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Investigation of the anti-body Concentration level Against *H. gallinarum* in two Local Chicken Genotypes in two Seasons From two Agro- Ecological Zones in Sudan

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Abstract

The study was aimed to determine the antibodies concentration level against *Heterakis gallinarum* of two genotypes of Sudanese local chickens (*n*=157), that raised under free-range laying hens system, and originated from two different agro-climatic zones in central and east of Sudan, namely Khartoum and Gadarif zones in wet and dry seasons where in each zone five villages were randomly considered. The plasma samples were obtained individually from the two genotypes Large Baladi (LB) (n=80) and Bare-neck (BN) (n=77), to measure antibodies concentration level via the enzyme linked immunoglobulin assay (ELISA) based on developed *Ascaridia galli* antigens. Worm burden was determined by worm count after slaughtering. Reliable and specific determination methods of the exposure of the chickens to *H. gallinarum* determine cutoff point (antibody titre = 71.09) between known infected and non-infected chickens. Receiver-operating characteristic (ROC) curve revealed an area under the curve (AUC) was 0.823±0.04, with an estimated sensitivity and specificity of the assay were 55% and 100%, respectively. Regarding the antibodies level of *H.gallinarum,* the variation in Khartoum and Gadarif States was (0.08), and (0.64) respectively between the two genotypes of chicken with significant variance (0.04) between the dry and wet seasons. Also the significant differences ($p<0.05$) between interaction of regions and seasons and between regions and genotype were observed with 0.20 and 0.30 respectively. The correlation between the antibody titre and worm burden showed no significant association between them (r= 0.034, P= 0. 701). We conclude that ELISA serves as a good and effective diagnostic tool, also the antibodies developed against *A. galli* can be used to identify *H. gallinarum* in infected chickens.

Keywords: *Heterakis gallinarum,* Antibody, Free-range, ROC analysis.

Introduction

Poultry production in most tropical countries is based mainly on scavenging production systems. It has been estimated that 80% of the total poultry population in Africa continent kept under free-range production systems and exposed to a wide variety of pathogens (Gueye, 2000). The mortality has been reported to be as high as 80-90% within the first year after hatching (Permin *et al*., 1997). The high mortality in indigenous chickens is believed to be caused by diseases, predators, malnutrition

and poor management including lack of good advisors.

Poultry production in Sudan is mainly based on the scavenging system in rural areas, and keeping plays a vital role in poor alleviation, improve the nutritional status and provide an important source of income to the smallholders farmers. The Sudanese indigenous chicken genotypes were classified into three types as described by Desai (1962).Control of infectious disease is essential to optimize the poultry production, achieve and enhance the animal welfare. The free-range poultry flocks face pressure from different pathogens including viruses, bacteria, parasites and others.

Helminth infections in free-range chickens are abundant even when they occur in low numbers, and may result in subclinical disease or can act as vectors for other disease infections because of their free access to outdoor areas e.g. *Escherichia coli* (Permin *et al.*, 2006).

Nematodes are considered the most important group of poultry helminthes, both in terms of number of species and pathology in poultry and birds (Ruff and Norton, 1997). *H.gallinarum* parasite does not affect the health of the bird seriously and has no marked symptoms or pathology that can be responsible for its presence, nevertheless inflammation and thickening of the cecum can be due to heavy infections (Taylor *et al*., 2007). *H.gallinarum* acts as transmitter for *Histomonas meleagridis*, a protozoan parasite that affects chicken, turkey, quails, pheasants and is the causative agent of histomoniasis or blackhead disease. This protozoan parasite is carried in the caecal worm egg and may remain viable for years in suitable conditions and transmitted from bird to bird through this egg which has a high morbidity and mortality in turkeys**.** The worm has direct life cycle and no intermediate host is needed, resembles other common roundworm (McDougald, 2005). The embryonated eggs are produced in the ceca containing infective L3-larvae, pass in the dropping of infected birds and then ingest by contaminated feed, water, or feces. *H.gallinarum* larvae hatch in the upper intestine the infective form in about two weeks. In cool weather, this may take longer. Eggs are very resistant to environmental conditions and will remain viable for long periods.

In the indigenous chicken the natural antibody levels are higher than other exotic and cross breeds in different ages which explain the ability of the indigenous chickens to survive disease pressure in the village poultry production systems (Wondmeneh *et al*., 2015).

The decrease of the immune response and synthesis of immunoglobulin might happen due to the loss of protein caused by parasitism (Tizard, 1996). Parasites control will results in better immune response and improve efficiency of vaccine against diseases e.g. Newcastle vaccination and reduction of stress caused by these parasites (Kemboi *et al.*, 2014).

Immune responses are affected by many factors prior exposure by the host such as: the stage of parasite development, the nutritional status of infected chickens, and the genetic character of the host (Yun *et al*., 2000) as the previous studies also stated that the strength of the immune response and the effect of the interaction of a host with a parasite are influenced by genetic and phenotypic characteristics, as well as by environmental variables (Colditz, 2008). Moreover, seasonal changes are one of the factors that affect the immune function during impact parasites on the breeding seasons of the host (Møller *et al*., 2003).

There are many studies indicated that there was a resistance variation between genotypes to gastrointestinal nematodes in various species of birds (Gauly *et al*., 2002; Kaufmann *et al*., 2011; Wongrak *et al*., 2015). In the other hand the resistance of some breeds can be explained by their location origin where the climate affects

the growth of gastrointestinal nematode larvae in the environment, also the indigenous genotypes that have prospered despite unfavorable environmental conditions which are more resistant than highly productive genotypes (Alba-Hurtado *et al*., 2010).

Material and Methods

Samples source

The study was carried out in different villages of two selected sites, representing different agro-climatic regions in central (Khartoum) and east (Gadarif) of Sudan. Khartoum lies between latitude 15°31'N and longitude 32°45'E, in the desert and semi desert climatic region. The annual rainfall is about 162.8 mm, and the relative humidity ranges 7%-17% in dry summer season and 27%-51% in the wet summer season, while the average temperature in dry summer range is 32 -41°C and the range in the wet season is 20-33°C. Gadarif lies between latitude 14°02'N and longitude 35°28'E in the semi-arid climatic zone. The annual rainfall is about 800.8 mm, and the relative humidity ranges between 23%-48% in the dry season and 65%-66% in the rainfall seasons, and the minimum and maximum temperature in the summer season is 23.5-41°C and the wet season is 21-34°C.

Birds

A total of 157 plasma samples were obtained individually from two genotypes of local chickens including: Large Baladi (LB) and Bare-Neck (BN) chickens which were bought directly from the farmers or from the open markets from two states (Khartoum state, N=95 and Gadarif state ,N=62).

Enzyme Linked Immunosorbent Assay (ELISA) and parasitic examination

The blood was collected during chicken slaughtering (neck bleeding) in 5 ml tubes with heparin, the plasma was separated by centrifugation at 13.000 rpm for 10 min, and frozen at -20 °C until serological evaluation. Antibodies level against *H.gallinarum* were measured by an enzyme-conjugated secondary antibody against chicken IgY as described by (Hennis *et al.*, 2013).The samples were thawed at room temperature (RT). The plasma was diluted in test buffer 1:2500 in two dilution steps. 100 µl from the diluted samples and standards were pipette into the wells of the microtiter plates (96 well flat-bottomed microtitre antigen coated plates). Then the plates were wrapped and incubated at room temperature. After 90 min of incubation, the plates rinsed using diluted wash solution by using a plate washer machine (TECAN) in order to remove unbound materials. Then 100 µl peroxidase-conjugated secondary antibody against chicken IgG (IgY) was added, then wrapped and incubated for 30 minutes on shaker machine at room temperature. After further washing steps, 100 µl (TMB) substrate (indicator dye) was added and incubated for 20 min under subdued light, inducing the development of the blue dye in the wells. The color development was terminated by the addition of 100 µl of the stop solution (1M hydrochloric acid). The optical density of the reacted solution was measured at 450 nm using ELISA reader with the reference wavelength at 650 nm wavelength (TECAN, Austria GmbH).

Worm burden:- All chickens were necropsied and their gastrointestinal tracts were examined following the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) guidelines. The gastrointestinal tracts were separated into esophagus, crop, proventriculus, gizzard, small intestine and caecum. Each part was opened in longitudinal section with scissors. The contents were washed separately under running tap water followed by scraping the mucosa of intestine and emptied into labeled petri-dishes using a sieve with a mesh aperture of 100 µm. *H. gallinarum* was identified and counted under stereomicroscope according to the procedure described by Permin and Hansen (1998).

Statistical analysis

The generated data was analyzed using three way ANOVA. The evaluation of ELISA was done using Receiver Operating Characteristic (ROC) curve and the area under the curve and the cut-off point were determined together with the sensitivity and specificity. The relationship between the antibody titre and the worm burden was determined by Pearson correlation analysis. SPSS software was used in data analysis.

Results

From the total of 157 birds were examined using IgG-ELISA protocol to detect antibodies against *H.gallinarum* there was no significant variation due to the region, bird's genotype and season. With the exception of the interaction between regions and seasons (P= 0.004). The correlation between the antibody titre and worm burden showed no significant association between them (*r*= 0.034, *P*= 0. 701) (Table, 1).

Table, 1: The effect of region, genotype and season on worm burden and antibody level of H. gallinarum

	N	Worm burden	Antibodies level
Overall mean	157	50.4	105.643
Regions			
Khartoum	95	59.7±1.7	92.1±74.6
Gadarif	62	32.9±1.4	104.4±85.6
P-value		0.018	0.073
Genotypes			
Large Baladi	80	48.9±1.6	107.99±76.4
Bare-neck	77	43.9±1.5	103.29±73.6
P-value		0.670	0.755
Seasons			
Dry summer	100	57.7±1.6	96.65±72.8
Wet summer	57	35.1±1.4	114.63±78.4
P-value		0.047	0.233
P (regions X genotype)		0.650	0.127
P (regions X seasons)		0.138	0.046
P (genotypes X seasons)		0.180	0.797
P(regions X genotypes X seasons)		0.607	0.298

Discussion

The present study was conducted with the objectives of evaluating ELISA in diagnosing *H.gallinarum and* seeking association between plasma antibody levels and burden of *H.gallinarum* in free-ranging local chickens in Sudan from different geographical regions in wet and dry seasons. The results showed no correlation between *H.gallinarum* antibody levels in plasma and total worm burden in the chickens.

The presence of the chickens that are freely roaming in different environments provide favorable condition of helminthes. However, the specific antibodies in plasma demonstrate previous parasite exposure, but it may not reflect the current infection status of the individual as antibodies can persist for a long time and a recent infection may not yet have induced the specific antibody response that is being measured.

The immunological changes and infections could be reversed by reverting to malnutrition (Chandra and Newberne, 1977). The association between the total worms burden and antibodies level were non-significantly different and that maybe refer to the same feed supplements gave to the chickens in both zones and cereals were the most dominant feed supplements as reported by Khalafallah *et al*., 2000).

Enzyme-linked immunosorbent assay (ELISA) is a diagnostic tool carried out commonly in parasitological studies to detect antibodies or antigens related to a specific parasite.

The different genotype is not differed significantly to association of worms and IgG levels between them which is counteractive finding from Gauly *et al*. (2002).

In general as discussed previously the antigen used was extracted from the *A.galli* parasite and possibly the ELISA could not detect the plasma antibodies or maybe due to cross reactivity with other genera of helminthes in the samples. However, the interpretation of the results from this study must be made with caution as these data were collected from a limited number of birds and statistical significance was difficult to achieve in this regard.

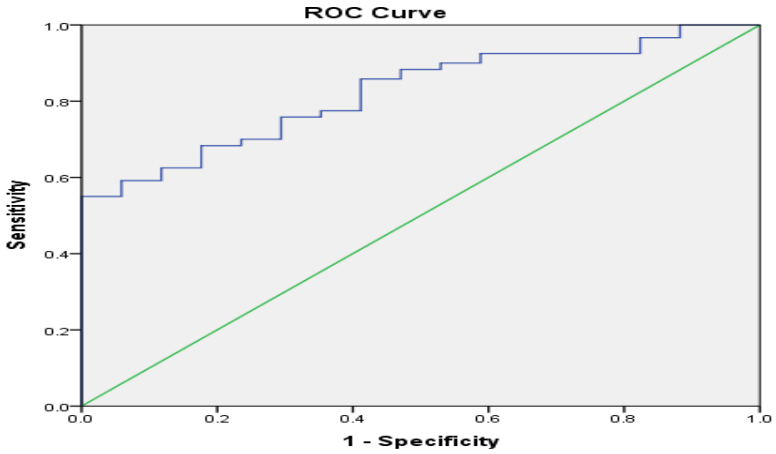

Figure (1): ROC analysis

Receiver-operating characteristic curve (ROC) analysis indicated an area under the curve (AUC) was 0.823±0.04, with an estimated sensitivity and specificity of assay were 55% and 100%, respectively.

Conclusion

The prepared IgY antibodies antigen against *A. galli* is allow to identify *H. gallinarum* infections

Finally, the immune response and the associated resistance can be modified by the type of antigen.

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التحقق من تركيز الاجسام المضادة ضد ديدان في طرزين محليين من الدجاج في موسمين ببيئتين زراعيتين بالسودان

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المستخلص

أجريت هذه الدراسة علي نوعين من الدجاج البلدي السوداني وهي تهدف إلى تحديد مستوى تركيز الأجسام المضادة ضد ديدان *Heterakis gallinarum* ، والتي نشأت في ظل نظام المرعي الحر، في منطقتين مختلفتين من حيث المناخ في وسط وشرق السودان وتحديداً منطقتي الخرطوم والقضارف في المواسم الرطبة والجافة حيث تم دراسة خمس قرى في كل منطقة بشكل عشوائي. تم الحصول على عينات البلازما بشكل فردي من كلا النوعين من الدجاج: البلدي الكبير (ن = 80) و عاري الرقبة (ن = 77) ، لقياس مستوى تركيز الأجسام المضادة عبر فحص المقايسة الأمتصاصية المناعية للانزيم المرتبط (ELISA) حيث تم استخدام انتجين دودة *Ascaridia galli*. تم تحديد كثافة وجود الدودة من خلال عدد الديدان بعد الذبح. تم تحديد النقطة الفاصلة لتعرض الدجاج لديدان (71.09) بين الدجاج المصاب وغير المصاب.

منحنى (ROC) أن منطقة تحت المنحنى (AUC) كانت 0.823 ± 0.04، مع حساسية وخصوصية تقديرية للمقايسة 55٪ و 100٪ على التوالي. فيما يتعلق بمستوى الأجسام المضادة لـ *H.gallinarum* ، كان التباين في ولايتي الخرطوم والقضارف (0.08)، و (0.64) علي التوالي بين كلا النوعين للدجاج مع تباين معنوي (0.04) بين الموسمين الجاف والرطب وكذلك الفروق المعنوية ($p<0.05$) بين المناطق والفصول الموسمية وبين المناطق وسلالتي الدجاج كانت 0.20 و 0.30 علي التوالي. لا يوجد أي ارتباط معنوي بين معيار الاجسام المضادة وكثافة تواجد الدودة ($r = 0.034$، $P = 0.701$). دلت نتائج هذة الدراسة أن أختبار ELISA جيد وفعال كأداة تشخيصية، كما يمكن استخدام الأجسام المضادة التي تم تطويرها ضد *A. galli* لتحديد *H. gallinarum* في الدجاج المصاب.