Pak. J. Agri. Sci., Vol. 52(3), 815-820; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

DESCRIPTIVE EPIDEMIOLOGY OF ENDO-PARASITIC FAUNA IN LAYER BIRDS (*Gallus domesticus*) OF CENTRAL PUNJAB, PAKISTAN

Tabassam Fatima[1], Muhammad Sohail Sajid[1,*], Muhammad Kashif Saleemi[2], Zafar Iqbal[1] and Rao Muhammad Siddique[1]

[1]Department of Parasitology, University of Agriculture, Faisalabad-38040, Pakistan; [2]Department of Pathology, University of Agriculture, Faisalabad-38040, Pakistan.
[*]Corresponding author's e-mail: drsohailuaf@hotmail.com

This paper describes a period prevalence and associated determinants of gastrointestinal (GI) parasites in layer (*Gallus domesticus*) population of district Faisalabad during March 2012 to February 2013. The live and/or dead birds (n=1996) brought to the central diagnostic facility of the Faculty of Veterinary Science and those screened from peri-urban regions, were screened for GI parasitic fauna through standard parasitological protocols. Relevant information regarding associated risk factors was recorded on a pre-designed closed-ended questionnaire. Overall prevalence of GI helminths and protozoa was recorded as 26.05% and 11.32% respectively. The prevalence was highest in the laying birds followed in order by brooding and growing birds. Commercially raised birds, conventional housing system, manual feeding and watering systems, crumbed-feed and soiled floor were found having positive statistical association with the GI parasitism in layer birds of the study area. This data will not only be helpful for the small holder poultry farmers to modulate their farming practices but also for the policy and decision makers to implement strategies which can minimize the risk of GI parasitism in commercial as well as backyard poultry rearing systems.
Keywords: Epidemiology, endoparasites, layer, *Gallus domesticus*, Pakistan.

INTRODUCTION

During the last few decades, poultry production has become an important sector of the livestock economy worldwide (Watt, 1996). In Pakistan, every rural family and almost every 5th urban family is associated with the poultry business (Anwar *et al*., 1991). A recent survey describes that about 66% of the Pakistan natives are protein deficient in their diets (Abedullah *et al*., 2007). Under this situation, ever increasing poultry production is encouraged to meet the daily protein requirements especially of the developing countries, in terms of eggs and meat (Nazir *et al*., 2014).

In Pakistan, poultry production is augmenting at an annual rate of 20-25%, in which a major share of 61% of the country's production is contributed by the Punjab province.

Gastrointestinal (GI) parasitism (caused by helminthes and/or protozoa) is a potential threat in layers (*Gallus domesticus*) causing huge economic losses in terms of impeded growth, reduced weight gain, lowered egg production (Anwar *et al*., 1991), loss of meat production, cost of labor, equipment, control and treatment. Clinical picture of GI parasitism includes: loose droppings, intestinal obstruction (heavy worm burden), anaemia, lowered Hb concentration, cachexia and nervous manifestations. Necropsy examination of infected birds reveal nodular, haemorrhagic and ulcerative enteritis, atrophy of villi, formation of granulomas in the duodenum and desquamation of epithelial cells (Shah *et al*., 1999).

Among helminths, cestodes (Platyhelminthes: Cestoda) are the most prevalent (52%), followed in order by nematods (Nemathelminthes: Nematoda) (16%) and trematodes (Platyhelminthes: Trematoda) (Ahmed and Sinha, 1993; Rabbi *et al*., 2006). Infections with protozoa are common in poultry that may cause mild to severe nature of the disease. Among GI protozoa, coccidian (Apicomplexa: Sporozoea) are of unquestionable significance. *Histomonas (Hm.) melagridis*; transmitted by cecal worm *Heterakis (H.) gallinarum* (McDougald, 1997), causes black head disease in turkeys and is well documented protozoan of GI tract in layers.

Round the globe, an overall parasitic fauna of layer GI helminths ranges from 1% to 85%. Factors associated with the GI parasitism include: species of host, rearing system, climatic conditions and the geographical location (Anwar *et al*., 1991).

Many researchers in Pakistan have reported the distribution of different GI parasites in the previous two decades; however, not even a single report has been found providing a complete data of all GI helminths and protozoa in the commercial and domestic layer population of district Faisalabad. Diversity of intermediate hosts in the transmission of helminth infections in layer birds and scarcity of data on the distribution of GI parasitism in layer population of district Faisalabad necessitates periodic monitoring of layer birds to determine the epidemiology of

the parasitic diversity and associated risk factors over the period of years for planning specific control measures.

MATERIALS AND METHODS

***Study area*:** Faisalabad stands in the rolling flat plains of the northeast Punjab, between longitude 73°74 East, latitude 30°31.5 North, with an elevation of 184 meters (604 feet) above the sea level. The city proper covers an area of approximately 1,230 km^2. The city of Faisalabad is situated in the center of the lower Rachana Doab, the area between Chenab and Ravi rivers. The topography is however marked by valleys, local depression and relatively high ground. This city bears harsh climatic conditions; May, June and July are the hotter months with highest temperature up to 50°C (122°F), whereas, December, January and February are the colder months with lowest temperature of −2°C (28°F). The average yearly rainfall lies only at about 300 mm (12 inch) and is highly seasonal with approximately half of the yearly rainfall in the two months July and August.

Faisalabad is ranked 1st in commercial while 2nd in rural layer farming of the Punjab province with population of about 8.66 million heads at 1270 farms in commercial production system while 4.83 million heads in rural/non-descript farming pattern under the available geo-climatic circumstances (www.poultry.punjab.gov.pk).

***Epidemiological investigations*:** The central diagnostic facility the Faculty of Veterinary Science was selected as a sentinel unit to conduct a passive surveillance of GI parasites in layers of the urban areas of district Faisalabad. In addition, convenient sampling was also done from peri-urban areas of Faisalabad over the period of one year from March 2012 to February 2013. A questionnaire containing open and closed-ended questions was designed about all the possible determinants associated with the host (age), agent (parasites) and environment (raising method, housing, feeding, feed type, watering and floor pattern). The questionnaire was tested through formal and informal testing procedures as recommended by Thrusfield (2007). According to age, birds were classified into three groups as 0-8 weeks (brooding), 9-24 weeks (growing) and 25-72 weeks (laying) of age. Participatory epidemiology and rapid rural appraisal techniques were adopted to record the relevant observations on the pre-designed questionnaire from the poultry farmers of the study area. Afterwards, data was numerically coded and maintained.

***Blood examination*:** Blood (5 mL) was collected from the wing vein of each bird examined. Blood was subjected to thin film preparation, Giemsa staining for identification of blood parasites. EDTA containing blood was subjected to erythrocyte count, total leukocyte count, differential leucocytes count (DLC), hemoglobin concentration and hematocrit (HCV) determination using the method described by Natt and Herrick (1952). Half of it was shifted and preserved in 0.5% EDTA coated tubes while rest was used for serum separation (Adam, 1971). Serum was then collected in Eppendorf tubes and stored at -20°C till further processing. Sera were processed spectrophotometrically for determination of total proteins, albumins and globulins (Benjamin, 1978).

***Examination of intestine/droppings*:** The live or dead birds were subjected to the collection of intestinal contents through standard protocols (Gresham and Ainsworth, 2011). After post mortem examination of birds, helminths were separated in the petri-plates containing normal saline. Before fixation, worms were fixed in 5% ethanol at room temperature. Later, specimens were transferred to 10% formalin for preservation. For the microscopic examination of droppings, centrifugal floatation method was used. The quantitative burden of GI parasites was determined through McMaster egg counting chamber (Zajac and Conboy, 2006).

***Statistical analyses*:** Multiple logistic regression and odd's ratio at 95% confidence level was used for the determinants influencing epidemiology of GI parasites in the layer population. Comparative haematology and serum biochemical profile of endo-parasite infected and non-infected layer birds were analyzed by student's T-test (Schork and Remington, 2010). All statistical parameters were performed using SAS package 2010 (Version: 6.12).

RESULTS AND DISCUSSION

A total of 349 farms (1996 birds) entered into the present epidemiological investigation from March 2012 to February 2013. Of these, 746 (37.37%) layer birds were found infected with GI parasitic fauna; however, no bird was found infected with blood parasites.

Four species of GI parasites viz; *Ascaridia (A.) galli, Heterakis (H.) gallinarum, Raillietina (R.) tetragona* and *Eimeria* (*E.*) *tenella* were observed in the study population (Table 1). Micrometery was used to differentiate the helminth and protozoan species from one another and their related species. Egg size was measured as 80-90×50-55µm, 65-75×40-45µm and 90-95×70-75µm for *A. galli*, *H. gallinarum* and *R. tetragona* respectively, while cyst of *E. tenella* were 45-50µm in length as standardized by Soulsby (1982).

A strong positive association (OR=1.56) of GI parasitism was observed with the season. The infection rate was found highest in the summer ($P<0.05$) followed in order by autumn, spring and winter. Same pattern was observed in monthly distribution being highest in the July (50.54%) and lowest in January (28.48%) (Figure 1).

Age of birds was found strongly associated with GI parasitism, where overall highest prevalence was found in the laying birds (46.89%; $P<0.05$; OR=1.85) followed in order by brooding and growing birds.

Table 1. Overall prevalence and associated risk factor of layer (*Gallus domesticus*) gastrointestinal parasitism in district Faisalabad, Punjab, Pakistan

Variables	Levels	Birds Screened (n)	Positive (N)	Prevalence (n/N×100)	95% C.I		Odds Ratio	P-Value
					Lower Limit	Upper Limit		
Parasitic Specie	*Ascaridia galli*	1996	425	21.29	19.54	23.13	10.90	0.000
	Eimeria tenella	1996	226	11.32	09.99	12.77	5.79	0.000
	Heterakis gallinarum	1996	56	2.81	02.15	03.60	1.44	0.085
	Raillatina tetragona	1996	39	1.95	01.41	02.63	-	-
Age of Birds	0-8 Week (Brooding)	1016	394	38.78	34.11	43.72	1.36	0.00
	9-24 Week (Growing)	498	126	25.30	18.38	33.54	-	-
	25-72 Week (Laying)	482	226	46.89	40.46	53.43	1.85	0.000
Bird Keeping	Commercial	1714	618	36.06	32.67	39.56	-	-
	Domestic	282	128	45.39	41.89	49.03	1.26	0.048
Feeding system	Manual	1606	627	39.04	35.55	42.55	1.28	0.032
	Automatic	390	119	30.51	19.04	43.59	-	-
Feed type	Crumbs	1336	495	37.05	32.80	41.29	-	-
	Mesh	666	251	37.68	32.00	43.97	1.02	0.851
Watering System	Manual	1278	490	38.34	34.14	42.74	1.08	0.420
	Automatic	718	256	35.65	30.98	42.76	-	-
Floor Pattern	Cemented	452	169	37.39	25.58	53.08	2.37	0.042
	Semi Cemented	1228	464	37.79	33.39	42.20	2.39	0.032
	Soiled	278	107	34.49	25.58	53.08	2.44	0.038
	Caged	38	6	15.79	0.83	59.09	1.16	-
Seasonal Prevalence	Summer	526	245	57.51	51.29	63.63	1.56	0.000
	Autumn	503	188	37.37	30.54	44.32	1.25	0.080
	Spring	489	170	34.76	27.83	42.10	1.16	0.255
	Winter	478	143	29.91	22.98	37.96	-	-

Rearing system was also having significant association, being higher in the domesticated birds (45.39%; P<0.05; OR=1.26) than commercial ones. Between the two feeding systems, the overall prevalence of GI parasites was higher in birds on manual system (39.04%; P<0.05; OR=1.28) compared with those on automated system. Feed type was also found non-statistically associated with the GI parasitism; where overall prevalence was higher in mesh type of feed (37.68%; P>0.05; OR=1.02). In case of watering system, no statistically significant association was found for GI parasites though overall prevalence was found numerically higher in manual system (38.34%; P>0.05; OR=1.08) as compared to automated one.

Floor pattern was found statistically associated with prevalence of GI parasitism where overall highest prevalence was observed on soiled floor pattern (34.49%; P<0.05; OR=2.44) followed by semi-cemented (37.79%; P<0.05; OR=2.39), cemented (37.39%; P<0.05; OR=2.37) and caged birds.

Results regarding the changes in blood profile of infected birds are given in Table 2. Parasitism is a renowned constraint in layer bird's productivity as it hampers the growth, meat and egg production (Anwar *et al*., 1991). Apparently, loose droppings, intestinal obstruction (heavy worm burden), anemia and nervous manifestations are observed while necropsy examination reveals nodules, haemorrhages, ulcerative enteritis, atrophy of villi, formation of granulomas and desquamation of epithelial cells of intestine (Shah *et al*., 1999). In Pakistan, both commercial (including open and closed farming patterns) and backyard (small scale and large scale) farming systems are practiced (Alam and Khan, 2000). Based on the review of literature, the overall prevalence of GI parasitism ranges from 1-85% as reported from various regions of Pakistan up to 2012 (Anwar *et al*., 1991; Shah *et al*., 1999; Tasawar *et al*., 1999; Sayyed *et al*., 2000; Bachaya *et al*., 2012).

Table 2. Haematology and serum biochemical profile of gastrointestinal parasite infected and non-infected birds.

Sr.	Parameter	Diseased Birds (Mean ± S.E.)	Reference Values
1.	PCV	17.10±2.1	22.0-35.0
2.	RBC	2.20±0.11	2.5-3.5
3.	WBC	0.39±0.09	1.2-3.0
4.	Heterophils (%)	39.07±3.01	15.0-40.0
5.	Lymphocytes (%)	57.71±3.07	45-70
6.	Monocytes (%)	1.45±0.47	5.0-10.0
7.	Eosinophils (%)	0.15±0.1	1.5-6.0
8.	Basophils (%)	00.00±00.00	Few
9.	Hb	5.19±0.77	7.0-13.0
10.	MCV	241±10.12	90.0-140.0
11.	MCH	75.40±3.00	33.0-47.0
12.	MCHC	337.48±1.7	26.0-35.0

Bird rearing systems have shown many improvements over the period of time. Variations in geo-climatic conditions also fluctuate the epidemiology of parasitism.

In this study, we found an overall prevalence of 37.37% in layers; however, variable reports ranging from 10% to 100% are available from different regions of the world including Trinidad, Turkey, Bangladesh, Ethiopia, Nigeria, Morocco, India, Ethiopia, Kenya, Zambia, Iran, Uganda and Tanzania (Ssenyonga, 1982; Yadav and Tandon, 1991; Eshetu *et al*., 2001; Magwisha *et al*., 2002; Hassouni and Belghyti, 2006; Rabbi *et al*., 2006; Mungube *et al*., 2007; Phiri *et al*., 2007; Yoriyo *et al*., 2008; Eslami *et al*., 2009; Kose *et al*., 2009; Baboolal *et al*., 2012; Molla *et al*., 2012).

Most favorable season for GI parasitism was summer which might be due to (a) hot and humid climatic conditions which impart significant role in the spread and maintenance of infection (b) abundance of intermediate host in summer (Soulsby, 1982).

Probable reasons of variation in the distribution of GI parasitism include: varying geo-climatic conditions (Buriro *et al*., 1989; Anwar *et al*., 1991), level of husbandry practices, sample origin either from intense conditions or rural areas, type of study population (broiler, layer or breeder), sample size, sampling design, bird rearing systems, availability of intermediate host and possibility of exposure to infection (Baboolal *et al*., 2012, Thrusfield, 2007). Higher prevalence in nondescript/backyard population could be due to scavenging properties of birds in day time (Permin *et al*., 1999).

Prevalence of *A. galli* is the highest in our study which is not different from earlier reports from different regions of the world (Jordan and Pattison, 1996; Magwisha *et al*., 2002; Delawi, 2007; Matur *et al*., 2010; Mwale and Masika, 2011). Probable reasons for highest prevalence of *A. galli* include: (a) ability of its eggs to withstand harsh climatic condition due to thick albuminous shells (Ashour, 1994) (b) direct life cycle and capability for early infection by L2 (Soulsby, 1982). *H. gallinarum* is not frequent in layer population of our study area which might be due to lack/scarcity of its intermediate host (earth worm) (Lund *et al*., 1966). Variable reports of *H. gallinarum* ranging from 0.9% to 39.62% are available from Ethiopia, Kenya, Tanzania, Trinidad and Turkey (Magwisha *et al*., 2002; Mungube *et al*., 2008; Kose *et al*., 2009; Bablool *et al*., 2012; Molla *et al*., 2012).

Ants act as intermediate hosts of *R. tetragona;* the distribution of which (reported from 12% to 65%, in Ethiopia and India) (Molla *et al*., 2012; Dar and Tanveer, 2013) depends upon that of former. Abundance of aunts depends upon environmental conditions and hydration status of soil (Davidson, 1944; Honek and Kocourek, 1988; Dar and Tanveer, 2013).

E. tenella is the only protozoa and most abundant parasite of GI tract in layer population. Its distribution, clinical effects, economic losses and associated determinants have extensively been studied round the globe (Hadipour *et al*., 2011; Rehman, *et al*., 2010; Awais *et al*., 2012) with prevalence ranging from 25.24% to 59.60% in layers causing approximately 3.53% economic losses in the developing countries like Ethiopia, Iran, Nepal and Pakistan (Razmi *et al.,* 2000; Adhikari *et al*., 2008; Amare *et al*., 2012; Bachaya *et al*., 2012). The success of settlement of *E. tenalla* is due to: (i) capacity of cyst formation (McDougald, 2003), (ii) addition of coccidiostats which reduce immunity of adults making them more prone to infection (Jordan *et al*., 2002; Vegad, 2004; Kahn, 2005) and (iii) resistance against anticoccidials (Abbas *et al*., 2011).

Among the risk factors associated with the GI parasites, age was found statistically associated i.e. highest prevalence in laying birds as reported earlier by Matur *et al.* (2010). This might be due to (a) voracious nature of laying birds towards feed though they may pick infected/contaminated feed which makes them more prone to GIT parasitism (Sonayia, 1990; Permin and Hansen, 1999; Soulsby, 1982) and (b) drop in the immune status of infected birds due to higher egg production (Permin and Hansen, 1999).

This manuscript probably provides the first report of association of feeding, watering and housing systems as well as feed types with the frequency distribution of GI parasites in layer bird population. Similarly, higher prevalence of GI parasitism in manual type feeding and watering systems may be due to (a) human interruption (labor) or fomites as a mean to assist in spread of eggs/cysts to the healthy birds and (b) lack of water disinfectants.

Type of feed also influenced the distribution of parasites because the feed stuff including grains, fruits and insects which may harbor infectious stages of parasites. Hence, the domesticated birds may become more prone to parasitic infection than commercialized birds (Oniye *et al*., 2001; Rabbi *et al*., 2006). Other reasons of higher GI parasitism in

domesticated layers include: feeding stress, longer life span of layers before human consumption (Matur *et al*., 2010).

In the current study floor pattern was found statistically associated with prevalence of GI parasitism which is not different from earlier reports (Morgenstern and Lobsiger, 1993; Wilson *et al*., 1994; Permin *et al*., 1999; Magwisha *et al*., 2002; Robel *et al*., 2003; Rabbi *et al*., 2006; Hassouni and Belghyti, 2006; Puttalakshamma *et al*., 2008; Kose *et al*., 2009. This may be due to unhygienic microclimate favoring the growth and propagation of non-parasitic developmental stages of GI parasites (Soulsby, 1982; Permin et al., 1999). Lowest prevalence of GIT parasitism in battery cage system was also observed by Zeller (1990) and Permin *et al*. (1999). Results of the current study do not match the findings of Kaufmann *et al*. (2011) who observed the higher worm prevalence in caged birds.

It is concluded that four different species of GIT parasites viz, *A. galli, H. gallinarum, R. tetragona* and *E. tenella* are prevalent in the layer bird population of district Faisalabad. Launching a wide scaled national extension program and conducting a country-wide survey of GI parasites of layers is needed. Special management and care is required for laying birds as they are more prone to GI parasites. Avoiding poor husbandry practices, domestic rearing system, manual feeding and watering systems and adapting environment control shed system can be helpful in reducing the risk of GI parasitism in poultry. Moreover, routine prophylactic chemotherapeutic administration remains the corner stone for reducing the distribution of GI parasitism especially before the onset of most favorable summer season.

***Acknowledgements*:** The authors would like to thank the personnel of the central diagnostic facility of the Faculty of Veterinary Science, farming community of peri-urban areas of Faisalabad and laboratory colleagues for their cooperation during the project.

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