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## **Abstract**

Indigenous chickens constitute over 81% of poultry in Kenya and produce 71% of eggs and poultry meat. Ecto- and haemoparasites limit production of these birds in the rural areas. However, there exists scanty information on these parasites infection in indigenous chicken. This study was conducted to determine and document the type and prevalence of haemoparasites affecting different ages and sex groups of free range indigenous chicken from two agro ecological zones: Lower highland 1 (LH1) in Embu District and Lower Midland 5 (LM5) in Mbeere District in Eastern Province, Kenya.

Of the 144 birds examined, 79.2% were infected with haemoparasites, with 62.3% single and 37.7% mixed haemoparasitic infections. *Plasmodium gallinaceum* was the most prevalent haemoparasite (53.5%) followed by *Leucocytozoon schoutedeni* (52.1%) and *Hemoproteus* spp., (3.5%). Grower birds had a prevalence of 83.3% for haemoparasites compared to 81.3% of adults, and 72.9% of chicks ( $p > 0.05$ ). Male birds had 83.3% prevalence, while female birds had 75.0% ( $p > 0.05$ ). LH1 was found to have a slightly high prevalence of 81.9% compared to LM5, 76.4% ( $p > 0.05$ ). *Hemoproteus* spp were isolated in chickens from LH1 but not from LM5. This study has documented a high prevalence of haemoparasites, hence further studies to determine the impact of infection on the health and productivity of these birds, and evaluation of cost benefit of various control strategies need to be undertaken.

**Key words:** Age, agro-ecological zones, free range, sex

## **Introduction**

Poultry production in most tropical countries is based mainly on scavenging production system. This system exposes birds to a range of parasites (Sehgal et al 2006). However, the most striking problem in relation to village poultry production is their high mortalities within the first year after hatching (Permin et al 2002). Newcastle disease, helminthosis, coccidiosis and ecto- and haemoparasites have been reported to be the main problems affecting indigenous chicken in Malawi (Njunga 2003). In Kenya, information concerning these parasites has not been recently documented. Such information will be useful in disease diagnosis and implementation of a disease control programme hence improvements in the productivity of indigenous free ranging chicken. The aim of

this study was therefore to determine the type and prevalence of haemoparasites infection in indigenous free-ranging village chickens in two different agro-ecological zones in Kenya.

## **Materials and methods**

### **Study design**

A total of 144 indigenous chickens with matching for age, sex and agro ecological zones were purposively randomly selected (purposive in that specific age groups, sexes and numbers were selected between each agro-ecological zones) and purchased from smallholder farms and transported alive in cages to the laboratories at the University of Nairobi, Kabete for examination. Three blood smears were prepared from each bird, processed and examined for haemoparasites. Slides were subjected to a microscopic examination and results recorded.

### **Study area**

Two agro-ecological zones in two neighboring districts were chosen for this study. The selection was based on the availability of free-ranging village indigenous rural poultry population in the areas and contrasting agro-ecological zones.

Of these two, one was a lower highland 1 (LH1) in Embu District. This is a high agricultural potential area where tea, maize, beans and various fruits are grown and free-range poultry and dairy cattle are kept. The area has a bimodal rainfall pattern of long rains between March and June, and short rains in October to December. It has an annual average rainfall of 1080mm. Altitude ranges from 1500 to 4500 meters above sea level. The temperatures range from 12 to 27°C (Onduru et al 2002).

The other study area was the lower midland 5 (LM5) in Mbeere District. This is a semi-arid area with livestock (beef cattle, sheep and goats), poultry, millet and green gram as the main agricultural activities. It has a bimodal and erratic rainfall pattern with average annual rainfall of 180mm per year. Altitude is 1200 meters above sea level and temperatures range from 20-30°C (Onduru et al 2002).

### **Study chickens**

Indigenous chickens were obtained from individual randomly selected homesteads and purposive sampling used. Calculated sample size was 144 birds which were purchased. Expected prevalence used in the sample size calculation was 50% and the maximum limits of error at 8.3% as per the following formula  $n=1.96^2pq/L^2$  (see comments above) where  $n$  is the sample size,  $p$  the prevalence,  $q=1-p$  and  $L$  the limits of error on the prevalence (Martin *et al* 1987).

The birds were categorized into three age groups as follows: Chicks (aged < 2 months), growers (2 to 8 months) and adult (aged > 8 months) according to Magwisha *et al* (2002)

with modification. A total of 72 birds were sampled per agro-ecological zone comprising 36 birds per sex group and 24 birds per age group. All the birds from the two agro-ecological zones were purchased from January to February 2007 in a one month period. They were transported alive in cages to the Department of Veterinary Pathology, Microbiology and Parasitology laboratories, Kabete for examination.

#### **Determination of chicken age**

Chicken ages were determined subjectively based on the size of crown, length of spur and flexibility of the xiphoid cartilage together with information from the farmers. They were classified as adults (cock or hen), growers (pullet or cockerel) and chicks (male and female) according to Magwisha *et al* (2002) and Maina (2005).

#### **Clinical examination, blood collection and necropsy of the chicken**

Before slaughter, each chicken was subjected to a thorough clinical examination and observation recorded. Birds were then killed by dislocation of the atlanto-occipital joint, followed by severing of the carotid arteries and jugular veins using a scalpel blade. Blood was collected in universal bottles containing Ethylenediaminetetraacetic acid (EDTA) to prevent clotting. Three blood films were prepared from each bird namely: a fresh thin blood smear, blood smear from EDTA blood and a buffy coat smear. Blood films were air-dried within 5–10 seconds after preparation. Slides were fixed in methanol for 5 minutes and then stained with 10% Giemsa for 15 minutes, washed with tap water, blotted and examined under the microscope for haemoparasites (Nemi 1986).

#### **Examination of Giemsa stained blood smears**

Examination of Giemsa stained blood smears and buffy coat smears was carried out as described by Nemi (1986). Blood films were examined for 10–15 minutes at low magnifications of X40, X63 and then 100 fields were studied at high magnification (X100). The haemoparasites detected were identified according to Soulsby (1982) and Valkiūnas (2005) and recorded.

#### **Data analysis**

Data from the study were entered in Ms-Excel, and later exported to Genstat® Discovery edition 3 for descriptive statistical analysis. To test differences in parasite-specific prevalence between the three chicken age groups, two sex groups and two agro-ecological zones, the two sample binomial test (Genstat® Discovery Edition 3) was used.

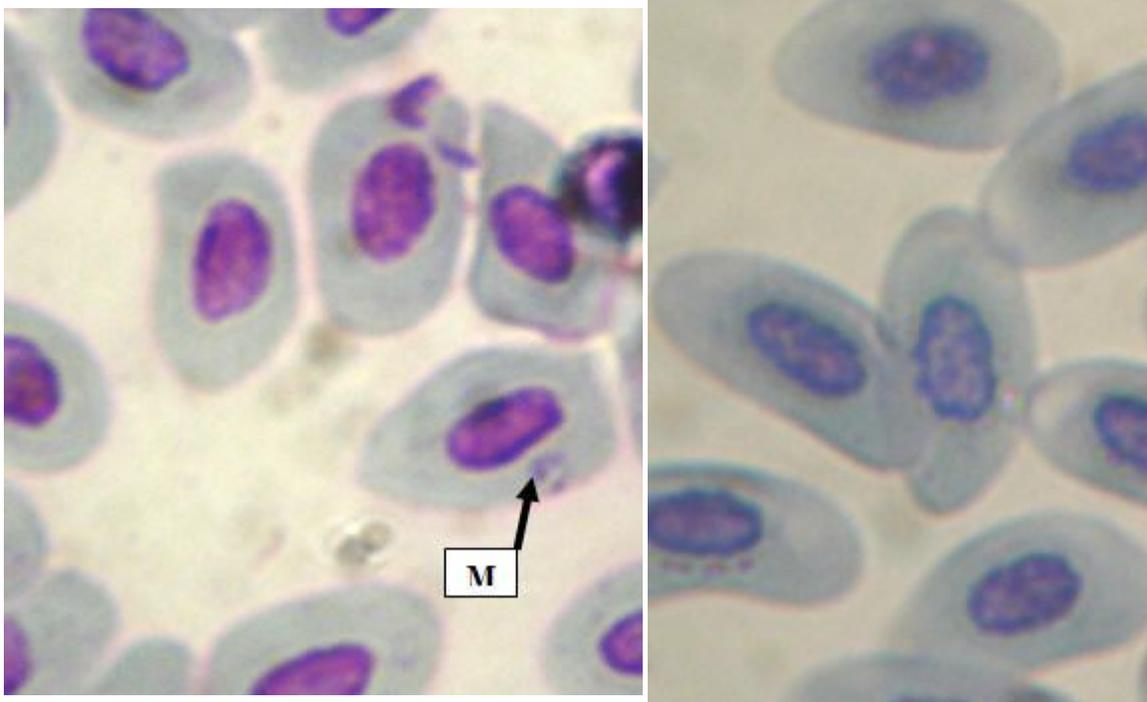
The prevalence of haemoparasitic infection was defined as the total number of birds infested with a particular parasite group/ species divided by the number of chicken examined at a point in time (Margolis *et al* 1982). A Kruskal Wallis one-way analysis of variance (Genstat® Discovery Edition 3) was used to analyze the influence of three age and two sex groups and two agro-ecological zones on the prevalence. A critical probability of 0.05 was adopted throughout as a cut-off point for statistical significance between groups compared.

## Results

Out of 144 birds examined, 114 (79.2%) were infected with haemoparasites. Three species of haemoparasites were found during this study. These were *Plasmodium* spp., *Leucocytozoon* spp., and *Haemoproteus* spp. *Plasmodium gallinaceum*, 53.7% (77/144) was the most prevalent haemoparasite, followed closely by *Leucocytozoon schoutedeni*, 52.1% (75/144) and lastly *Haemoproteus* spp., 3.5% (5/144). Of the 114 infected birds, 71 (62.3%) had single infection, while 43 (37.7%) had more than one genera of haemoparasites (**Table 1**).

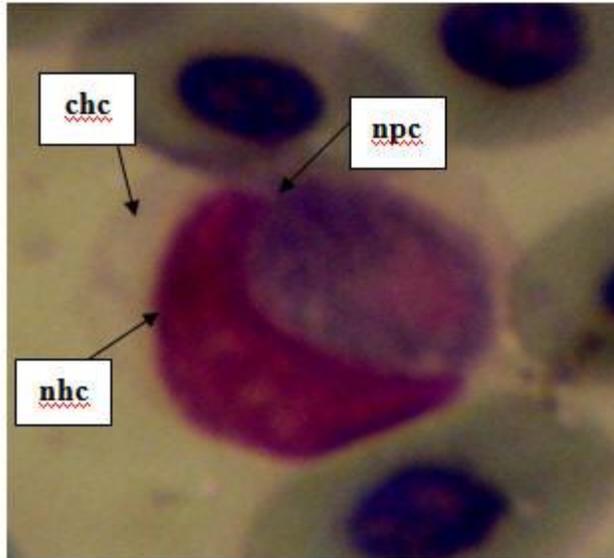
Among the age groups, grower birds showed a slightly higher rate of occurrence of 83.3% (40/48) compared to adults, 81.3% (39/48) and chicks, 72.9% (35/48). Male birds had a slightly higher rate of 83.3% (60/72) than female birds, 75.0% (54/72). Between the two agro ecological zones, LH1 (Embu) was found to have a higher prevalence rate of 81.9% (59/72) compared to LM5 (Mbeere), 76.4% (55/72). The rate of occurrence among bird age groups and sexes and between the agro ecological zones was not statistically significant.

*Plasmodium gallinaceum* was found to infect 77 (67.5%) of the 114 birds infected with haemoparasites. *Plasmodium* gametocytes were observed in erythrocytes. They appeared as yellow, brown or black intra-cytoplasmic inclusions. The gametocytes were round to irregular, relatively small, and some parasites tended to be in contact with the host cell nucleus. The merozoites had a “signet-ring” appearance due to a large vacuole that forced the parasite nucleus to one pole (Figures 1 and 2).



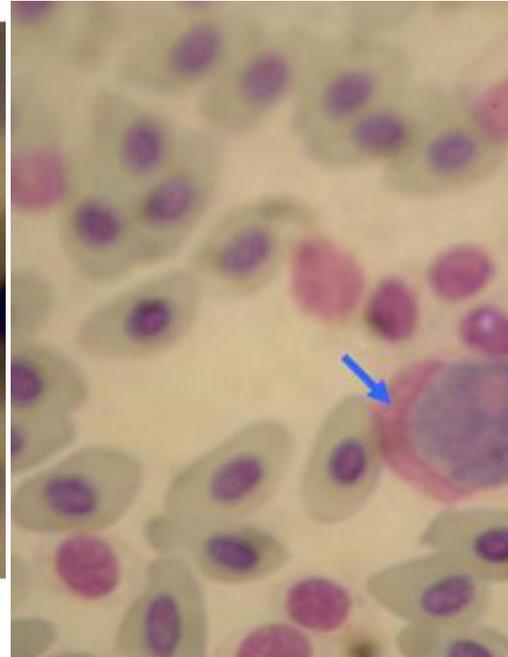
**Figure 1.** Blood smear showing a red blood cell (R) infected with a “signet-ring” merozoites (M) of *Plasmodium gallinaceum* (Giemsa stain, magnification X 1000).

**Figure 2.** Blood smear showing a red blood cell infected with *Plasmodium gallinaceum* (arrow) (Giemsa-stained, Magnification X 1000).



**Figure 3**

**Figure 3 and 4.** Chicken blood smears showing football-like *Leucocytozoon schoutedeni* (cytoplasm of host cell- chc; nucleus of host cell-nhc; cytoplasm of parasite cell- cpc; nucleus of parasite- npc) (Giemsa-stained, magnification X1000)



**Figure 5.** A Chicken blood smear showing an elongated host cell nucleus (arrow) forming a long thin dark crescent band along one side of parasitized cell (Giemsa stained, magnification X 1000)

The occurrence rate of *Plasmodium* was the same (56.3%) in adult and grower birds, but slightly lower in chicks (47.9%). Males had a slightly higher rate of infection with *Plasmodium* (56.9%) compared to female birds, 50%. Between the agro ecological zones, Lower highland 1 was found to have a slightly higher rate of infection. 61.1% (44/72) than the Lower midland zone 5, 45.8% (33/72). The difference in the rate of occurrence of *Plasmodium gallinaceum* among the bird age groups and sexes, and between the agro ecological zones was not statistically significant.

Out of the 114 birds infected with haemoparasites, 75 (65.8%) had *Leucocytozoon schoutedeni*. This parasite was found to infect the leukocytes. Parasites were spherical, ovoid or spindle-shaped containing one to four elongated deeply staining structures called host cell nucleus or lateral bars. Gametocytes caused marked enlargement and distortion of the infected cell producing a football-like appearance (Figure 3 and 4). Nucleus of the host cell was elongate and formed a long thin dark crescent along one side of parasitized cell (**Figure 5**). Macrogametes stained dark blue with Giemsa, and the nucleus was compact and had several vacuoles occurring in darkly stained cytoplasm. Microgametes

were slightly smaller than macrogametes, their cytoplasm stained less deeply, usually pale blue in color, and the nucleus was diffuse and stained pale pink.

This parasite showed an increase in prevalence rate with increase in age of the chicken. Adult birds had a slightly higher prevalence of 58.3% (28/48) compared to growers, 50.0% (24/48) and chicks 47.9% (23/48). Male birds had a higher prevalence 54.2% (39/72) than in females, 50% (36/72). There was a slight difference in occurrence of *L. schoutedeni* between Lower LM5 (Mbeere) 52.8% (38/72) and LH1 (Embu), 51.4 (37/72). The rate of *L. schoutedeni* occurrence among bird age groups and sexes, and between the agro ecological zones was not statistically significant.

Out of 114 birds that had haemoparasites, 5 (4.4%) had *Haemoproteus* spp., occurring either singly or with mixed infections (Table 1). In Giemsa stained blood smears, the gametocytes of *Haemoproteus* spp., appeared elongate, sometimes horseshoe shaped cells embracing the erythrocyte nucleus. Cytoplasm of gametocyte contained pigment granules accumulating as result of incomplete digestion of hemoglobin.

**Table 1.** Prevalence of haemoparasites in birds (single or mixed infection)

Haemoparasite occurrence in birds	Number of birds infected with haemoparasites (x)	Percentage prevalence rate (x/144)
<i>Plasmodium</i> species	34	29.8
<i>Leucocytozoon</i> species	36	31.6
<i>Haemoproteus</i> species	1	0.9
<i>Plasmodium</i> species and <i>Leucocytozoon</i> species	39	34.2
<i>Plasmodium</i> species and <i>Hemoproteus</i> species	4	3.5
<i>Leucocytozoon</i> species and <i>Hemoproteus</i> species	0	0.0

Among age groups, this parasite was found in chicks 6.3% (3/48) and grower birds 4.2% (2/48), with no cases isolated from the adult birds. Between sexes, the female birds had a slightly higher prevalence, 4.2% (3/72) compared to males, 2.8% (2/72). This parasite was only found in chickens obtained from Lower highland zone 1 (Embu), 6.9% (5/72). The difference in rate of occurrence of *Haemoproteus* spp., among age groups and sexes was not significant statistically ( $p > 0.05$ ), while that between the agro ecological zones was statistically significant.

## Discussion

Results obtained from examination of blood smears revealed the presence of three haemoparasites (*Plasmodium* spp, *Leucocytozoon* spp and *Haemoproteus* spp) that were found to infect poultry in the study zones. Mixed infections with two of these haemoparasites were encountered. The findings of this study are consistent with the study by Sadiq *et al* (2003) in Ibadan, Nigeria, who reported the same three haemoparasites (*Plasmodium* spp, *Leucocytozoon* spp and *Haemoproteus* spp) during their study. In other studies in Malawi, Njunga (2003) reported the occurrence of *Plasmodium gallinaceum*,

*P. juxtannucleare* and *Aegyptinella pullorum*. However, observation on haemoparasitism indicated that the indigenous chicken samples examined in this study lacked *Aegyptinella* spp., which is frequently encountered in birds in Africa (Poulsen *et al* 2000, Permin *et al* 2002 and Njunga 2003).

Prevalence of haemoparasites in birds in this study was found to be 79.2%. This high prevalence of blood parasites is comparable to the studies done by Valkiūnas *et al* (2005) who reported the prevalence of avian blood parasites in Uganda to be 61.9%, while Njunga (2003) in Malawi found the prevalence of haemoparasites in chicken to be 71%. There were no differences in occurrences among bird age groups, and across sexes and agro ecological zones. Findings among age groups were consistent with the findings of Permin *et al* (2002), who compared the prevalence between young and adult free range birds. All infected chickens in Zimbabwe (Permin *et al* 2002) had a low parasitaemia (<1% of erythrocytes were infected). However, there were no documented studies on the comparisons between sexes, and agro ecological zones. The reason for similar prevalence between bird's age groups and across sexes and agro ecological zones were not clear hence should be studied further.

*Plasmodium gallinaceum* occurred in 77 of 144 birds (53.7%) examined and was the most prevalent haemoparasite encountered during our study. This high prevalence was in contrast to results found in a study in Zimbabwe where, 14 of 94 chickens (14.9%) harbored *Plasmodium gallinaceum* (Permin *et al* 2002), and that in Ghana where 27 of 100 birds (27.0%) harbored *Plasmodium juxtannucleare* (Poulsen *et al* 2000). This variation can be adequately attributed to variation between agro climatic conditions Fallis *et al* (1973), Adene and Dipeolu, (1975) and Permin *et al* (2002) indicated that *Leucocytozoon* spp. may be the most common hematozoan in these birds in Africa. This was however in contrast to the findings of the current study and that by Sadiq *et al* (2003), where *Plasmodium* spp., was found to be the most prevalent.

Of the three haemoparasites encountered, *Plasmodium gallinaceum* is the most pathogenic. Soulsby (1982) cited progressive emaciation, anaemia, enlargement of spleen and liver. Paralysis may be observed where there are massive numbers of exoerythrocytic forms in the endothelia cells of the brain capillaries and death in untreated cases. In Zimbabwe, Permin *et al* (2002) found that the differences in prevalence of *Plasmodium gallinaceum* were not significantly different between the bird's ages (young and adult). This report was similar to our findings. However, there were no previous reports on comparison of occurrences of *Plasmodium* spp. between bird's sexes and agro ecological zones. The differences in prevalence of *Plasmodium* spp. in this study is most likely connected to abundance and variations in appearance of vectors (Permin *et al* 2002).

*Leucocytozoon schoutedeni* was found in 75 (52.1%) of 144 birds examined. This prevalence was comparable to studies by Fallis *et al* (1973) who reported that of 150 chickens tested in Tanzania, more than 50% were infected with *L. schoutedeni*. Sehgal *et al* (2006) in their studies in Uganda found that the prevalence of *L. schoutedeni* was 31.0%, a prevalence that was lower than these findings. In Zimbabwe, 4 of 94 (4.3%) chickens' harbored *Leucocytozoon sabrazesi*, but in Ghana, no *Leucocytozoon* infection

was detected (Poulsen *et al* 2000). Earlier studies showed *Leucocytozoon* spp. infected 55 of 163 (34%) examined chickens in Ibadan, Nigeria (Adene and Dipeolu 1975). This variation in prevalence and distribution of various *Leucocytozoon* spp. can be attributed to agro climatic variation which affects vector distribution and adaptation of the *Leucocytozoon* spp., in different agro climatic zones. *Leucocytozoon* spp infection causes anaemia, thickened oral discharge and paralysis of legs (Sadiq *et al* 2003).

On the other hand, *Haemoproteus* was the least found haemoparasite during this study. This haemoparasite was found to infect birds from LH1 zone only, and has not been recently reported in other African countries. Variation in prevalence between LH1 and LM5 is likely due to a difference in geographical and climatic factors between the study areas. *Haemoproteus* infection is not particularly pathogenic in domestic chicken (Soulsby 1982).

## Conclusion

- This study revealed that there is a high prevalence of haemoparasitic infection in healthy looking indigenous chicken. However, there is a need to do further studies to assess the intensity of this infection and subsequent levels that can cause clinical disease. Predisposing factors to haemoparasite infection need to be examined. Also, the only test used in this study was microscopy which has a very low specificity and therefore more invasive tests need to be used as such can reveal a higher level of prevalence than the one reported here.

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