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Seasonal Newcastle disease antibody titer dynamics in village chickens of Mbeere District, Eastern Province, Kenya

D C Kemboi, H W Chegeh, L C Bebora, N Maingi, P N Nyaga, P G Mbuthia, L W Njagi and J M Githinji*

University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya
kemboidc@gmail.com
** Ministry of Livestock, Central veterinary laboratories, Private Bag-00625, Nairobi, Kenya*

Abstract

This study was conducted in Mbeere district, Eastern Province Kenya, to establish Newcastle (ND) antibody titre levels in healthy, non-vaccinated village chicken chicks, growers and adults, in wet and dry seasons.

In wet season, all ages, many birds had higher titers in comparison to the dry season. There was, thus, a statistically significant difference (p=0.05) between the two seasons with a decrease on the number of birds with protective titer from 100% (wet season) to 83% (dry season). These results show continued endemicity of the disease in the area. Reason for not being able to isolate the virus from swabs and tissues of the birds may be presence of the high ND antibody titers; reducing the viral titers to levels not easily detectable by the serological test used. (Meaning that a more sensitive test was needed for detection). From the serological results, therefore, it is advisable to target vaccination at the beginning of the dry season, so as to boost immunity in these birds.

Key words: *hemagglutination inhibition, indigenous chicken, seasonality, seroprevalance*

Introduction

Free-range poultry keeping is the most common type of poultry production system in Kenya. These birds, however, have low production levels, compared to their exotic counterparts. Diseases are reported to be the main constraint to poultry production, especially Newcastle disease (ND) which causes mortalities as high as 100% (Njagi et al 2010a). Studies by (Kasiiti 2000) and (Njagi et al 2010a) showed that the ND virus (NDV) is present in healthy village chicken and that hens that survive outbreaks or have antibodies from previous exposure to Newcastle disease may maintain the virus endemicity in the village chicken. Thus, carrier chicken, village poultry population dynamics, other poultry species, wild birds and heterogenecity of the virus are some of the risk factors that have been associated with the maintenance of NDV (Awan et al 1994, Njagi et al 2010b). Management practices, including confinement, mode of disposal of poultry waste and carcasses and recovery rates of chicken

from disease outbreaks also favour maintenance of virus in village populations (Njagi et al 2010b). Nyaga et al (1985) indicated that Newcastle disease outbreaks are reported during the cold and dry periods of the year with peaks in April, June-July and September-November periods meaning that antibody titers to NDV virus can be found in birds all year round. The aim of the study was therefore to determine the prevalence of antibodies to NDV in naturally exposed, non- vaccinated multi-age village chickens in the wet and dry seasons in Mbeere District as an indicator of Newcastle disease endemicity. It was based on the hypothesis that season does not affect the immune response to NDV in village chicken in Mbeere District, Kenya.

Objective

- To determine antibody titers to Newcastle disease in chicken and recover Newcastle disease virus in dry and wet seasons.

Materials and Methods

Study area

Mbeere district has a human population of 219,220 and a large population of free-range chicken of 202,410 (KNBS 2009). These birds are kept for income, food and socio-cultural purposes. Other agricultural activities practised in the district include cattle, sheep and goats keeping; and millet, green grams, sorghum and cotton production. The district lies between latitude 0^o 20' and 0^o 50' South and longitude 37^o 16' and 37^o 56' East, at altitude 500 to 1200 metres above sea level. Long rains fall between mid-March and June while short rains occur October to December. Dry periods are between January and early March; and between August and September. The daily temperature ranges from 20 - 30 ^oC (Onduru et al 2002).

Experimental design

The birds were purchased from farms in Mbeere district in Eastern province. The study was cross-sectional and sampling was purposive and convenient (based on reachable willing owners, regardless of the number of chicken kept; so long as the birds were kept on free-range system, had no history of ND vaccination, and no parasite control/treatment was exercised).

Forty eight chicken (24 birds of both sexes each in dry and wet season) consisting of 7 chicks, 8 growers and 9 adults (wet season) and 9 chicks, 8 growers and 7 adults (dry season) with no previous history of Newcastle disease vaccination or parasite control. The wet season was in November while the dry season was in March. The chicks were less than 2 months old; growers were between 2 to 8 months; and adults, above 8 months of age (Sabuni 2009). All birds were labelled and transported in cages to Kabete, University of Nairobi campus for sampling. Collected serum samples were tested for NDV specific antibody by hemagglutination inhibition (HI) test while cloacal and oro-pharyngeal swabs were processed for NDV isolation (OIE 2000).

Collection and processing of blood and swabs

Blood was collected from the jugular vein at post-mortem by severing the neck and collecting the blood into universal bottles, without anticoagulant. Serum was separated from respective clotted blood samples by centrifugation at 3000 rpm for 10 minutes, decanting the serum into vials and keeping the vials frozen at -20^{o}C until hemagglutination –inhibition (HI) test was performed.

Swabs were taken from the oro-pharynx and cloaca using sterile cotton swabs and placed in 2ml viral transport medium comprising minimum essential medium, with penicillin (2000 international units/ml) and streptomycin (2000µg/ml). The swabs were expressed, centrifuged at 3500 rpm for 10 minutes and the supernatant transferred to a sterile bijoux bottle. All samples were stored at -20^{o}C until virus isolation was done.

Serology

Presence of NDV antibody was detected by hemagglutination inhibition test as described by OIE (2000). A cut off titer of 1:8 was considered specific indicating that the birds had been previously exposed to the virus, while titers less that this value were considered non specific. The validity of the results was assessed against a negative control serum included in the test. The HI titers were determined in all chicken, and the geometric mean titter (GMT) of each group calculated.

Virus isolation

The processed swabs and tissues were inoculated into embryonated eggs, incubated and harvested as previously described by Nyaga et al (1985) and the ND virus presence from the swabs and tissues was checked using hemagglutination test. The samples were passaged only once.

Statistical analysis

Data on antibody titers from hemagglutination inhibition test results per group was analysed using Genstat Discovery edition 3 for descriptive statistics. The mean geometric titer (GMT) per group were calculated and used in the analysis. The titers were compared across the various age groups and seasons. A critical probability of $P< 0.05$ was adopted as cut off point for statistical significance.

Results and discussion

Seasonality of antibody titers against Newcastle disease

Wet season

A total of 24 indigenous village chickens (7 chicks, 8 growers and 9 adults) of all sexes were examined for antibodies against Newcastle disease virus in their serum using hemagglutination inhibition. All of the 24 birds tested positive for antibodies against Newcastle disease with the titers ranging from 1:16 (2^4) to 1:256 (2^8) (Table 1).

Table 1: Hemagglutination Inhibition titers of different age group of chicken during the wet season

Age groups	No. of	Antibody Titer

	samples	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	GMT
Chicks	7	-	-	-	1	-	4	1	1	70.7
Growers	8	-	-	-	1	2	4	-	1	53.8
Adults	9	-	-	-	-	1	5	3	-	74.7
TOTAL	24	0	0	0	2	3	13	4	2	65.8

Key: GMT- Geometric mean titer (GMT = $\sqrt[n]{x_1x_2x_3...x_n}$*)*

All of the serum samples were found to be positive for antibody against Newcastle disease virus. The chicks had a titer ranging from 1:16 to 1:256 while growers had titer ranging from 1:16 to 1:256 with majority having 1:64. Adults had a titer ranging from 1:32 to 1:128 with majority (5) having titers of 1:64.

There was a significant difference ($P<0.05$) between the lower geometric mean antibody titers in growers (53.8), than in chicks (70.7) and adults (74.7) but no significant difference between chicks and adults ($P>0.05$).

All the birds showed serological evidence of specific immunity (Table 2). that is the level of antibody titer that show the bird has been in contact with ND virus a titer of 1:8 (2^3) and above (Allan and Gough 1974). A titer of between 1:16 to1:128 is considered protective whereby the bird is protected from developing Newcastle disease. Using this criterion, 100% of the birds in the wet season had protective levels of antibodies (Table 5).

Table 2: Serum samples of chicken showing immune response to Newcastle disease virus using hemagglutination inhibition during the wet season.

Age groups	Total Samples	Specific immunity	Non specific immunity	Percentage specific immunity
Chick	7	7	-	100
Growers	8	8	-	100
Adults	9	9	-	100
Total	24	24	-	100

Key: No. – Number of serum samples; GMT – Geometric mean titers

Dry season

A total of 24 indigenous village chicken (9 chicks, 8 growers and 7 adults) of all sexes were examined for antibodies against Newcastle disease virus using hemagglutination inhibition test. All the 24 birds tested positive for antibodies against Newcastle disease with the titer ranging from 1:4 (2^2) to 1:128 (2^7) (Table 3).

Table 3: Hemagglutination inhibition titers of different age group of chicken during the dry season.

Age groups	No. of Samples	Antibody Titer								
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	GMT
Chicks	9	-	1	1	2	4	1	-	-	20.2
Growers	8	-	-	-	2	-	3	3	-	58.7
Adults	7	-	-	2	2	-	2	1	-	26.3
TOTAL	**24**	**0**	**1**	**3**	**6**	**4**	**6**	**4**	**-**	**31.1**

The chicks had antibody titer ranging from 1:4(2^2) to 1:64 (2^6) while growers had titers ranging from 1:16 (2^4) to 1:128 (2^7). Adults had titers ranging from 1:8 (2^3) to 1:128 (2^7).

The geometric mean antibody titer of the different age groups during dry season was 31.1.

There was a significant difference between the higher geometric mean antibody titers in growers (53.7), and that in chicks (20.2) and adults (26.3) ($P<0.05$) but no significant difference between chicks and adults ($P>0.05$).

95.8 % of the birds showed serological evidence of specific immunity with titers of 1:8 (2^3) and above (Allan and Gough 1974), with 100% of the growers and adults and 88.8% of the chicks (Table 4). A titer range of 2^4-2^7 is considered protective and using this criterion 83% of the birds had protective levels of antibodies (Table 5).

Table 4: Serum samples of chicken showing immune response to Newcastle disease virus using hemagglutination inhibition during the dry season.

Age groups	Total Samples	Specific immunity	Non specific immunity	Percentage specific immunity
Chick	9	8	1	88.8
Growers	8	8	-	100
Adults	7	7	-	100
Total	24	23	1	95.8

There was a significant difference ($P<0.05$) in the mean antibody titers between the two seasons. Adults and chicks had significant higher antibody titers in the wet season than dry season ($P<0.05$) while growers had no significant difference in levels of antibody titers between the two seasons.

Table 5: Protective Newcastle disease antibody levels for the dry and wet season

Age groups	Wet season		Dry season	
	Protective NDV Ab titer ($2^4$to 2^7)	Non protective NDV Ab titer	Protective NDV Ab titer ($2^4$to 2^7)	Non protective NDV Ab titer
Chicks	100%	-	77.8%	21.2%
Growers	100%	-	100%	-
Adults	100%	-	71.4%	28.6%
Total	100%	-	83%	17%

Key: NDV-Newcastle disease, Ab-Antibody

Isolation of NDV

No virus was isolated from swabs and tissues from the birds from one passage in embryonated eggs.

Overall the birds had a higher levels of antibody titer that is unexpected in unvaccinated birds during both the wet and the dry season yet the owners did not vaccinate there birds confirming the endemicity of the virus in village chicken in Mbeere as previously reported by Njagi et al (2010a) in the same region, Otim et al (2004) in Uganda and Zeleke et al (2005) in Southern and Rift Valley districts in Ethiopia. Using Allan and Gough (1974) criterion that states that a titer of 1:8 and above is generally accepted as indicative of specific immunity, most birds had specific immunity meaning they had come in contact with the NDV. This wide range of NDV titer may be due to natural infection which is known to produce higher antibody titers than vaccination (Luc et al 1992). The continued hatching of chicks and the presence of birds that survived previous ND outbreaks mean there will always be susceptible chicken in free range chicken to which infected birds can transmit the disease (Martin 1992). This may have been the case during the wet season where the farmers reported an outbreak of a disease similar to Newcastle and serum samples from the birds showed high ND antibody titer. This implies that chicken in the village get infected at different times producing a near

cyclic pattern of the disease hence maintaining an endemic situation throughout the year (Otim et al 2004, Njagi et al 2010a). Free range management system that allows the uninterrupted cycle of infection as the virus passes from one age to another may also be a cause of this endemicity as suggested by Zeleke et al (2005). The chicken are also prone to acquire infections from wild birds and in some instances ducks that some farmers kept together with chicken that have been shown to harbor and shed the NDV without showing any clinical signs of the disease (Njagi 2008).

Both cold and hot seasons have been associated with ND outbreaks in Kenya (Nyaga et al 1985) but start of wet season has been associated more with outbreaks (Jintana, 1987); although in Vietnam (Nguyen 1992) and Uganda (Mukiibi 1992) higher seasonal incidence and severity of ND is reported in dry season. This may be the reason behind the higher antibody titers that were demonstrated during the wet as compared to the dry season in this study; a ND outbreak may have occurred during the wet season. This is in line with Martin (1992)'s suggestion that outbreaks are often associated with change in season especially between wet and cold weather. It is also supported by Awan et al (1994)'s conclusion that ND is associated with periods of stress, which could be due to change in climate and lowered resistance at the beginning of wet season, due to inadequate feed. The decrease in antibody titers observed in the dry season in the current study may have been an indication of lowered resistance. Moreover, concentration of antibodies has been reported to decline within 3-4 months of non-stimulation (Otim et al 2005). This may then lead to outbreak of the disease in the susceptible birds. From this study, the maintenance of the cyclicity of the disease in Mbeere chicken can, therefore, be linked to two factors; availability of susceptible population of chicken and lowered immunity, as manifested by low antibody titres. Part of this cyclicity was observed during the study period where in the wet season (November 2011), the number of birds per homestead was low, with some homesteads having as few as two birds; most of them being adults. This low number could be attributed to an outbreak of Newcastle disease that had caused high mortalities, all the 24 birds that were screened for Newcastle disease antibodies, within this season, turned positive with high antibody titers. Contrary to this, during the dry season (March 2012), the number of birds had increased significantly, the flock composition constituted mostly growers and chicks and most of the hens were either brooding or incubating. This could be explained by the fact that dry season was the harvesting time; there was, therefore, abundant grain harvest. With improved nutrition, there was increased egg laying and hatching; enabling the farmers to restock their flocks that had gone down during the wet season. Restocking resulted in increased number of chicks which ended up being susceptible to the disease, as observed during the study in November 2011. This fuelled ND outbreaks and maintained the virus within the recovered chicken; which became a source of infection for the next cycle. The maternal antibodies, if any were passed to the chicks, waned off within 3-4 months (Otim et al 2005). Village chicken scavenge for their feed with little supplementation and this scavenging behaviour encourages the spread of ND. Otim et al (2005) associated socio-cultural activities in rural households with ND outbreak. The ND dynamics appear to depend on regional and community activities and control measures need to take this into account.

Adults and chicks had a significantly higher titer compared to growers during the wet season and a significantly lower titer during the dry season with the finding in dry season similar to that of Njagi et al (2010a). This is because chicks and juveniles get maternal antibodies from immunized hens through the eggs or through contact with infected discharges and excretions during feeding and drinking (Mwakapuja 2009). The low antibody levels in the two groups during the two seasons may be due to the low level of antibodies in adults that will

correspond to the low levels in eggs and hence chicks.The actual cause of the apparent low levels of antibody titer seen in the grower group in comparison to the other groups during the wet season could not be identified; further study, therefore, needs to be done on this.

The lack of isolation of the virus from the birds may be due to neutralization of the virus by the high levels of protective antibodies (Alexander 2003). Healthy looking birds may harbour virulent NDV but if they have high antibody titer this may prevent them from having clinical disease (Njagi et al 2010a). Njagi et al (2012) suggested that ducks with protective levels of antibodies ($2^4$to 2^7) may not develop clinical disease but instead remain virus carriers and when immunosupressed they have been shown to shed the virus. This may also happen in chickens and the birds with antibodies may shed and act as source of infections to other susceptible birds during periods of stress.

Conclusions

Based on the results of this research it is concluded that:

- In all seasons birds have high antibody titers, and the antibodies tend to wane off during the dry season making the birds susceptible to introduction of velogenic strain of NDV.

- That vaccination is recommended during the start of dry season to maintain high levels of antibodies and prevent outbreaks and especially in chicks and adults;

- That age has influence on seropositivity of Newcastle disease with adults and chicks having a higher titer during the wet season than growers while in the dry season growers had a higher antibody titer than adults and chicks.

- Flock owners need to be educated on disease transmission and prevention and they be discouraged from restocking their farms with chicken from the market since these birds, though healthy looking, maybe harbouring the Newcastle disease virus.

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