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Association Between Helminth Burden, Helminth Species Richness, Body Condition, Haematological Profile and Spleen Morphometrics in Domestic Guinea Fowl (*Numida meleagris*)

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Abstract: The economic, physiological and immunological costs of endo-parasitism are documented to be higher in poultry with greater parasite intensities and diversity. The current study was undertaken to determine the effects of helminth burden in domestic guinea fowls at quantified natural infestation levels on body condition, haematological profile and spleen morphometrics. We hypothesized that the spleen size and haematological parameters would be correlated to both total helminth burdens and species richness per host. All guinea fowls were infected with gastrointestinal nematodes (147/147). Mean intensity of nematode infection was 113.7 (CI 98.9-128.6) and the average species richness was 4.17 species/host (Range 1 to 7). Although helminth species richness was positively associated with spleen size and weight, the study showed no compelling evidence ($p < 0.05$) of association with total worm count per host. There was no association between body weight and helminth species richness but a positive association between total worm counts per host and body weight with heavier guinea fowls having more worms per host. Packed cell volume (PCV) was mildly negatively associated with helminth species richness but total worm count was paradoxically positively associated with PCV in male guinea fowl. All haematological values at different worm burdens and species richness were however, within reference values. Collectively, these findings may suggest that the most common helminths at natural infection levels in free-living guinea fowl populations have minimal or symbiotic effect on the immune system of the host. However, experimental trickle infections with different helminth species to reach natural infestation levels, up to clinical disease levels, are required to better understand the progression of immune responses to helminths in guinea fowls and evaluate the potential consequences on their health. Research studies leading to a comprehensive understanding of the effects of helminths on the host's immune system will have a bearing on the pre-requisite preparations preceding and the responses to, immunization in poultry.

Key words: Domestic guinea fowl (*Numida meleagris*), helminth burden and richness, spleen correlation, haematology profiles, body condition

INTRODUCTION

Endo-parasitism in poultry is reported to have detrimental effects on the host as the host responds to try and counteract these negative effects (Hoste, 2001). The avian pathology associated with helminth parasitism includes the sequela of direct nutrient deprivation by feeding helminths or the indirect costs as the host invests resources, which include nutrients, to mount an immune response and/or repair tissues that have been damaged. Although parasite burden is critical, parasite pressure resulting from species diversity is also an important factor to be considered (Bordes and Morand, 2009).

Chickens have similar characteristics to other poultry species and several aspects of the biology of chickens

have been extrapolated and imposed on other species such as guinea fowl and quails. However, there are differences in haematological, electrolyte and serum biochemical parameters in these species (Durai *et al.*, 2012; Simaraks *et al.*, 2004). It is important therefore, to define some of these differences and determine how they could alter the response of guinea fowls to helminth infestation. This would help shed more light on how different birds have evolved to cope with helminth infestation. Our recent work has shown that natural-level helminth infestations of clinically healthy village chickens have insignificant effect on the humoral immune responses of the chickens (Saasa *et al.*, 2014). The effect of pathogens on the host modifies the way the host responds to the invasion. The changes usually

affect the immune system from the cellular level and consequently the organ as a whole. This gives rise to changes in the morphology and other parameters in the components of the immune system such as the spleen and lymph nodes.

The spleen is the primary organ for immunity and resistance to diseases and parasitic infestation in birds. The situation in the avian species is in sharp contrast with the main splenic function of erythropoiesis and storage of blood in mammals. Though contradictory results have been published (Brown and Brown, 2002; Shutler *et al.*, 1999), the avian spleen has been frequently used in studies of avian parasitology to infer immune system strength (Smith and Hunt, 2004). It has also been correlated to both ecto- and endo-parasitic infections and studies have been done to relate ecto-parasitism, nutrition and immune organs, spleen and bursa of Fabricius (Blanco *et al.*, 2001). Other studies have shown a correlation between spleen size and helminth burdens that is sex restricted to males only (Figuerola *et al.*, 2005). Seasonal changes in spleen size have also been demonstrated (Moller *et al.*, 2003; Silverin *et al.*, 1999) with some researchers linking this variation to seasonal exposure to parasitism (John, 1994b). A multi-avian species study by Morand and Poulin (2000) also showed that bird species with larger spleens harboured more species of nematodes than those with smaller spleens. A negative correlation between spleen size and helminth burden has been shown in mammals; Vicente *et al.* (2007) showed that in wild red deer, the lungworm *Elaphostrongylus cervi* burden was negatively correlated with spleen size.

Age and sex related differences in spleen weights in parasitised bird have also been shown in poultry where young female partridges harbouring *Cheilosporira gruvelli* have heavier spleens than those that are non-parasitised (Millan *et al.*, 2004). To further exemplify the importance of the spleen, molecular evidence from expressed sequence tags (ESTs) resulted in a number of spleen-related genes being identified in red grouse responding to nematode infection (Webster *et al.*, 2011). Haematological variations related to parasitism have also been documented in chickens with haematological parameters change in *Gallus gallus domesticus* infected with cestode parasites showing lowered haematological values (Aade *et al.*, 2011) and related to immune status. Shutler *et al.* (2012) however, showed little evidence for a relationship between cestodes and body condition in wild geese. Understanding the role of the leucocyte profile and immune organs in host-pathogen interactions can lead to a better understanding of the development of immunity to pathogens.

Very few studies have assessed relationships between avian body weights and helminth burdens. Previous studies have reported a negative effect of helminths on body condition in yellow-legged gulls (*Larus cachinnans*)

(Bosch *et al.*, 2000), roseate spoonbills (*Ajaia ajaja*) (Sepúlveda *et al.*, 1994) and willow ptarmigan species (*Lagopus lagopus*) (Holmstad and Skorping, 1998) all three cited by Shutler *et al.* (1999). The current limited number of studies correlating helminth burdens and body condition make it difficult to assess the generality of relationships between these two factors thus, clearly demonstrating the need for more research, particularly on free-range poultry, that are likely to harbour more helminths.

This study was thus, designed to elucidate the effect of varying helminth burdens and species richness on immune organs as well as haematological profiles in guinea fowls naturally infected with helminths.

MATERIALS AND METHODS

Study area: The present study was conducted in the Namwala District in Southern Province of Zambia. Namwala District, like the rest of Zambia, has a distinct warm, wet rainy season between November and April of the following year. This is followed by a cooler dry season (May-July) and, finally, a hot dry season that precedes the rainy season. Clinically healthy guinea fowls were bought from villages around the Namwala District of the Southern Province of Zambia.

Study design: The study was a cross-sectional study that involved monthly sampling of domestic guinea fowls (*Numida meleagris*) from November 2010 to October 2011 to determine the gastrointestinal helminth fauna.

Sampling and laboratory analysis: A total of 147 domestic guinea fowls were obtained and sampled. The guinea fowls that were bought at monthly intervals, from villages were transported to the University of Zambia, School of Veterinary Medicine for processing. After acclimatization, the birds were subsequently euthanized humanely and processed as described elsewhere (Nalubamba *et al.*, 2010).

Each of the collected birds was weighed and examined macroscopically for any gross lesions. Body condition was determined by the ratio of body mass controlled for structural body size using the tarsus length. The sex of each guinea fowl was positively determined by either cloacal examination of live birds and/or post-mortem gonad examination of euthanized birds.

Necropsy and helminth processing: Necropsy proceeded by dissecting the birds and extracting the entire gastrointestinal tract (GIT). As soon as the GIT was removed from the body cavity, individual sections of the crop, proventriculus, gizzard, small intestines, caecae and colon were tied off with a 0.45 mm diameter nylon ligature to prevent transfer of parasites from one site to the other. Post-mortem for thorough examination of viscera and identification of helminths was carried out.

All the nematode helminths recovered from each GIT section were identified and counted individually to determine the worm burdens. Helminths were identified using the taxonomic keys previously described (Permin and Hansen, 1999; Rahman *et al.*, 2009; Rahman and Manap, 2014; Soulsby, 1982). Briefly, the nematodes were sorted and processed according to their species, then identified using taxonomic keys and enumerated for total worm count per host and species richness/diversity (i.e., the total number of parasite species). Identification was performed using lactophenol wet mounts on cover slip depression slides. For the cestodes, these were not counted but identified as being present or not because their scolices were often absent.

Haematology and differential leucocyte determination:

For the haematology, packed cell volume (PCV) was determined by microhaematocrit method using duplicate blood samples spun at 12,000 g. Haemoglobin was determined using a spectrophotometer at 540 nm using Drabkin's solution. Total plasma protein (TPP) levels were determined by using a refractometer. The differential leucocytes profile was determined from May Grunwald-Giemsa stained thin smears after counting 200 leucocytes under 400 X magnification and identifying individual cells according to Campbell (1995). Erythrocyte indices were calculated using standard formulae.

Spleen measurements: The spleen was removed from carcass and weighed after dissecting connective tissue around the organ. The weight of the organ was recorded in grams. The length and width of each spleen was measured in millimetres with Vernier callipers. The spleen volume was measured as for an ellipsoid using the formula:

$$\text{Spleen volume} = (\pi \times \text{length} \times \text{width} \times \text{width}) / 6$$

Data analysis and statistical analysis: Data was entered and stored in Microsoft Excel[®] (Microsoft Ltd) while Minitab[®] version 14 (Minitab Inc., Pennsylvania, USA) was used for statistical analysis and graphing. Data was checked for normality using the Ryan-Joiner test. Pearson's correlation coefficient was used to determine correlation between variables. Differences were considered significant when $p \leq 0.05$ at 95% confidence interval.

RESULTS

All the guinea fowls in this study were infested with at least one gastrointestinal helminth and the mean intensity of infection for nematodes was 113.7 worms per host and helminth species richness ranged from 1 to 7 with an average of 4.17 species per host. The helminths species identified were; *Raillietina*

echinobothrida, *R. tetragona*, *R. cesticillus*, *Ascaridia galli*, *Allodapa sucturia*, *Gongylonema ingluvicola*, *Tetrameres* spp, *Heterakis* spp, *Acuarria spiralis*, *Syngamus trachea* and *Streptocara pectinifera*. There was no significant association between helminth species richness and total worms per host ($r = 0.083$, $p = 0.322$). There was also no significant association ($p < 0.05$) between individual helminth species burden and spleen morphometrics or individual helminths species burden and haematological indices.

There was a positive significant correlation between spleen mass and spleen volume ($r = 0.905$, $p < 0.001$) but there was no significant association between body weight and spleen volume ($r = -0.062$, $p = 0.458$) or spleen mass ($r = 0.071$, $p = 0.395$) (Table 1 and Fig. 1). Both overall spleen weight and spleen volume were positively associated with species richness. This association was mildly positive in both male and female guinea fowl (Table 1). There was however, no association between total worm counts per host and overall and sex segregated spleen volume and spleen weight. There was a positive association between body weight and total worm count per host in both male and female guinea fowl (Overall- $r = 0.419$, $p = 0.000$), Male- $r = 0.366$, $p = 0.001$), Female- $r = 0.310$, $p = 0.009$) but no association between body weight and species richness (Overall- $r = -0.069$, $p = 0.410$).

Furthermore, no significant association was established between the overall haematological indices, white blood cell count (WBC), heterophil/lymphocyte (H/L) ratios and TPP with either total helminth burden per host or species richness (Table 1 and Fig. 2). There was however, a mild negative association between species richness and PCV ($r = -0.240$, $p = 0.004$) in both male ($r = -0.280$, $p = 0.016$) and female ($r = -0.245$, $p = 0.041$) guinea fowl and a positive association between total worm count and PCV and RBC count in only the male guinea fowl (PCV $r = 0.309$, $p = 0.007$, RBC $r = 0.398$, $p = 0.002$).

DISCUSSION

The clinical manifestation of helminthic infections range from asymptomatic to severe disease condition and during these clinical phases of disease, there are subtle to severe changes that take place in the body which can only be measured by looking at the various possible changes that could affect the cellular components or if severe the entire organ of the host organism. In the present study, the effects of helminths burden were evaluated based on the correlation with haematological indices, body weight and spleen morphometric data (weight and volume).

We found no significant association between the haematological indices, total white blood cell counts, H/L ratios and total plasma protein with either total helminth burden per host or species richness. There was however, a mild negative association between the

Table 1: Pearson's correlation coefficients of species richness and total worm counts per host and selected morphometric and haematological indices in domestic guineafowl (*Nuntdia meleagrts*) in Zambia (Overall and by sex)

Variable	Species Richness (Pearson's r, p value)			Total worm count per host (Pearson's r, ps value)		
	Overall	Male	Female	Overall	Male	Female
Spleen wt (g)	(r = 0.199, P = 0.016)**	(r = 0.318, P = 0.005)**	(r = -0.004, P = 0.971)	(r = -0.058, P = 0.488)	(r = -0.009, P = 0.938)	(r = -0.084, P = 0.487)
Spleen volume (cm ³)	(r = 0.172, P = 0.038)**	(r = 0.279, P = 0.015)**	(r = 0.001, P = 0.995)	(r = -0.035, P = 0.670)	(r = 0.017, P = 0.882)	(r = -0.038, P = 0.752)
Body wt. (kg)	(r = -0.069, P = 0.410)	(r = -0.180, P = 0.122)	(r = -0.016, P = 0.897)	(r = 0.419, P = 0.000)**	(r = 0.366, P = 0.001)**	(r = 0.310, P = 0.009)**
Corrected body wt.	(r = -0.042, P = 0.618)	(r = -0.063, P = 0.594)	(r = -0.044, P = 0.718)	(r = 0.412, P = 0.000)**	(r = 0.271, P = 0.018)**	(r = 0.291, P = 0.014)**
Spleen % of body wt.	(r = 0.208, P = 0.012)**	(r = 0.328, P = 0.004)**	(r = -0.004, P = 0.972)	(r = -0.118, P = 0.154)	(r = -0.047, P = 0.688)	(r = -0.139, P = 0.248)
WBCs (x 10 ⁹ /L)	(r = 0.059, P = 0.491)	(r = 0.159, P = 0.180)	(r = -0.061, P = 0.625)	(r = -0.038, P = 0.653)	(r = -0.157, P = 0.183)	(r = -0.010, P = 0.939)
PCV (%)	(r = -0.240, P = 0.004)**	(r = -0.280, P = 0.016)**	(r = -0.245, P = 0.041)**	(r = -0.090, P = 0.283)	(r = 0.308, P = 0.007)**	(r = -0.075, P = 0.536)
TPP (g/dl)	(r = -0.116, P = 0.169)	(r = -0.246, P = 0.036)	(r = -0.013, P = 0.914)	(r = 0.060, P = 0.477)	(r = 0.130, P = 0.269)	(r = -0.113, P = 0.347)
RBCs (x 10 ¹² /L)	(r = -0.176, P = 0.072)	(r = -0.150, P = 0.264)	(r = -0.245, P = 0.093)	(r = 0.021, P = 0.830)	(r = 0.398, P = 0.002)**	(r = -0.052, P = 0.725)
Hb (g/dL)	(r = -0.202, P = 0.060)	(r = -0.134, P = 0.364)	(r = -0.493, P = 0.001)**	(r = -0.068, P = 0.530)	(r = 0.238, P = 0.100)	(r = -0.088, P = 0.587)
H/L ratio	(r = -0.22, P = 0.260)	(r = -0.310, P = 0.16)	(r = -0.215, P = 0.34)	(r = 0.052, P = 0.413)	(r = 0.063, P = 0.604)	(r = 0.039, P = 0.688)

Packed cell volume (PCV), Total plasma protein (TPP), heterophil/lymphocyte (H/L), Haemoglobin (Hb), weight (wt.), Red blood cells (RBCs), White blood cells (WBCs). **Significant Association (p = 0.05)

PCV and species richness in both male and female guinea fowl. Despite, there being no overall association between total worm counts per host and PCV, there was a paradoxical positive association between these two factors in the male guinea fowl. This can be speculated to be attributed to the documented higher PCV seen in male guinea fowl (Nalubamba *et al.*, 2010), especially the large breeding males. These are the same ones that this study has also shown to harbour more worms per host. The effect of worms on the haematological parameters has been used to evaluate the effects of the infestation of the host. However, studies have shown that a heavy helminth burden does not have much effect probably due to the ability of the host to balance the trade-offs between nutritional requirements and losses through helminth infestation (Cripps *et al.*, 2014; Moore and Hopkins, 2009). This study did not demonstrate any association between H/L ratios and helminth burdens and species richness. Though H/L ratios are a measure of immune stress, the wide variation of leucocyte numbers and profiles that has been shown in guinea fowl (Nalubamba *et al.*, 2010), would likely affect the possibility of significant differences in this parameter at varying levels of helminth infestation where study populations are small.

In this study there was no significant association between body weight and either spleen mass or spleen volume. This finding is in contrast with findings by Morand and Poulin (2000) in a multi-avian species study. In addition, there was no significant correlation between individual helminth species burden and spleen morphometric or individual helminths species burden and haematological indices. Furthermore, we found no significant association between body weight and spleen volume or body weight and spleen mass. However, there was a positive significant correlation between spleen mass and spleen volume. It is expected that the cost of helminths on the host's immune system is likely to be affected by the species richness and therefore, affect spleen morphometrics. However, the lack of a fixed spleen size under various physiological situations can affect the relationship of the organ during helminth infestation (Crivellato *et al.*, 2004).

Although more interest has been placed on understanding the role of the spleen in avian immunology, the comprehensive function of avian spleen is poorly understood (John, 1994a; John, 1995; Smith and Hunt, 2004). In domestic poultry, most of the work on the spleen has also only been done on domestic chickens (*Gallus gallus domesticus*) (Davison, 2008; Smith and Hunt, 2004). Similar to findings by Shutler *et al.* (1999) in geese, this study did not find any significant correlation between helminth burden and spleen morphometrics. Mallory *et al.* (2007) also showed that neither contaminant burden nor helminth parasites affected the spleens of Northern

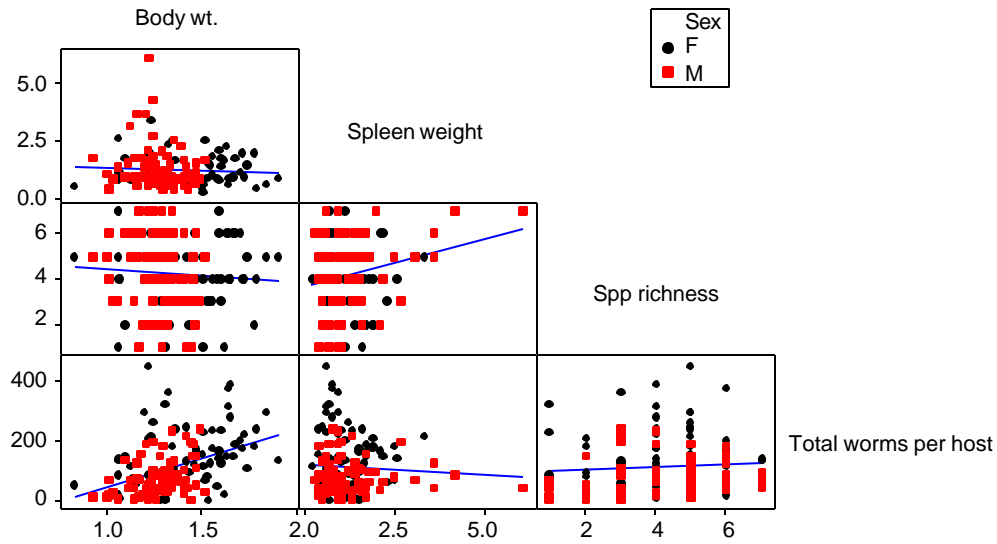


Fig. 1: Matrix Plot with regression line of Body Weight (wt.), Spleen weight, Species (Spp) Richness and Total worm count per host in domestic guinea fowl (*Numida meleagris*) in Zambia

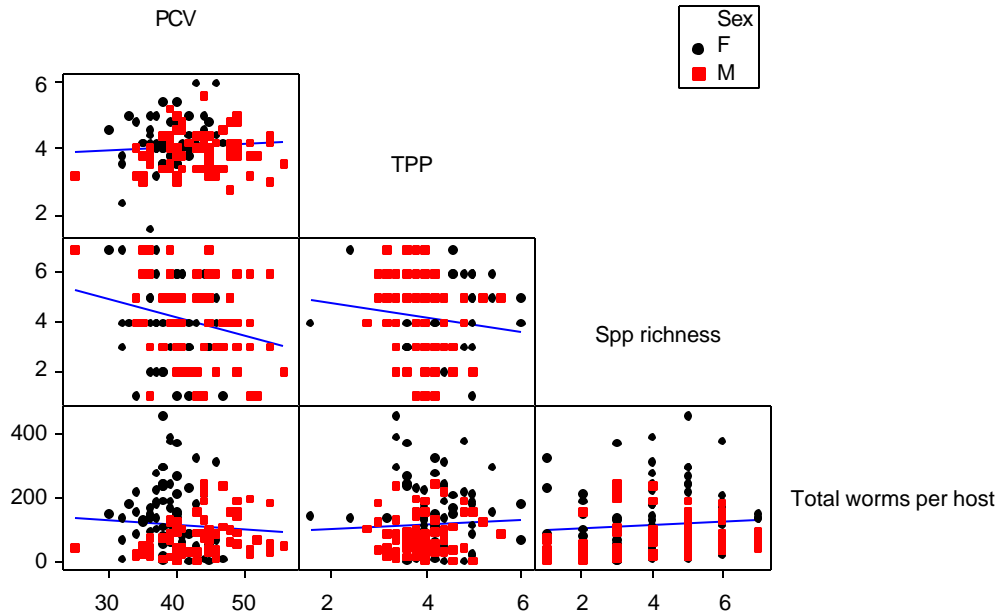


Fig. 2: Matrix Plot with regression line of Packed cell volume (PCV), Total plasma protein (TPP), Species (Spp) Richness and Total worm count per host in domestic guinea fowl (*Numida meleagris*) in Zambia

Fulmars (*Fulmarus glacialis*) from the Canadian high Arctic. Shutler *et al.* (1999) further showed that when uninfected individuals were removed from the analysis, the spleen size did not correlate with helminth burdens. Similarly, Robinson *et al.* (2008) did not find any sex bias or helminth load correlation with the spleen morphometrics in cormorants (*Phalacrocorax auritus*).

This study did not show any significant negative correlation between helminth burdens and species richness with either haematological or body weights in both male and female guinea fowl. This is in contrast with findings by Figuerola *et al.* (2005) who found a significant interaction between body condition and nematode abundance in male greylag geese but not in females. However, this study demonstrated a para-

oxical positive association between body weight and total worm count per host. This can only be possibly explained that larger hosts harbour more GI helminths because they eat more and thus more likely to ingest more infective stages of GI helminths. Morand and Poulin (2000) also found an association between body weight and nematode species richness in a multi-avian species study. Another research group did not find any differences in immune responses between dewormed raptures and those that were not (Hanssen *et al.*, 2013). This would further strengthen the argument that at natural infestation levels, helminths do not have any effect on immune responses in the avian species and thus immune organ morphometrics and immune cell profiles are expected to be similar. Despite the varying levels of helminth burdens in the guinea fowl in this study, all haematological values were within normal reference values.

Species richness has been considered a more stable measure of parasitism than prevalence (Morand and Poulin, 2000). Species richness was not associated with total helminth burden in the guinea fowl in this study. Even when segregated for sex, the female guinea fowl did not show any significant correlation between parasitism and helminth burden despite other studies showing this relationship in juvenile partridges (Millan *et al.*, 2004). Species richness was however, more likely to be associated with PCV and spleen volume in both male and female guinea fowl than the total worm count per host in our study. The findings from this study partly agree with the findings of Figuerola *et al.* (2005) who found that there was a positive association of helminth burden and spleen size in males only.

In order to further understand the interaction between helminths and various physiological parameters and indices of health, a study that can be performed is looking at parasitically naive young fowl and those that are trickle infected with nematode infective larvae (single and/or mixed infections) compared with un-infected controls. Their haematological findings as well as their immune organ morphometrics over the infection course until full establishment of the helminth infestation with overt clinical disease can then be followed and compared over time. Studying haematological findings as well as their immune organ morphometrics in adult guinea fowl that are naturally infested with helminths compared with those that have been dewormed can also be performed as a complimentary study to further elucidate the interaction between helminths and the aforementioned health indices. An immune response genes, functional genomics study would further contribute towards mapping the immune response at a molecular level. It is likely that age could be an important factor influencing the response of guinea fowls to helminth infestation as previously determined (Magwisha *et al.*, 2002). Since most of the birds were adults, at this age an optimal balance could have been

reached resulting in undetectable changes on both organ morphometric and other blood parameters in response to existing helminth infestations.

Thus, this study expands the hitherto limited work on the avian spleen in another domestic poultry species, the guinea fowl. It shows that in guinea fowl that are naturally infected with GIT helminths, there is minimal interaction between helminth burdens, helminth species richness and body condition, spleen morphometrics or haematological findings. This study further stimulates research questions into possible research areas that once undertaken will contribute towards a greater understanding of avian immuno-parasitology.

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 studies or patient data.

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REFERENCES

- Aade, U., H. Wankhede and K. Kaldate, 2011. Haematological parameters change in *Gallus gallus domesticus* infected with *Cotugnia digonopora*. Recent Res. Sci. Technol., 3: 16.
- Blanco, G.U.I.L., J.D.L. Puente, M.E.R.C. Corroto, A.R.T.U. Baz and J.O.R.D. Colus, 2001. Condition-dependent immune defence in the Magpie: how important is ectoparasitism? Biol. J. Linn. Soc. Lond, 72: 279-286.
- Bordes, F. and S. Morand, 2009. Parasite diversity: an overlooked metric of parasite pressures? *Oikos*, 118: 801-806.
- Brown, C.R. and M.B. Brown, 2002. Spleen volume varies with colony size and parasite load in a colonial bird, Proc. R. Soc. Lond B. Biol. Sci., 269: 1367-1373.
- Campbell, T.W., 1995. Descriptions of abnormal leukocytes in avian peripheral blood, in Avian Haematology and Cytology, 2nd edn, Iowa State University Press, pp: 3-29.
- Cripps, J., I. Beveridge, R. Ploeg and G. Coulson, 2014. Experimental manipulation reveals few subclinical impacts of a parasite community in juvenile kangaroos. Int. J. Parasitol. Parasites Wildl, 3: 88-94.
- Crivellato, E., A. Vacca and D. Ribatti, 2004. Setting the stage: an anatomist's view of the immune system, Trends Immunol., 25: 210-217.

- Davison, F., 2008. The Importance of the Avian Immune System and its Unique Features, in Avian Immunology, D. Fred, K. Bernd and A.S. Karel, eds., Academic Press, London, pp: 1-11.
- Durai, P.C., T. Muruthai, S. Arumugam and O. Venugopa, 2012. Haematological profile and erythrocyte indices in different breeds of poultry. Int. J. Livest. Res., 2: 89-92.
- Figuerola, J., J. Torres, J. Garrido, A.J. Green and J.J. Negro, 2005. Do carotenoids and spleen size vary with helminth load in greylag geese? Can. J. Zool., 83: 389-395.
- Hanssen, S.A., J.O. Bustnes, L. Schnug, S. Bourgeon, T.V. Johnsen, M. Ballesteros, C. Sonne, D. Herzke, I. Eulaers, V.L.B. Jaspers, A. Covaci, M. Eens, D.J. Halley, T. Moum, R.A. Ims and K.E. Erikstad, 2013. Antiparasite treatments reduce humoral immunity and impact oxidative status in raptor nestlings, Ecol. Evol., 3: 5157-5166.
- Hoste, H., 2001. Adaptive physiological processes in the host during gastrointestinal parasitism. Int. J. Parasitol., 31: 231-244.
- John, J.L., 1994a. Nematodes and the spleen: An immunological relationship. Cell Mol. Life Sci., 50: 15-22.
- John, J.L., 1995. Parasites and the avian spleen: helminths. Biol. J. Linn. Soc., 54: 87-106.
- John, J.L., 1994b. The Avian Spleen: A Neglected Organ. Q Rev. Biol., 69: 327-351.
- Magwisha, H.B., A.A. Kassuku, N.C. Kyvsgaard and A. Permin, 2002. A Comparison of the Prevalence and Burdens of Helminth Infections in Growers and Adult Free-Range Chickens. Trop. Anim. Health Prod., 34: 205-214.
- Mallory, M.L., J.D. McLaughlin and M.R. Forbes, 2007. Breeding status, contaminant burden and helminth parasites of Northern Fulmars *Fulmarus glacialis* from the Canadian high Arctic. Ibis, 149: 338-344.
- Millan, J., C. Gortazar and R. Villafuerte, 2004. Ecology of nematode parasitism in red-legged partridges (*Alectoris rufa*) in Spain. Helminthologia, 41: 33-37.
- Moller, A.P., J. Erritzoe and N. Saino, 2003. Seasonal Changes in Immune Response and Parasite Impact on Hosts. Am. Nat., 161: 657-671.
- Moore, I.T. and W.A. Hopkins, 2009. Interactions and trade-offs among physiological determinants of performance and reproductive success. Integr. Comp. Biol., 49: 441-451.
- Morand, S. and R. Poulin, 2000. Nematode parasite species richness and evolution of spleen size in birds. Can. J. Zool., 78: 1356-1360.
- Nalubamba, K.S., N.B. Mudenda and M. Masuku, 2010. Indices of Health; Clinical Haematology and body weights of free-range Guinea Fowl (*Numida meleagris*) from Southern Province of Zambia. Int. J. Poult. Sci., 9: 1083-1086.
- Permin, A. and J. Hansen, 1999. The epidemiology, Diagnosis and Control of Parasites in Poultry FAO, Rome.
- Rahman, A.W., H. Salim and M.S. Ghouse, 2009. Helminthic Parasites of Scavenging Chickens (*Gallus domesticus*) from villages in Penang Island, Malaysia. Trop. Life Sci. Res., 20: 1-6.
- Rahman, W.A. and N.H. Manap, 2014. Description on the morphology of some nematodes of the Malaysian domestic chicken (*Gallus domesticus*) using scanning electron microscopy. Malaysian J. Vet. Res., 5: 35-42.
- Robinson, S.A., M.R. Forbes, C.E. Hebert and J. Daniel McLaughlin, 2008. Male-biased parasitism by common helminths is not explained by sex differences in body size or spleen mass of breeding cormorants *Phalacrocorax auritus*. J. Avian Biol., 39: 272-276.
- Saasa, N., J. Siwila, E. M'kandawire and K.S. Nalubamba, 2014. Antibody response in non-dewormed and dewormed village chickens to sheep red blood cells. Int. J. Poult. Sci., 13: 353-356.
- Shutler, D., R.T. Alisauskas and Daniel J. McLaughlin, 2012. Associations between body composition and helminths of lesser snow geese during winter and spring migration. Int. J. Parasitol., 42: 755-760.
- Shutler, D., R.T. Alisauskas and J.D. McLaughlin, 1999. Mass dynamics of the spleen and other organs in geese: measures of immune relationships to helminths? Can. J. Zool., 77: 351-359.
- Silverin, B., R. Fnnge, P.A. Viebke and J. Westin, 1999. Seasonal Changes in Mass and Histology of the Spleen in Willow Tits *Parus montanus*. J. Avian Biol., 30: 255-262.
- Simarak, S., O. Chinrasri and W. Aengwanich, 2004. Hematological, electrolyte and serum biochemical values of the Thai indigenous chickens (*Gallus domesticus*) in northeastern, Thailand. Songklanakarin. J. Sci. Technol., 26: 425-430.
- Smith, K.G. and J.L. Hunt, 2004. On the use of spleen mass as a measure of avian immune system strength. *Oecologia*, 138: 28-31.
- Soulsby, E., 1982. Helminths, Arthropods and Protozoa of Domesticated Animals Bailliere Tindall, London.
- Vicente, J., L. Perez-Rodriguez and C. Gortazar, 2007. Sex, age, spleen size and kidney fat of red deer relative to infection intensities of the lungworm *Elaphostrongylus cervi*. Naturwissenschaften, 94: 581-587.
- Webster, L.M.I., L.V. Mello, F. Mougeot, J. Martinez-Padilla, S. Paterson and S.B. Piertney, 2011. Identification of genes responding to nematode infection in red grouse. Mol. Ecol. Resour., 11: 305-313.