this activity is not prioritized by veterinary services in the province. Our study therefore highlights the importance of village chickens as a potential source of emerging virulent strains of ND virus due to the lack of vaccination and biosecurity. Village chicken may serve as amplification hosts which increases the probability that virulent NDV could spill over into commercial poultry flocks due to large amounts of circulating virus (Brown and Bevins, 2017). Vaccinated chickens can also play a role as a reservoir for virulent strains of NDV because they can become infected with virulent strains following vaccination and shed infectious virus in the absence of clinical disease (Miller et al., 2010).

The overall seroprevalence of AI in the province was found to be 1.8% (95% CI 0.2-3.4%) (Table 2 and Figure 3) but varied from 0% to 8% at the District level. This was in agreement with a recent work which reported a varied regional prevalence in Sub-Saharan Africa ranging from 1.1% to 7.1% (Kalonda et al., 2020). AI ELISA-positive samples were tested using HIs against the H5, H6 and H7-subtypes, but only H6-specific antibodies were detected. It was found that these H6-specific antibodies were circulating in chickens from Alfred Nzo District

which had a highest prevalence of AI. This is not surprising since this is the closest District to KwaZulu-Natal Province where an outbreak of H6N2 occurred: South Africa's H6N2 epidemic in chickens began in 2001. The progenitor was traced to a reassortment between viruses that infected commercial ostriches in the Western Cape Province in the mid to late 1990's notably an H6N8 virus and an H9N2 virus. The disease later spread to KwaZulu-Natal (Camperdown area) and to other provinces (Abolnik et al., 2007). The movement of infected chickens between Alfred Nzo and its neighbouring District in KwaZulu-Natal could explain this high prevalence.

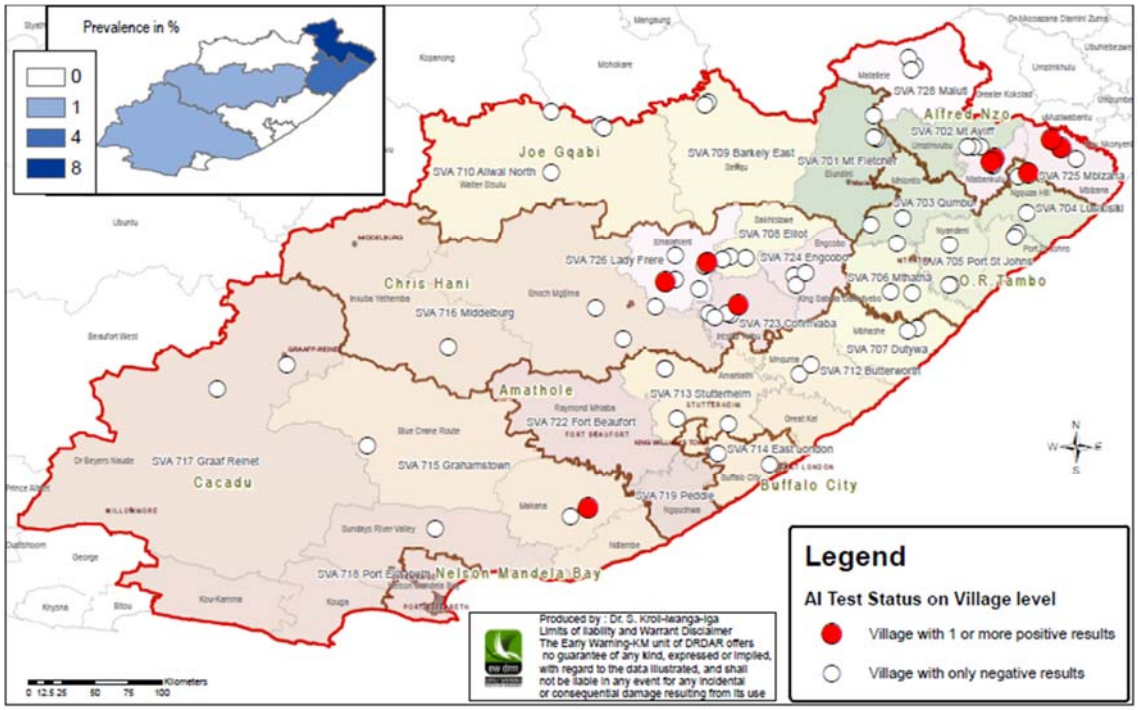

Figure 3: Apparent prevalence of H6 avian influenza at district level, ECP, from August 2019 to February 2020

The threat of poultry-origin H6 avian influenza viruses to human health emphasizes the importance of monitoring their evolution. The true incidence and prevalence of H6N2 in the country has been difficult to determine, partly due to the continued use of an inactivated whole virus H6N2 vaccine and the inability to distinguish vaccinated from non-vaccinated birds on serology tests (Abolnik et al., 2019). A recent study found that the H6N2 viruses in South

African chickens are mutating and reassorting amongst themselves but have remained a genetically pure lineage since their emerging. Greater efforts must be made by government and industry in the continuous isolation and characterization of field strains for use as HI antigens, new vaccine seed strains and to monitor the zoonotic threat of H6N2 viruses (Abolnik et al., 2019).

All sampled poultry were free of respiratory symptoms at the time of sampling and the majority of farmers did not confirm the use of IB vaccine during the interview (0.9%). The apparent prevalence of IB found in this study [78.5% (95% CI 74.9-82%)] (Table 3 and Figure 4) was higher than reported by Thekisoe et al. (2003) in QwaQwa in South Africa. Variations in prevalences between other SADC countries were also noticed. The highest prevalence (86%) was found in backyard chicken flocks of Chitungwiza, Zimbabwe (Kelley et al., 1994) whereas in Botswana, the seroprevalence of IB in backyard chickens was found to be 34.78% (Mushi et al., 2000). The difference in seroprevalence between various region might be explained by different types of biosecurity, management practices, vaccination status, environmental factors as well as the sample size. Although the present study could not identify different strains of IB, the range and magnitude of the serological results provided evidence to suggest exposure of the birds to IBV circulating within the local chickens. A QX-like IBV strain has been isolated in the province (Knoetze et al., 2014) but it is not clear whether it was the same strain circulating among village chickens. Ideal management which include strict isolation, high biosecurity and repopulation following the cleaning and disinfection of the poultry house and equipment as well as immunization in an attempt to prevent production losses (Jackwood and de Wit, 2013) would be of great importance.

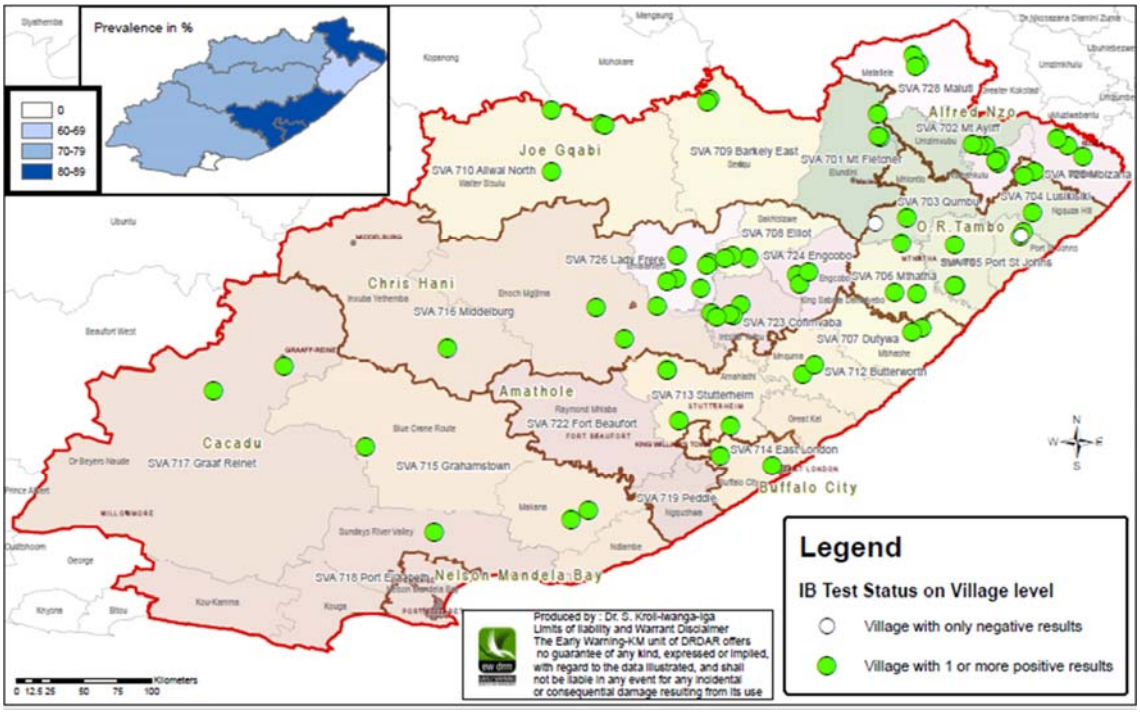

Figure 4: Apparent prevalence of avian infectious bronchitis at district level, ECP, from August 2019 to February 2020.

The overall seroprevalence of *Mycoplasma gallisepticum* in this study was found to be 55.8% (95% CI 41.3-70.3%) (Table 3 and Figure 5) at the provincial level and varied between 36% and 92.9% at the district level. Based on these results, it appears that MG infection may be endemic in the village chickens of Eastern Cape Province and since it can be egg transmitted, its control may be difficult. The survey showed that farmers didn't have enough knowledge on the respiratory diseases of chickens, and the use of the vaccine was very limited. Prevention and control programs, which may include surveillance (isolation and identification, serology, molecular detection and characterization), vaccination, and eradication of infected breeding stock should be prioritized if policy-makers want to improve the rural poultry sector in the province.

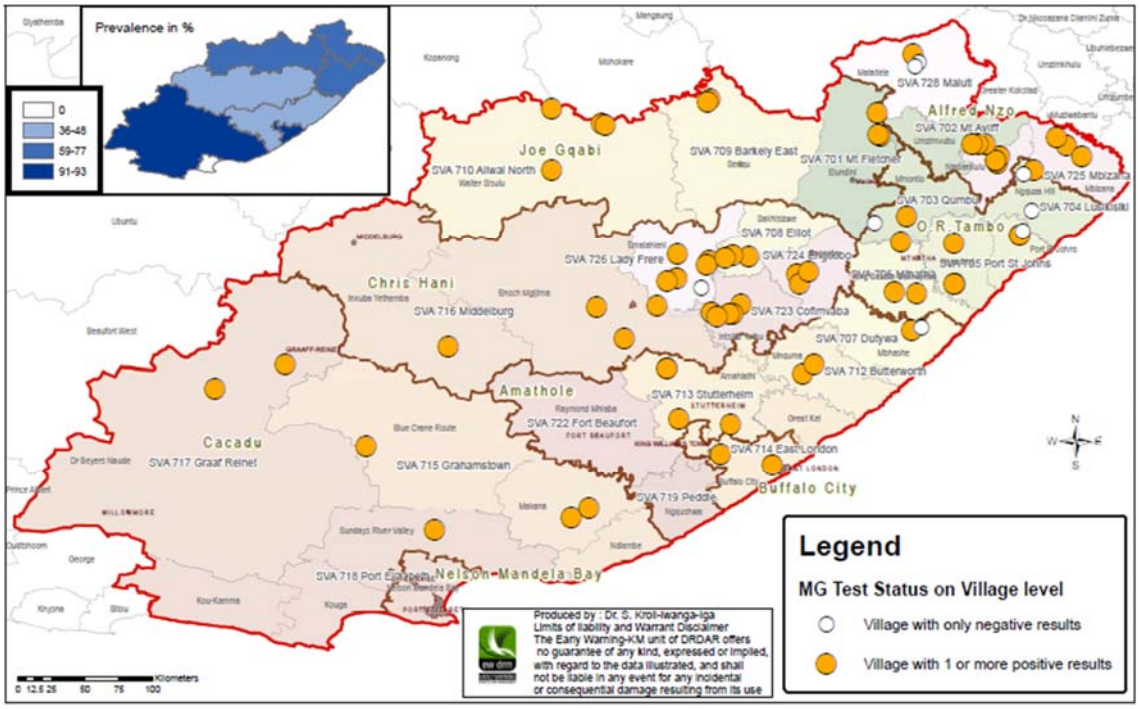

Figure 5: Apparent prevalence of *M. gallisepticum* at district level, ECP, from August 2019 to February 2020

5. Limitation

The limitation of serological tests, as used in this study to confirm exposure to ND, is they cannot differentiate antibodies induced by an infection from those induced by vaccination with live or inactivated vaccines (Thayer and Beard, 2008). Hence prevalence estimates will be influenced by this but due to low vaccination rates in this study the bias is likely to be small. As with all prevalence studies, the time when chickens were exposed to the agent cannot be accurately determined in this study. Another limitation is that the questionnaire interview took almost 5 months to be completed (From February to June 2019). The serological survey started one month later. By the time the serological survey started, not every household interviewed had chickens to be used in the survey (some were consumed or sold) hence the targeted number of 250 households in the study design could not be reached. This study could not establish any seasonal patterns of the selected chicken diseases as the study was designed to measure the point prevalence of disease and not incidence over time.

6. Conclusion

This is the first serological survey done in the village chickens of Eastern Cape Province, which determined the seroprevalence of ND, AI, IB and MG infections. The study found a high seroprevalence of ND, IB, and MG infections in village chickens. However, the economic impact of these infections on the growth of local poultry sector still needs to be determined. This study has also identified antibodies against the H6N2 subtypes of AI circulating in these chickens. These viruses were responsible for the 2002 chicken outbreak in KwaZulu-Natal and due to their zoonotic threat, efforts must be made to monitor their evolution. The survey found that village chickens were susceptible to virulent NDV because of the lack of vaccination and biosecurity. They may therefore serve as amplification hosts which increases the probability that virulent NDV could spill over into commercial poultry flocks due to large amounts of circulating virus. The use of ikhala (*Aloe*) in the prevention of chicken diseases was confirmed through the questionnaire interview but its efficacy on these selected diseases was not specified. The availability and use of antibiotics by untrained farmers was another concern found as this could be contributing to anti-microbial resistance (AMR). The findings highlight the importance of village chickens as a social health care system through income generation. Although this study presented some limitations, it provides important baseline information on the prevalence and significance of selected infectious diseases in village chickens and the importance of sociological and environmental factors that contribute to the emergence of diseases and antimicrobial resistance within village communities.

7. Ethical considerations

Permission to conduct this study was obtained from the Directorate of Veterinary Services, Department of Rural Development and Agrarian Reform in the Eastern Cape Province of South Africa. Ethical approvals to use live chickens and to interview village chicken farmers were

obtained from the University of Pretoria: animal use and care committee (V038-18) and the Faculty of Humanities (GW20180835HS).

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Appendix 1: List of villages sampled and number of chickens per village, ECP, from August 2019 to February 2020

District	Village's name	Household	Local Municipality	Number of chickens sampled
Chris Hani	Bengu	3	Emalahleni	7
	Machubeni	1		2
	Mtsheko	7		14
	Hala 1	3		38
	Hala 2	3		42
	Kavara	7		90
	Tsazo	3	Ngcobo	8
	Beyele	3		12
	Khalinyanga	4		9
	Tshamazimba	2	Intsika Yethu	10
	Woodhouse	3		35
	Deckert's Hill	1		8
	Qamata	4		72
	Tsengiwe	1	Sakhisizwe	7
	Upper Indwana	1		6
	Stokwe's basin	1		8
	Machibini	4	Enoch Mgijima	7
	Zola	3		9
	Tambo	2		8
	Cradock	2	Inxuba Yethemba	19
Alfred Ndzo	Ramatli	1	Matatiele	6
	Nchodu	2		9
	Zwelitsha	3		6

	Nomlacu	1	Mbizana	8
	Nikwe	2		8
	Nkantolo	2		6
	Yandlala	1	Ntabankulu	8
	Dambeni	2		9
	Mpisini	2		6
	Goso	1	Umzimvubu	7
	Saphukanduku	1		6
	Rode	3		9
Joe Gqabi	Mzamomhle	2	Walter Sisulu	5
	Maize field	2		7
	Aliwal North	2		8
	Mogesi	2	Senqu	9
	New Rest	2		6
	Zava	3		8
	Ezingonyameni	2	Elundini	9
	Luzi Port	1		7
	Luzi	2		7
Metropolitan	Qalashe	3	Buffalo City	17
	Restini	2		17
OR Tambo	Kambi	2	KSD	12
	Nkalane	1		5
	Mqanduli	2		5
	Bala	1	Ingquza Hill	12
	Malangeni	2		6
	Mhlanga	1		1
	Moyeni	3	Nyandeni	12
	Mgojweni	3		9
	Lujizweni	2		3
	Godzi	3	Mhlontlo	14

	Gungqwana	3		10
	Mbinja	1		2
	Mazizini	2	Port St. Johns	4
	Goqwana	1		1
Sarah Baartman	Pearston	1	Blue Crane	13
	Aberdeen	1	Dr Beyers	18
	Graaf Reinet	1		11
	Bhishibha	1	Sunday's River Valley	5
	Tanki	1	Makana	20
	Wynek	1		17
Amathole	Qeto	1	Nqushwa	19
	Nyaniso	1		4
	Ndabakazi	3	Mnquma	52
	High Hill	1		7
	Gwiligwili	2	Amahlathi	28
	Kie Road	8		67
	Shinira	1	Mbashe	2
	Xuba	6		49
	Total: 71	**158**		**1007**

Appendix 2: Cross-HI test results for ND ELISA positive samples

Sample	Genotype VII antigen (virulent field strain) Log_2 HI titre	Genotype II antigen (avirulent vaccine) Log_2 HI titre
CDA1	9	10
CDA6	8	10
CDA7	7	9
CBA13	0	2
CBA16	2	3
CDC2	8	9
CDC14	5	6
YAA18	6	7
YAA19	5	7
YAA31	6	7
IBC10	2	4
IBA9	3	3
GBA2	7	9
EAD2	6	6
FAB3	5	6
GAA5	6	8
OCB2	6	7
OBA3	0	5
OAA2	6	6
PAA11	7	8
TAA10	2	4
UAA10	5	6
NAB1	0	2
LCA2	10	12
NBA1	7	8
KCB1	3	5
WBA3	7	8

WAA15	8	8
UBA11	2	3
BBB1	7	7
CAA1	4	4
ACA4	9	10
BCD1	1	2
AAC2	6	8
JAA6	6	7
KAB2	8	10
HAA8	1	2
GCB1	1	1

Appendix 3: HI Test results (Log_2 titre) for ELISA AI positive samples

Sample number	H5N1 antigen	H5N2 antigen	H5N6 antigen	H5N8 antigen	H6N2 antigen	H6N8 antigen	H7N1 antigen	H7N7 antigen
ADA1	0	7	0	0	11	9	0	0
AFB18	0	0	0	0	0	0	0	0
AFC11	0	0	0	0	0	0	0	0
AFD11	0	0	0	0	0	0	0	0
AFE6	0	0	0	0	0	0	0	0
CAA1	0	2	3	0	6	4	0	0
HAA5	0	3	2	0	9	9	0	0
HCA1	0	3	1	0	9	7	0	0
ICA1	0	4	3	0	7	7	0	0
ICB2	0	2	0	0	4	5	0	0
PAA2	0	4	2	0	8	8	0	0
PAA4	0	3	1	0	7	5	0	0
PAA9	0	3	1	0	11	7	0	0
PAA10	0	4	2	0	9	5	0	0

Appendix 4: Remedies used by village chicken farmers in the ECP

Remedies	**Active ingredient**	**Usage by farmers (%)**
Traditional:		
Aloe ferox Mill.	Cape Aloe Ferox Gel. Vitamin C or Ascorbic acid (Water Soluble) Vitamin B5 or Pantothenic acid. Vitamin A palmitate. Vitamin E or Tocopherol (Oil Soluble) Vitamin B6 or Pyrodoxine (Oil soluble) Vitamin B2 or Riboflavin.	28.23
Zifozonke	Sodium permanganate	5.71
Mthuma*	Not found	0.41
Fish oil		0.55
Sugar		0.48
Salt		0.95
Epsom salt	Magnesium sulfate	0.59
Engine oil		1.31
Jeyes fluid	p-chloro-m-cresol, Tar acids, Propan-2-ol, Terpineol	0.48
Karbadust	Carbaryl (Carbamate)	0.48
Blue Death	Carbaryl	0.76
Ashes		0.48
Sniff		0.95
Garlic with vinegar		0.37
Madubula		0.78
Mbanga-mbanga	Not found	0.28
Vicks		0.68

Deadline	Flumethrin	0.22
Parafin		0.74
Sibabile		2.70
Total usage		**47.15**
Sulpha products:		
Cosumix Plus	Sulphachloropyridazine & Trimethroprim	6.23
ESB3	Sulphachloropyrazine sodium	1.9
Coliprim	Sodium Sulphachloropyridazine & Trimethroprim	1.43
Sulfazine 16%	Sulphadimidine Sodium	0.95
Triple Sulfa	Na-sulphamerazine, Na-sulphamethazine, Na-sulphathiazole sesquihydrate	0.95
Norotrim	Sulphonamide	0.55
Total usage		**12.01**
Tetracyclines		
Oxytetracycline	Oxytetracycline HCl	0.48
Terramycin powder	Oxytetracycline HCl	10.75
Hi-Tet	Oxytetracycline HCl	3.33
Doxysyrup	Doxycycline hyclate	0.95
Terramycin Liquid	Oxytetracycline HCl	1.43
Doxymycin	Oxytetracycline, sodium sulphacetamide, cetrimide	0.48
Total usage		**17.42**
Vaccines		
Newcastle (Lasota)		6.91
Gumboro		4.80
IB		0.90
Total usage		**12.61**

Supplements		
Stresspac	Vitamins and Minerals	10.33
SE Care powder	Vitamin E and Selenium	0.48
Total usage		**10.81**

* *Solanum aculeastrum*