Effect of parasite control on immune response to Newcastle Disease vaccination in village chicken, Mbeere sub county

D C Kemboi, H W Chegeh, L C Bebora, P N Nyaga, L W Njagi, N Maingi, P G Mbuthia 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

The aim of this study was to determine the effect of parasitism on village chickens' immune response to Newcastle disease (ND) vaccination. Seventy two chickens, from a population that was confirmed to be infected with ecto- and endo-parasites, from Mbeere District, were bought and divided into 8 groups which were variously treated for parasites: Groups 1 and 8 were controls, while Groups 2 and 5, 3 and 6, 4 and 7 were duplicate treated for endo-parasites only, ecto-parasites only and for both endo- and ecto-parasites, respectively. Groups, 5, 6 and 7 were also vaccinated with ND vaccine; the duplicate unvaccinated groups served as respective controls. Since these birds were also found to carry coccidian, all groups except Group 8 were also treated for coccidiosis; this was to give all the treated groups a baseline start, free of coccidia. After respective vaccination, all the birds were monitored serologically for six weeks; their antibody titres were determined on weekly basis, using hemagglutination inhibition (HI) test.

Upon vaccination, groups 5, 6 and 7 showed a significant rise (P<0.05) in Newcastle disease antibody titer from the start to the end of the experiment compared to the baseline one in the non vaccinated groups 2, 3 and 4. For the vaccinated group, after the 3rd week post vaccination up to the end of the experiment, group 7 had a significantly higher antibody titer (P< 0.05) than the other 2 vaccinated groups 5 and 6. Titres for Groups 1 and 8 continuously dropped over the experimental period. The study has, thus, shown that parasite control resulted in improved immune response to ND by the experimental birds; total parasite treatment giving better results than partial treatments (for ecto- or endo-parasites only). Farmers should, therefore, be advised to practice total parasite control before vaccination.

Key words: *antibody titer, ectoparasites, endoparasites, foraging, scavenging*

Introduction

Free-range poultry keeping is the most common type of poultry production system in Kenya. Though they have low production levels compared to their exotic counterparts (Yongolo et al

1997), these birds are more preferred for their taste and believed freedom from drug residues; in the market, their eggs sell at higher price than exotic ones.Newcastle disease (ND) is documented among the most important diseases in the world, causing devastating losses in both commercial and village chickens. It is capable of causing mortality rates of up to 100% of the flock and, as a viral disease; it can only be effectively controlled through vaccination. The disease has been shown to be endemic in village chickens in Eastern Province of Kenya. On the other hand, parasites, which have a tendency of causing stress to the birds through nutrient consumption, blood sucking and irritation, have also been isolated at high levels from chickens from this area. These chickens have a great diversity of ecto-parasites, endo-parasites and hemoparasites with studies putting the prevalence at a range of 90 – 96% (Maina 2005; Sabuni 2009).This, coupled with a wide range of bacterial and viral conditions; in addition to poor nutrition and walking over long distances in search of food, induces stress, which has been reported to cause immunosuppression (Njagi et al 2010a). Parasitism has been reported to cause absolute loss of proteins (Tizard 1996) and this may lead to reduced synthesis of immunoglobulin and hence a reduced immune response.

Knowing that stress is associated with immune-suppression, this study was carried out to check on the extent to which these parasites (ecto- and endo-) may suppress immune response to ND vaccination. This was done through monitoring of antibody titers after selective parasite treatments followed by ND vaccination.

Objective

- To determine the effect of endo- and ecto-parasite control on the chicken's immune response to Newcastle disease vaccination.

Materials and Methods

Source of birds for the Study

The birds were sourced from Mbeere district. Mbeere district has a human population of 219,220and 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 birds

Seventy two (72) chickens from a population that was confirmed to be infected with ecto- and endo-parasites, with no previous history of vaccination or worm control, were used in this study. They were bought from the market; growers of all sex were used for the study. Growers were those from 2 months to 8 months (Sabuni, 2009). They were transported alive in cages approved by the animal welfare committee of the University of Nairobi to the Department of Veterinary Pathology, Microbiology and Parasitology for the controlled study. The birds were housed in standard approved cages, fed and watered *ad libitum*. They were given two weeks to acclimatize, during which period the pre-experiment parasitic situation was established by sacrificing eight (8) randomly selected birds and carrying out post-mortem examination; this was to ascertain that the birds had parasites. Serum samples collected from these birds were used to establish the initial ND antibody titres; this was done using hemagglutination inhibition test.

The birds were found to harbour lice, helminths and coccidia and this was used as a basis to divide the remaining birds into eight different groups of 8 birds each, as given in Table 1 based on the treatments to be administered.

Groups 2 to 4 were the controls for treatment groups while Group 1 overall control group treated for coccidia, and Group 8 was overall control group not treated for coccidia.
Birds were wing tagged for identification.

Groups 5 to 7 were treatment groups that were used to determine the antibody responses to ND vaccine after ecto-parasite treatment, endo-parasite treatment, and combined treatment, respectively.

All the birds were bled for serum, from the wing vein at intervals of 1 week for 6 weeks.

Table 1: Experimental groups, with respect to parasite treatment, coccidia treatment and vaccination

Group of chicken	No. of chicken	Endoparasites treatment	Ectoparasites treatment	ND vaccination	Coccidia treatment
1	8	-	-	-	Intracox® (Tolrazuril)
2	8	Albendazole	-	-	Intracox® (Tolrazuril)
3	8	-	Sevin+permethrin	-	Intracox® (Tolrazuril)
4	8	Albendazole	Sevin+permethrin	-	Intracox® (Tolrazuril)
5	8	Albendazole	-	+	Intracox® (Tolrazuril)
6	8	-	Sevin+permethrin	+	Intracox® (Tolrazuril)
7	8	Albendazole	Sevin+permethrin	+	Intracox® (Tolrazuril)
8	8	-	-	-	-

KEY: *ND: Newcastle disease; No. : Number; + given or done; - not given or not done*

Treatment and vaccination

One locally available antihelmintics; Albendazole, a coccidia drug (Intracox®) and an ecto-parasite drug [combination of Sevin (Cabaryl) and pemethrin] were used in this study. Albendazole was administered one week prior to vaccination at a dosage of 20mg/kg body weight via drenching (i.e. 0.2ml per bird); calculation based on weight of the heaviest chicken. This was repeated after four weeks. Intracox® (Tolrazuril 25mg) was administered at a dose of 1ml per 1000ml of drinking water for 2 days to all the groups except control group eight. Combination of sevin (Cabaryl) and permethrin were administered by dusting individual birds and the cages and this was repeated after a month to prevent re- infection.
Coccidial treatment was done so as to give all the treated groups a baseline start, free of coccidia. Control group 8 was used to monitor changes that may have been caused by the coccidial infection.

Vaccination was done using a locally available vaccine (Avivax-F from Kenya Veterinary Vaccine Production Institute) following the manufacturer's recommendation. A layers vaccination regime was used, i.e. primary vaccination on day 0, a booster 14 days later, followed by another booster 1 month later as scheduled in Table 1.

Hemagglutination inhibition (HAI) test

Hemagglutination inhibition was carried out as described by OIE (2000) with the source of the reference virus being the department of Veterinary, Pathology, Microbiology and Parasitology, University of Nairobi. The HI titers were determined using serum samples from all chickens, and the geometric mean titter (GMT) of each group calculated.

Examination and identification of the parasites

All the helminths were examined under light microscope and were identified on the basis of helminthological keys described by Soulsby (1982) and Permin and Hansen (1998).

Ectoparasites were examined and identified following the method and criteria described by Sabuni (2009).

Statistical analysis

The data collected were stored in a spreadsheet program (excel).

Analysis was done to assess the immune status of the birds and comparison was done using Analysis of Variance (ANOVA).

Results

Parasitology results

Pre-treatment examination results

The 8 birds sacrificed before the start of the experiment showed the following: (1) that the birds had 62.5% ecto-parasite prevalence; lice the only ecto-parasite found (Table 2), and. (2) the sacrificed birds had 100% endoparasite prevalence; tapeworms (62.5%), caecal worms (100%) and *Gongylonema* species (37.5%) being the species found (Table 3).

Results of ecto-parasite treated groups

Groups 2, 4, 5 and 7 were treated for ecto-parasites using a combination of permethrin and sevin (Cabaryl). At post-mortem, all the birds in the treated groups had no ecto-parasites. Untreated groups 1, 3, 6 and 8 still had significantly higher prevalence of ecto-parasites than the treated group ($P<0.05$) (Table 2)

Table 2: Ecto parasites prevalence before and after treatment

Group	Overall prevalence	*M.stramineus*	*M.gallinae*	*G.gallinae*
Preliminary findings				
	62.50%	100%	0	0
Non vaccinated				
1	100%	100%	25%	37.50%
2	0	0	0	0
3	100%	100%	30%	0
4	0	0	0	0
8	60%	100%	0	0
Vaccinated				
5	0	0	0	0

6	100%	100%	0	0
7	0	0	0	0

Key: M.stramineus – Menacanthus stramineus, M. gallinae – Menopon gallinae, G.gallinae – Goniocoites gallinae

Results of endo-parasite treated groups

Groups 3, 4, 6 and 7 were treated using Albendazole (20mg/kg body weight), after treatment all the birds had no helminths with the exception of *Gongylonema inguivicola* species that was embedded in mucosa of the crop giving the overall prevalence of endoparasites as shown on the table 3. The non treated groups still had varied helminths species with the overall prevalence significantly higher than the treated ($P<0.05$) (Table 3).

Table 3: Endoparasites prevalence prior and after treatment

Group	Overall prevalence	TA	G.I	S.S	H.I	R.E
Preliminary findings						
	100%	0	37.50%	100%	0	62.50%
Non Vaccinated						
1	100%	0	37.50%	100%	0	87.50%
8	100%	20%	80%	60%	60%	60%
2	80%	20%	40%	100%	60%	80%
3	0	0	0	0	0	0
4	33.30%	0	33%	0	0	0
Vaccinated						
5	100%	0	33%	100%	33%	100%
6	20%	0	20%	0%	0	0
7	50%	0	50%	0	0	0

Key: TA: Tetrameres americana GI: Gongylonema inguivicola S.S: Subulura suctoria HI: Heterakis isolonche RE: Raillietina echinobathrida

HI test results

All the birds were positive for specific NDV antibodies pre-vaccination with the individual GMT of the group shown in table 4. Throughout the vaccination period, the vaccinated groups 5, 6 and 7 showed a significant rise ($P<0.05$) in the antibody levels compared to the control groups. . Thus, while the antibody titers in the control group were dropping, titers in the vaccinated groups were rising. From week 3 post vaccination, group 7 had a significantly higher antibody level ($P<0.05$) than the other vaccination groups, this extended all the way to the end of the experiment (Figure 1 and 2). The vaccinated groups showed a steady rise up to week 3, where group 7 acquired a higher level compared to the other two groups 5 and 6. Group 6 had a lower level than the other two vaccinated group but was level with group 5 from week 4 to week 6.

Control groups 1, 2, 3, 4 and 8 had a drop in the pre-vaccination NDV antibody titers to week 6 though this was not statistically significant ($P>0.05$); Table 4**)**.

Table 4: Geometric mean antibody titers to NDV for the experimental groups

Group	GMT	Pre-vaccination	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	G1	90.5	90.5	53.8	64	53.8	53.8	45.25
2	G2	128	111.4	73.5	73.5	84.4	73.5	48.5
3	G3	80.63	64	40.31	40.31	40.31	40.31	32
4	G4	111.4	111.4	55.71	64	55.71	42.22	42.22
5	G5	32	256	512	776	891.4	891.4	1024

6	G6	64	256	512	676	891.4	891.4	1024
7	G7	57.01	228.07	512	912.28	912.28	1024	1149.4
8	G8	73.5	73.5	64	64	42.22	42.22	32
Key								
G7	Grp 7	Ecto and Endoparasitetreatment+Vaccination						
G5	Grp 5	Endoparasite treatment+ Vaccination						
G6	Grp 6	Ectoparasitetreatment+Vaccination						
G4	Grp 4	Ecto and Endoparasite treatment						
G3	Grp 3	Ectoparasite treatment						
G2	Grp 2	Endoparasite treatment						
G8	Grp 8	Control group						
G1	Grp 1	Control group						

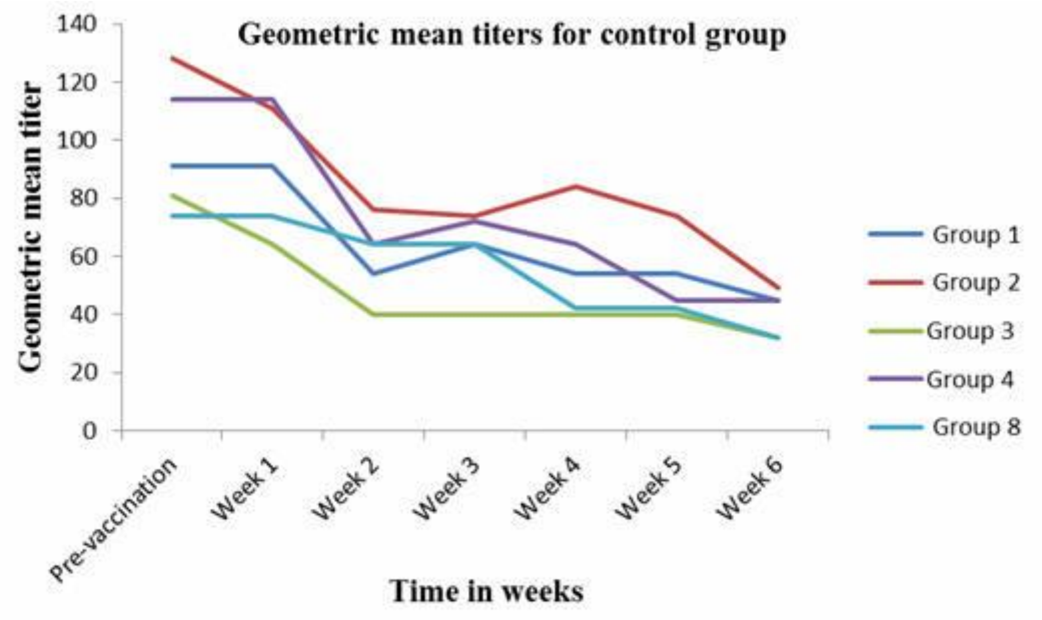

Figure 1: GMT of control groups in relation to time

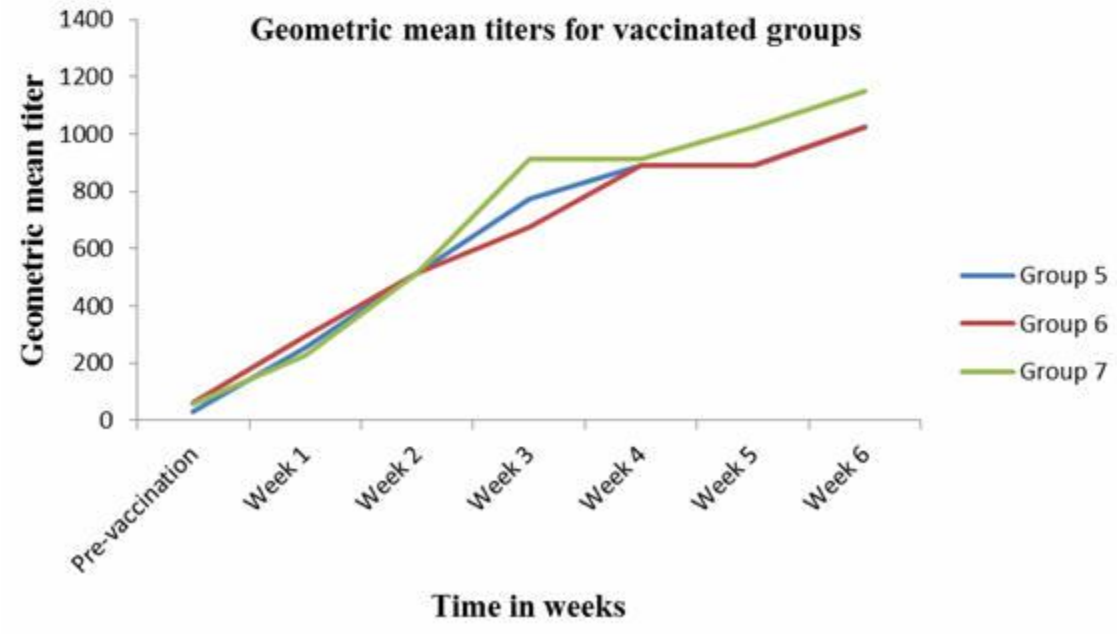

Figure 2: GMT for vaccinated groups with respect to time post vaccination. The HI titer for group 7 was significantly higher than the other groups at week 6

Discussion

This study showed that the indigenous chickens from Eastern province had a wide range of parasites, similar to what Maina (2005) and Sabuni (2009) documented. Various species of lice,*Menacanthus stramineus, Menopon gallinae* and *Goniocoites gallinae,* were the only ecto-parasites found on the birds. This does not quite match what Sabuni (2009) found: apart from lice, he also documented fleas and mites from chickens in Eastern province. The chickens, in the current study, also had a high prevalence of endoparasites (100%), similar to the findings of Maina (2005); she reported a prevalence of 90% with *Heterakis, Gongylonema, Tetrameres, Acuria, Ascaridia* species and *Railletia echinobathrida* (a cestode) being the most prevalent. In this study, *Railletia echinobathrida*(cestode*),Heterakis isolonche*, *Subulurasuctoria*, *Tetrameres americana* and *Gongylonema inguivicola*(roundworm species) were recovered. *Acuria* and *Ascaridia galli* were not recovered; this may be attributed to the fact that piperazine (Ascarex®) is the most commonly used drug in the area (unpublished questionnaire study results). Piperazine has a narrow range of activity, acting on *Ascaridia galli* and a few other round worms and not on tapeworms and caecal worms (Arends 2003).

On treatment, by dusting, of the ecto-parasites using a combination of sevin®(Carbamates) and permethrin, all the ecto-parasites were cleared; this underscores the effectiveness of the combined drug method. Application of the treatment on monthly basis should be recommended to avoid re-infection. Albendazole at a dose of 20mg/kg body weight, repeated after a month, was shown to be effective and safe against most of the helminths, both nematodes and cestodes. This is in accordance with a finding by Tucker et al (2007) who showed it to work against *Ascaridia galli*, *Heterakis gallinarium*, *Capillaria obsignata* and *Raillietina cesticillus.* The drug did not act against *Gongylonema inguivicola* species, meaning it has a little or no activity against this parasite. The drug may not have reached where the parasite was, since *Gongylonema inguivicola* is normally found under the mucosa of the crop; the mucosa may have protected it.

Biosecurity cannot be effectively implemented in domestic village chicken hence vaccination is the ideal control method (Otim 2005), however certain factors affect immunity of the chicken and may adversely affect post vaccination immune response. Immunosuppressant such as Infectious bursal disease, Aflatoxicosis and parasites can affect immune response and can lead to vaccination failure (Otim 2005, Permin1998). There was a significant difference between the humoral response of the vaccinated and respective non vaccinated groups indicating that vaccination boosts the immune response of the birds and is the sure method of controlling NDV (Alders and Spradbrow 2001). Where treatment was done, separately or combined, the HI titre was significantly higher than that of the control birds. Also, combined ecto- and endo-parasite treatment resulted in chickens that had significantly higher HI titres than in cases where ecto- and endo-parasite infections were treated separately. This is similar to findings by Hørning (2003) who showed control of helminth parasite improved NDV vaccine immune response. It emphasizes the importance of parasite prevalence of 90-100% in lowering the humoral immunity as reported by Permin et al (1998). The synthesis of immunoglobulins is reduced in animals severely affected by parasites, owing to an absolute loss in protein (Tizard 1987). This might result in reduced antibody response as seen in this study and that by Hǿnning et al (2003).There was no significant difference between chickens that had only ecto-parasite control and those that had only endo-parasite control, indicating that ecto- and endo-parasites may have similar effect on the immune response.

Local chicken ecotypes may have divergent responses towards ND vaccine; with some demonstrating high selection for antibody response to vaccine as well as early response (Beard and Hanson1984) hence these may be the cause of variation to vaccination among individual birds in the vaccinated groups.

A decline in the levels of antibody titers for the control groups is a natural phenomenon, since not vaccinated; this has also been reported to take about 3-4 months by Otim (2005)

Faecal samples taken at the beginning of the experiment showed that all the birds had coccidiosis at a lower level (+) but later there was a flare up leading to some mortality. This flare up may be due to confinement that leads to stress as reported by Alexander (1998) since the birds had been used to free range system. Other factors that may contribute to this infection may be transport, change in weather, change in feed and handling during vaccination and bleeding (Alexander 1998). On treatment with Tolrazuril all coccidia were cleared from the birds as indicated from their faecal samples hence the drug is quite effective against coccidiosis in poultry

This study has shown that parasite control results in improved immune response to ND vaccination by the experimental birds; total parasite treatment giving better results than partial treatments (for ecto- or endo-parasites only). If regular total parasite control is exercised by the farmers, their birds will respond well to ND vaccination, hence be protected from the disease; they will be free from stress, caused by the parasites; and their productivity will improve. The resultant effect of this will be more income to the farmers, which will eventually translate to improved economy for the country as a whole. The farmers and their families will also get enough proteins, will be healthier, and hence be able to work better to build the country's economy.

Conclusion

- Parasite control results in improved immune response to ND vaccination; total parasite treatment giving better results than partial treatments (for ecto- or endo-parasites only).

- If regular total parasite control is exercised birds will respond well to ND vaccination, hence be protected from the disease; they will be free from stress, caused by the parasites; and their productivity will improve. The resultant effect of this will be more income to the farmers, which will eventually translate to improved economy for the country as a whole. The farmers and their families will also get enough proteins, will be healthier, and hence be able to work better to build the country's economy.

- Albendazole at a dosage of 20mg/kg body weight repeated monthly is both safe and effective in elimination of most helminths with the exception of *Gongylonema inguivicola* and hence a preparation for poultry could be reconstituted for use.

- Treatment, by dusting, of the ecto-parasites using a combination of sevin® (Carbamates) and permethrin, all the ecto-parasites is effective. Application of the treatment on monthly basis should be recommended to avoid re-infection.

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