

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Antibody Response of Non-Dewormed and Dewormed Village Chickens to Sheep Red Blood Cells

Ngonda Saasa¹, Joyce Siwila², Ethel M'kandawire¹ and King Shimumbo Nalubamba²
¹Department of Disease Control, ²Department of Clinical Studies,
University of Zambia, School of Veterinary Medicine, P.O. Box 32379, Lusaka 10101, Zambia

Abstract: The economic activities of rural communities rely on small scale enterprises such as raising village chickens. However, the impact of many infectious diseases has hampered the activities of these rural communities necessitating the use of vaccines to control and mitigate the negative effects of the diseases. Understanding the effect of helminths on the immune system and ultimately on the outcome of vaccinations is important. In this study, the effect of deworming on the immune response was evaluated by antibody titres to sheep red blood cells using direct hemagglutination in 41 naturally helminth-infested village chickens. We found that the immune response of both the control and dewormed groups of chickens, respectively, to sheep red blood cells were similar at primary (mean titres 726.74 and 819.58; $p = 0.78$) and secondary (mean titres 1822.24 and 1792.92; $p = 0.95$) levels. The results show that helminth infestation of clinically healthy chickens may not grossly affect the immune response of village chickens, suggesting that naturally infested chickens can be immunized without deworming.

Key words: Anti-sheep red blood cells (anti-SRBC), village chicken, immunity, helminths, hemagglutination, deworming, Zambia

INTRODUCTION

The rural communities of Zambia rely on various small scale agricultural activities to sustain their livelihoods. These include rearing cattle, small ruminants such as goats and sheep as well as free-range chickens, often referred to as village chickens. Chickens are handy when small amount of income is required, as compared to large livestock due to their ease of sale. However, chickens are prone to infection with different kinds of pathogens ranging from viral, bacterial and helminths. These invasions result in disease onslaughts that lower the economic returns on the chicken rearing enterprises through weight loss, morbidity and mortality. In this regard, vaccination is an important intervention towards improving the productivity of poultry farming in the rural areas (Henning *et al.*, 2013).

In Zambia, the poultry industry comprises the commercial poultry and the village poultry. Under the commercial system, chickens are obtained from hatcheries and reared on commercial feed and in properly designed chicken structures or houses. Strict vaccination schedules for vaccines such as lentogenic LaSota/46 Newcastle disease vaccine strain are followed. In contrast, under the rural set up, village chickens are reared on free range and do not usually undergo regular vaccination or deworming. This lack of regular interventions gives rise to various kinds of infections and worm/helminth infestations that are normally observed. Factors such as helminth infestation are known to affect the growth rate of chickens (Katoch

et al., 2012). However, since all village chickens are infested by gastrointestinal helminths (Phiri *et al.*, 2007), it is not known whether efficacious immune response or vaccination failure due to lowered immune response can result from this natural infestation.

Although there is evidence that helminths have an effect on the chicken humoral and cell-mediated immune responses after vaccination, (Pleidrup *et al.*, 2014), the modulatory effect of helminths on the immune system of chickens in the rural settings still needs to be investigated. The knowledge of the modulatory effects of helminths on the immunity of village chickens could provide valuable information such as the importance of deworming before administration of vaccines. Immunization of chickens with sheep red blood cells (SRBCs) is one way of evaluating the immune response. Since village chickens are usually pre-exposed to many kinds of infections, stimulation with SRBC is a convenient way of evaluating the immune response. The SRBC stimulation test is widely used to evaluate modulatory effects of various elements on the immunity of chickens (Boa-Amponsem *et al.*, 2000; Parmentier *et al.*, 1998; Ruiz-Feria and Abdukalykova, 2009).

The aim of this study was therefore, to determine whether naturally occurring helminth infestation has a modulatory effect on the chicken immune response using SRBC stimulation, by measuring the strength of antibody response after deworming.

MATERIALS AND METHODS

Study design and study area: This was a case control study conducted at the University of Zambia, School of Veterinary Medicine in Lusaka. The village chickens used in the study were obtained from Namwala district of Southern province of Zambia located about 330 km south of the Capital city of Zambia, Lusaka. The district has approximately 102,866 people (CSO, 2012) and the main activities are livestock farming, mainly cattle and occasionally goats. Apart from cattle, farmers keep village chickens in large numbers.

Chickens and housing: For this study, a total of 41 clinically healthy adult village chickens were acquired from local farmers and transported to the University of Zambia. The chickens were housed at the institutional animal facility for three weeks before commencement of the experiment to acclimatize them to the environment. During this period, all the chickens were placed in one room without any barriers, with *ad libitum* food and water. All the chickens were uniquely colour tagged using leg bands for individual identification.

Deworming of chickens: After the acclimatization period, the list of identities was prepared and the chickens were selected and allocated into two groups using a simple random method. The chickens were placed into two adjacent demarcations separated by a wire mesh. Group one (control, n = 20) was not dewormed while group two (experimental group, n = 21) was dewormed. The experimental group was dewormed twice, seven days apart using Piperazine at 20 g in 10 L of drinking water for 24 h. Prior to deworming, water and food were withdrawn for 4 h for both groups, but the control group was not given any treatment.

Preparation of SRBCs and immunization of chickens: Briefly, sheep blood collected into Alsevier's solution was washed three times with sterile normal saline and resuspended at a concentration of 2.5% in normal saline. A fresh preparation of SRBC was made on every day of immunization. Preliminary experiment showed that intravenous administration of SRBCs produced optimal antibody response over intramuscular and intraperitoneal routes. All the chickens (both control and experimental) were immunized twice (Day 0 and day 7) intravenously using the wing vein with 0.5 mL of 2.5% SRBC suspension in sterile normal saline. Blood was collected in plain vacutainer tubes on day 0 before the first inoculation (pre-immune), 7 and 14 and serum was prepared and stored at -20°C until use.

Direct hemagglutination assay: Direct hemagglutination assay was carried out to determine the antibody response of both the control and experimental chickens

to SRBC. The preparation of SRBCs was similar to the procedure for immunization. The standard procedure for direct hemagglutination was followed (Cheema *et al.*, 2003). Fifty microlitres of phosphate buffered saline (PBS) was added to the round-bottomed 96-well microplate. Then 50 µL of serum was added and serially double diluted. Then 50 µL of 2.5% SRBC in PBS was added to all wells to make a 100 µL final volume. The plates were shaken for 1 min and incubated for 2 h at 37°C followed by 24 h at 4°C and the agglutination titres were determined. The well preceding a distinct SRBC button was considered as the endpoint titre for agglutination.

Euthanasia and post-mortem: The body weights of the chickens were taken and recorded. The chickens were humanely sacrificed by cervical dislocation. The heart, liver and spleen were collected and weighed.

Data handling and statistical analysis: The antibody titres and organ weights were recorded for all the measurements. Data was entered into a Microsoft Excel® Spreadsheet. Statistical analysis using STATA version 13 (Stata Corp, College Station, Texas, USA) was carried out to determine whether there was any significant difference in the means for the two groups (control and dewormed group) using the One way ANOVA as well as between the sexes. All results were considered significant at $p \leq 0.05$.

RESULTS

Distribution of study units: A total of 41 chickens were included in the study. Out of these, 80.5% (33/41) were female while 19.5% (8/41) were males. The dewormed group had 21 members while the control group had 20 chickens (one chicken died before commencement of the experiment). Of the dewormed group, 16 were female and five were males while the control group had 17 females and three males.

Direct hemagglutination assay: Agglutination titres were obtained for all the chickens in the control and dewormed groups. Table 1 show the means and standard deviations for the two groups. All the chickens had no previous exposure to sheep RBC (mean titre = 3.00). There were no differences in the mean anti-SRBC titres between the primary and secondary immune response in the control and the dewormed group (day 7 ANOVA $F_{1,40} = 0.08$, $p = 0.779$ Mean control \pm SD = 726.74 \pm 1221, Mean dewormed \pm SD = 819.58 \pm 748; Day 14 ANOVA $F_{1,40} = 0.00$, $p = 0.949$ Mean control \pm SD = 1822.24 \pm 1601, Mean dewormed \pm SD = 1792.92 \pm 1192). There were also no differences between sexes in both control and dewormed groups for both the primary and secondary immune response (Fig. 1a and b).

Table 1: Summary of means and standard deviations (std dev) for the control and dewormed groups of chicken showing the mean antibody titre at days 7 and 14, body weight (kg) and organ weights (g)

Measurement	Control (n = 20)		Dewormed (n = 21)		p-value
	Mean	SD ^a	Mean	SD ^a	
Day 7 ^b	726.74	1221.36	819.58	747.77	0.78
Day 14 ^b	1822.24	1601.16	1792.92	1191.61	0.95
Bwt (kg) ^c	1.99	0.58	2.14	0.35	0.35
Liver (g) ^d	41.93	13.90	47.41	7.82	0.15
Heart (g)	9.81	3.69	10.71	2.65	0.41
Spleen (g)	3.91	1.63	3.64	1.09	0.57

^aStandard deviation ^bAntibody titre ^cBody weight Kg: Kilogram ^dg: grams taken at the end of the experiment

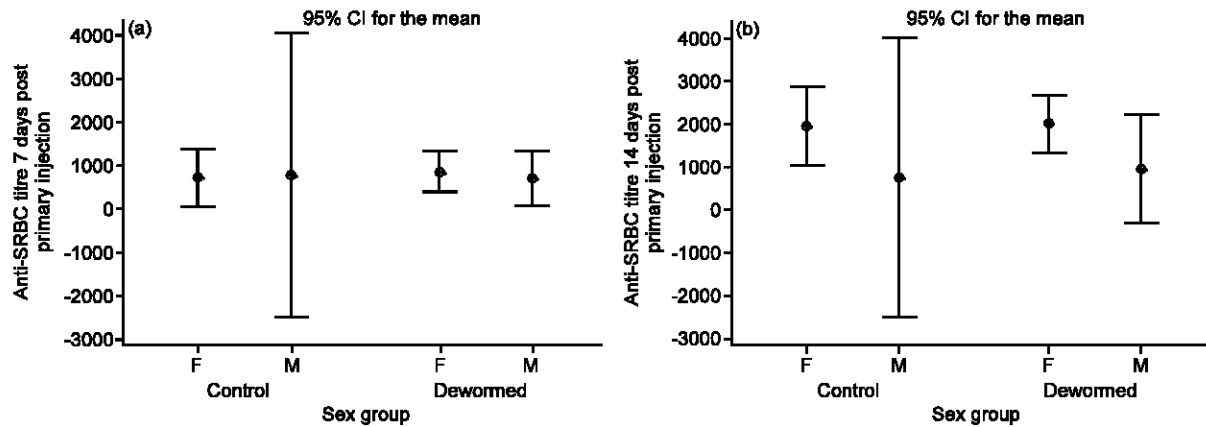


Fig. 1: Interval plot of anti-sheep RBC titres in the male and female village chickens in naturally helminth infected vs dewormed subjects after primary (a) and secondary (b) SRBC challenge (n = 41)

As indicated in the Table 1, the weights of all the measured organs from the chicken in both the control and dewormed groups were not significantly different. According to sex of the chickens, mean titres were similar for both the primary (p = 0.90) and secondary (0.08) responses (overall mean titre primary immune response ± SD (95% CI) = 773.10 ± 999.97 (444.48, 1101.84); Overall mean titre secondary immune response ± SD (95% CI) = 1806.30 ± 1379.30 (1339.59, 2272.97). There were differences in body weight (p = 0.0034), heart (p = 0.0008) and spleen (p = 0.05) between the males and females.

DISCUSSION

The immune system plays a central role in protecting the host against the onslaughts of various invasions by bacteria, viruses and helminths. Most infections result in the lowering of the immunity towards other infection (Horning *et al.*, 2003). In order to assess whether natural helminth infestation has a modulatory effect on the immune response, we dewormed clinically healthy chickens and immunized them with SRBCs and measured the antibody titre. We found that there was no significant difference in the response in the control and dewormed groups of chickens.

The rural communities in Zambia are striving to earn an income through various small scale enterprises

including small scale businesses and rearing free range chickens. The rampant outbreaks of viral infections such as Newcastle disease and other infection result in high morbidities and sometimes mortalities reaching 100%. Although vaccination of chickens in rural areas has not been regularly carried out, it has become important that village chickens be vaccinated against the common poultry diseases. As such a clearer understanding of factors that influence the efficacy of vaccines such as strains affecting different part of the country and host immune-related factors is critical.

Our study found that there was no difference in antibody response after deworming the chickens. These findings suggest that natural infection with helminths does not cause a difference in the antibody response of village chickens to immunization at least with SRBCs. Therefore, this observation would suggest that deworming of clinically healthy village chickens does not have to be undertaken prior to vaccination.

Other studies have observed that deworming chickens gives rise to higher antibody titres compared to non-dewormed chickens (Horning *et al.*, 2003). The differences could be attributable to the fact that the studies used experimental infection of chickens with worms that could potentially cause a drastic and heavier burden of worms on the chickens as compared to

natural trickle-infection. The response of chickens to Newcastle Disease Virus (NDV) could produce a more pronounced immune response to the vaccine because these viral infections are common in chickens. Better still, challenge experiments could provide additional information on the effect of helminths by measuring the natural response challenge infection (Juhl and Permin, 2002). In this study, use of SRBCs was opted for because the chickens showed varying extent of pre-exposure to Newcastle disease virus. The response of chickens to SRBC showed that deworming prior to vaccination did not have a detectable modulatory effect on the immunity of the chickens. These findings suggest that deworming clinically healthy village chickens prior to vaccination does not have a beneficial effect on the immune response to antigen stimulation of chickens, a cost that can be excluded.

Although our approach targeted the humoral immune response of the chickens to SRBCs, it is possible that the true response to vaccination or infection may be altered by helminth infestation. It would be interesting to investigate this aspect in a natural environment where the chickens can roam freely; deworm one group, leave one naturally helminth infested and vaccinate with NDV and thereafter, challenged with virulent strains of NDV during an outbreak. A further experiment to assess the anti-SRBC response in village chickens trickle-infected with different burdens of helminths will help elucidate at what helminth burden the anti-SRBC response would be affected by helminth infestation. This would shed more light on the level of helminth burden that would translate into immune interfering infestations. It is also still not clear how the commercial breeds of chicken would respond to the effects of deworming after infestation with worms.

Conclusion: In conclusion, although helminths may interfere with the general well-being of the free-range village chickens, vaccination can be carried out without prior deworming in clinically healthy chickens. This is important more so in the resource poor part of the nation where purchasing of dewormers may hamper the efforts to purchase vaccines against economically important diseases such as Newcastle disease.

Conflict of interest statement: The authors of this study declare that none of them have any financial or personal relationships with individuals or organizations that would unacceptably bias the content of this study. The manuscript does not contain clinical or patient data.

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