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Therapeutic efficacy of metronidazole and Sulphamethoxazole-Trimethoprim in managing trichomoniasis in black kites (*Milvus migrans*) and domestic pigeons (*Columba livia domestica*) with reference to Hematological alterations

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Abstract
Trichomoniasis, caused by *Trichomonas gallinae*, is a significant protozoan disease affecting a wide range of avian species, including domestic pigeons (*Columba livia domestica*) and black kites (*Milvus migrans*). The present study evaluated the therapeutic efficacy of Metronidazole and Sulfamethoxazole-Trimethoprim, with emphasis on associated haematological alterations in naturally infected birds. In pigeons, haemoglobin (Hb) concentrations significantly decreased from 12.6 g/dL to 10.2 g/dL, accompanied by a marked reduction in packed cell volume (PCV) from 38.4% to 31.2%. Heterophil counts significantly increased from 58.8% to 65.2%, whereas lymphocyte counts declined from 29.6% to 21.3%, yielding an elevated heterophil-to-lymphocyte (H/L) ratio of 3.07. Black kites exhibited comparable trends, with Hb decreasing from 13.2 g/dL to 10.9 g/dL, heterophils increasing to 64.6%, and lymphocytes decreasing to 23.7%, resulting in an H/L ratio of 2.72. Therapeutic evaluation revealed that Metronidazole was markedly superior, achieving 100% recovery in mild infections, 75% improvement in moderate cases, and 50% efficacy in severe trichomoniasis. Birds receiving Metronidazole also demonstrated more substantial haematological normalization. Conversely, Sulfamethoxazole-Trimethoprim exhibited limited efficacy, with only 50% improvement in mild cases and negligible response in moderate or severe infections. These findings underscore the clinical value of Metronidazole as the preferred therapeutic option for avian trichomoniasis and highlight the utility of haematological indices as indicators of disease severity and treatment response.

Keywords: *Trichomonas gallinae*, black kite, domestic pigeon, Haematology, H/L ratio, metronidazole, Sulfamethoxazole-trimethoprim

Introduction
Domestic pigeons (*Columba livia domestica*) and black kites (*Milvus migrans*) are two widespread urban species frequently exposed to infectious agents due to congregation, scavenging behaviour, and close association with human environments (Opara *et al.*, 2012; Kumar *et al.*, 2019) [1, 2]. India hosts over 1,300 bird species, providing a diverse ecological interface for host-parasite interactions (Grimmett *et al.*, 2011; Praveen *et al.*, 2019) [3, 4].
Trichomonas gallinae is a widespread important protozoan pathogen of the upper digestive tract in columbids and raptors, producing caseous oropharyngeal lesions and, in severe cases, systemic pathology (Stockdale *et al.*, 2015; Borji *et al.*, 2011) [5, 6]. Transmission occurs through contaminated feed, water, crop milk, and predation, making pigeons primary reservoirs and raptors frequent spillover hosts (McDougald, 1992; Robinson *et al.*, 2010) [7, 8]. Although trichomoniasis is well documented in pigeons, data on natural infections in wild raptors, including black kites, remain limited (Krone *et al.*, 2005; Chavatte *et al.*, 2019) [9, 10].
Haematological profiling is a sensitive measure of disease impact in avian species, reflecting anaemia, inflammatory responses, and stress-related leukocyte shifts. However, comparative haematological evaluation of *T. gallinae* infection across domestic and wild birds is lacking. Metronidazole remains the standard therapy for avian trichomoniasis, whereas

sulphonamide-trimethoprim combinations are occasionally used but with limited evidence of efficacy in raptors (Miller *et al.*, 2017) [11].

The present study characterises the haematological alterations associated with trichomoniasis in domestic pigeons and black kites and compares the therapeutic outcomes of Metronidazole and Sulphamethoxazole-Trimethoprim with reference to post-treatment haematological recovery.

Materials and Methods

The research work was conducted at the Department of Veterinary Clinical Medicine in collaboration with Jivdaya Charitable Trust (JCT), Panjarapole, Ahmedabad.

Study Population

A total of 200 birds were examined, comprising 60 domestic pigeons (*Columba livia domestica*) presented from urban flocks and 140 black kites (*Milvus migrans*) admitted to Jivdaya Charitable Trust, Panjarapole, Ahmedabad. Birds showing clinical signs of upper digestive tract infection (dysphagia, salivation, caseous oral lesions, inappetence) were subjected to parasitological screening.

Diagnostic Methods

1. **Hanging Drop Method:** Fresh oral swabs were microscopically examined for motile *T. gallinae* trophozoites.
2. **PCR Confirmation:** DNA was extracted from oral swabs and subjected to PCR amplification targeting the ITS1-5.8S-ITS2 region of *T. gallinae*.

Experimental Grouping & Therapeutic Protocol

A total of 200 birds were screened for Trichomonas infection during the study period, out of these, 36 birds (18 black kites and 18 domestic pigeons) were randomly selected for the therapeutic study. Six black kites and six domestic pigeons were selected as healthy controls, while the remaining 24 birds (12 black kites and 12 domestic pigeons) were randomly selected for therapeutic management based on clinical signs suggestive of Trichomonas infection. The 24 infected birds were randomly divided into four groups (Groups A, B, C, and D), each consisting of six birds.

Healthy Control: (n = 6) Domestic pigeons

Group A: (n = 6) Trichomonas infected Domestic pigeons treated with Metronidazole @ 30 mg/kg BW twice daily PO for 5 days.

Group B: (n = 6) Trichomonas infected Domestic pigeons treated with Sulfamethoxazole-trimethoprim @ 30 mg/kg BW twice daily PO for 5 days.

Healthy Control: (n = 6) Kites

Group C: (n = 6) Trichomonas infected Black kites treated with Metronidazole @ 30 mg/kg BW twice daily PO for 5 days.

Group D: (n = 6) Trichomonas infected Black kites treated with Sulfamethoxazole-trimethoprim @ 30 mg/kg BW twice daily PO for 5 days.

Haematological analysis

Blood sample (1 ml) was collected from the wing vein in vials containing K3EDTA from suspected cases on day 0 and thereafter from birds under the therapeutic trial on the 7th day. Anticoagulated blood collected from apparently healthy as well as from sick birds were analysed in an Automated blood cell counter for estimation of Haemoglobin concentration (g/dl), Packed cell volume (%). Immediately after collection thin blood smears were prepared on grease free clean slides, smears were dried at room temperature and fixed in alcohol. Later on, blood smears were stained in field's stain, allowed to dry and examined microscopically for differential leukocyte counts under oil immersion objective (100x). Heterophil to Lymphocyte Ratio was calculated manually from DLC obtained data.

1. Haemoglobin(g/dl),
2. Packed Cell Volume (PCV) %
3. Heterophil %
4. Lymphocyte %
5. Eosinophils %
6. Heterophil Lymphocyte Ratio (HLR)

Blood samples were also collected from birds in the healthy control group only on day 0 for haematological analysis to compare the findings with Trichomonas positive birds.

Statistical Analysis

The data obtained through haemato-biochemical examination in the research work were statistically analyzed and clinical variants were subjected to one way analysis of variance (ANOVA), independent t-test and paired t-test by employing SPSS software 21.0. The value $p>0.05$ were considered as non-significant, $p<0.05$ were considered as significant and $p<0.01$ were considered as highly significant.

Results and Discussion

A total of 200 birds (140 black kites and 60 domestic pigeons) were screened for Trichomoniasis, out of them 70 birds were found positive by the gold standard test PCR with an overall prevalence of 35.00 per cent. In pigeon, the prevalence was recorded as high as 75.00 per cent (45/60) and in black kite, it was 17.86 per cent (25/140). Statistically, the percentage prevalence of Trichomoniasis in domestic pigeon was highly significant ($p < 0.001$) by Chi square analysis.

1.0 Haematological changes in trichomonas affected domestic pigeons (Day 0)

In present study, whole blood samples were collected from 6 healthy Pigeons and 12 Trichomonas infected Pigeons initially for haematological examinations. The findings are presented in Table 1.

Table 1: Haematological observation in healthy and trichomoniasis affected domestic pigeons on days 0 (Mean ± SE)

Parameters	Healthy Pigeons (N = 6)	Trichomonas affected Pigeons (N = 12)	p-value
Hb (g/dl)	17.8±0.66	13.45±0.34**	0.001
PCV %	46.83±1.54	39.17±0.67**	0.003
Heterophils %	37.33±1.36	53.67±0.48**	0.001
Lymphocytes %	62.33±1.59	45.67±0.45**	0.001
Eosinophils %	0.33±0.21	0.33±0.14	1.000
Monocytes %	0.33±0.21	0.33±0.14	1.000
Heterophil to Lymphocyte Ratio	0.6±0.04	1.18±0.02**	0.001

$p>0.05$ = non-significant, $p<0.05$ = significant and *$p<0.01$ = highly significant **

The study revealed highly significant differences in haematological parameters between healthy pigeons and Trichomonas-affected pigeons, indicating the systemic impact of the infection. Haemoglobin (Hb) levels were highly significantly reduced in affected pigeons (13.45±0.34 g/dl) compared to healthy ones (17.8±0.66 g/dl). This reduction reflects anaemia, commonly associated with parasitic infections due to nutrient depletion or damage to erythrocytes. Packed Cell Volume (PCV) was highly significantly lower in affected pigeons (39.17±0.67%) compared to healthy pigeons (46.83±1.54%).

The heterophil percentage increased highly significantly in Trichomonas-affected pigeons (53.67±0.48%) compared to healthy ones (37.33±1.36%), indicating an inflammatory response to infection. In contrast, the lymphocyte percentage decreased highly significantly in affected pigeons (45.67±0.45%) compared to healthy pigeons (62.33±1.59%), reflecting a stress-induced immune shift. These changes resulted in a highly significant elevation of the heterophil-to-lymphocyte (H/L) ratio in affected pigeons (1.18±0.02) compared to healthy ones (0.6±0.04), indicating a marked stress response in the infected group. No significant changes were observed in eosinophil (0.33±0.14%) and monocyte percentages (0.33±0.14%) between the two groups.

1.1 Haematological changes in trichomonas affected black kites (Day 0)

In present study, whole blood samples were collected from 6 healthy Black kites and 12 Trichomonas infected Black kites initially on day 0 for haematological examinations. The findings are presented in Table 2

Table 2: Haematological observation in healthy and trichomoniasis affected black kites on days 0 (Mean ± SE)

Parameters	Healthy Black kites (N = 6)	Trichomonas affected Black kites (N = 12)	p-value
Hb (g/dl)	12.51±0.27	10.84±0.08**	0.001
PCV %	34.33±0.56	41.08±0.43**	0.001
Heterophils %	53.00±0.58	62.83±0.71**	0.001
Lymphocytes %	46.17±0.65	36.5±0.71**	0.001
Eosinophils %	0.33±0.21	0.33±0.14	1.000
Monocytes %	0.33±0.21	0.33±0.14	1.000
Heterophil to Lymphocyte Ratio	1.15±0.03	1.73±0.05**	0.001

The haematological parameters of healthy and Trichomonas-affected black kites showed highly significant differences. Haemoglobin (Hb) levels were highly significantly reduced in affected black kites (10.84±0.08 g/dl) compared to healthy ones (12.51±0.27 g/dl), indicating anaemia, which is commonly observed in parasitic infections. Packed Cell Volume (PCV) was highly significantly elevated in affected black kites (41.08±0.43%) compared to healthy kites (34.33±0.56%). This elevation is atypical and might be attributed to haemoconcentration or dehydration secondary to infection in affected birds.

The heterophil percentage was highly significantly increased in Trichomonas-affected black kites (62.83±0.71%) compared to healthy kites (53±0.58%), reflecting an inflammatory response to the infection. Conversely, the lymphocyte percentage decreased highly significantly in affected kites (36.5±0.71%) compared to healthy ones (46.17±0.65%), indicating a stress-induced immune shift. These changes resulted in a highly significant elevation of the heterophil-to-lymphocyte (H/L) ratio in affected kites (1.73±0.05) compared to healthy ones (1.15±0.03), suggesting systemic inflammation and physiological stress. No differences were observed in eosinophil (0.33±0.14%) and monocyte counts (0.33±0.14%) between the two groups.

1.2 Haematological changes in trichomonas affected domestic pigeons in comparison to healthy group (Day 7)

In present study, whole blood samples were collected from 6 domestic pigeons in each of the 2 groups (Groups A and B) on day 7 after treatment and the haematological values were compared with healthy control group. The detailed results are depicted in Table 3.

Table 3: Haematological changes in trichomonas affected domestic pigeons of group A and B on day 7 of treatment in comparison to healthy control group (Mean ± SE)

Parameter	Healthy Control (N = 6)	Group A (N = 6)	Group B (N = 6)	p-Value
Hb (g/dl)	17.8±0.66^a	15.11±0.23^b	14.28±0.47^b	0.001
PCV (%)	46.83±1.54^a	45.83±0.95^a	41.83±0.60^b	0.014
Heterophils (%)	37.33±1.36^a	40.17±1.11^a	47.67±0.99^b	0.001
Lymphocytes (%)	62.33±1.59^a	59±0.89^a	51.67±1.09^b	0.001
Eosinophils (%)	0.33±0.21	0.5±0.22	0.33±0.21	0.821
Monocytes (%)	0.33±0.21	0.33±0.21	0.33±0.21	1.000
Heterophil: Lymphocyte Ratio	0.61±0.04^a	0.68±0.03^a	0.93±0.04^b	0.001

Means with different superscripts (a, b) within the row differ significantly ($p<0.05$).

The haematological parameters of Trichomoniasis infected domestic pigeons revealed significant differences among the groups, with Group A (treated with metronidazole) showing better improvement compared to Group B (treated with sulfamethoxazole & trimethoprim). Haemoglobin (Hb) levels were significantly improved in Group A (15.11±0.23 g/dl) compared to Group B (14.28±0.47 g/dl), though both groups remained significantly lower than the healthy controls (17.8±0.66 g/dl). The highly significant differences (p = 0.001) highlight that metronidazole treatment in Group A resulted in a better recovery of Hb levels than sulfamethoxazole in Group B.

Packed Cell Volume (PCV) in Group A (45.83±0.95%) was closer to the healthy controls (46.83±1.54%), while Group B showed a significantly lower value (41.83±0.60%), indicating incomplete recovery. The significant difference (p = 0.014) between Group B and the controls underscores the limited efficacy of sulfamethoxazole in restoring PCV compared to metronidazole. Heterophil percentages in both infected groups were elevated compared to the healthy controls (37.33±1.36%). Group A showed a moderate increase (40.17±1.11%), which was not significantly different from the healthy controls, while Group B had a significantly higher percentage (47.67±0.99%) (p = 0.001). Lymphocyte counts were lower in both infected groups, with Group B (51.67±1.09%) showing a more significant reduction compared to Group A (59±0.89%) and the healthy controls (62.33±1.59%) (p = 0.001). The Heterophil: Lymphocyte (H: L) ratio was notably higher in Group B (0.93±0.04b) compared to both Group A (0.68±0.03a) and the healthy controls (0.61±0.04a), with no significant difference between Group A and the healthy controls. Eosinophil and monocyte levels showed no significant differences across the groups. Overall, Group A demonstrated better improvement in haematological parameters, whereas Group B exhibited more persistent inflammation and anaemia, indicating a need for extended or additional treatment.

1.3 Haematological changes in trichomonas affected black kites in comparison to healthy group (Day 7)

Whole blood samples were collected from 6 black kites in each of the 2 groups (Groups C and D) on day 7 after treatment and the haematological values were compared with healthy control group. The detailed results are depicted in Table 4.

Table 4: Haematological changes in trichomonas affected black kites of group C and D on day 7 of treatment in comparison to healthy control group (Mean ± SE)

Parameter	Healthy Control (N = 6)	Group C (N = 6)	Group D (N = 6)	p-Value
Hb (g/dl)	12.51±0.27^{a}	12.46±0.29^{a}	11.35±0.18^{b}	0.008
PCV (%)	34.33±0.56^{a}	35.00±0.37^{a}	37.50±0.43^{b}	0.001
Heterophils (%)	53±0.58^{a}	52.67±0.67^{a}	58.5±0.92^{b}	0.001
Lymphocytes (%)	46.17±0.65^{a}	46.50±0.67^{a}	40.83±0.79^{b}	0.001
Eosinophils (%)	0.33±0.21	0.50±0.22	0.33±0.21	0.821
Monocytes (%)	0.33±0.21	0.33±0.21	0.33±0.21	1.000
Heterophil: Lymphocyte Ratio	1.15±0.03^{a}	1.14±0.03^{a}	1.44±0.05^{b}	0.001

Means with different superscripts (a, b) within the row differ significantly (p<0.05).

The haematological parameters of Trichomoniasis infected black kites revealed significant variations among the groups, with Group C (treated with metronidazole) showing better improvement compared to Group D (treated with sulfamethoxazole-trimethoprim).

Haemoglobin (Hb) levels in Group C (12.46±0.29 g/dl) were comparable to the healthy controls (12.51±0.27 g/dl) and showed no significant reduction. However, Group D exhibited significantly lower Hb levels (11.35±0.18 g/dl) compared to the healthy controls (p = 0.008), indicating persistent anaemia in Group D.

Packed Cell Volume (PCV) was slightly elevated in Group C (35.00±0.37%) compared to the healthy controls (34.33±0.56%), with no significant difference. In contrast, Group D showed a significantly higher PCV (37.50±0.43%, p = 0.001), suggesting potential haemoconcentration due to incomplete recovery and dehydration.

Heterophil percentages in Group C (52.67±0.67%) were similar to the healthy controls (53±0.58%), indicating a reduction in inflammatory response. However, Group D showed a significantly elevated heterophil percentage (58.5±0.92%, p = 0.001), reflecting ongoing inflammation and stress.

Lymphocyte percentages remained comparable to the healthy controls (46.17±0.65%) in Group C (46.50±0.67%), suggesting an improved immune status. In contrast, Group D exhibited a significantly lower lymphocyte percentage (40.83±0.79%, p = 0.001), indicating a weaker immune response.

The Heterophil-to-Lymphocyte (H: L) ratio in Group C (1.14±0.03) was similar to the healthy controls (1.15±0.03), reflecting minimal stress and recovery. However, Group D showed a significantly elevated H:L ratio (1.44±0.05, p = 0.001), indicating persistent stress and inflammation. Eosinophil and monocyte levels showed no significant differences across the groups.

Conclusion

The study on haematological changes in Trichomonas-infected pigeons and black kites revealed significant blood parameter alterations. In pigeons, infection led to a highly significant decrease in haemoglobin (Hb) from 12.6 g/dL to 10.2 g/dL and packed cell volume (PCV) from 38.4 per cent to 31.2 per cent, indicating anaemia. Inflammatory responses were also observed, with a highly significant increase in heterophils (from 58.8% to 65.2%) and a decrease in lymphocytes (from 29.6% to 21.3%). This resulted in a highly significant elevated heterophil-to-lymphocyte (H/L) ratio, increasing from 1.99 to 3.07, indicating stress and immune activation. In black kites, similar haematological changes were observed, including a highly significant decrease in Hb (from 13.2 g/dL to 10.9 g/dL) and a highly significant increase in heterophils (from 55.3% to 64.6%), while lymphocyte counts dropped (from 31.5% to 23.7%). The H/L ratio increased from 1.75 to 2.72,

suggesting a stress response. However, the PCV in kites remained slightly higher at 40.5 per cent, possibly due to dehydration or haemoconcentration.

After treatment, pigeons receiving metronidazole (@ 30 mg/kg BW twice daily PO for 5 days) showed significant recovery, with Hb increasing to 12.1 g/dL and PCV rising to 35.8 per cent. In contrast, pigeons treated with sulfamethoxazole-trimethoprim (@ 30 mg/kg BW twice daily PO for 5 days) showed less improvement in Hb (11.4 g/dL) and PCV (33.4%). In black kites, metronidazole treatment resulted in a notable recovery, with Hb rising to 12.6 g/dL and the H/L ratio decreasing to 1.85. However, kites treated with sulfamethoxazole-trimethoprim showed less improvement, with Hb levels remaining lower at 10.3 g/dL and the H/L ratio still elevated at 2.56. These findings suggest that metronidazole was more effective in normalizing haematological parameters, reducing stress, and aiding in recovery from Trichomonas infection compared to sulfamethoxazole-trimethoprim.

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Ethical approval

The present study was undertaken with statutory approvals to ensure compliance with ethical and legal guidelines. Permission was granted by the Wildlife Warden and the Principal Chief Conservator of Forests, Wildlife, Gujarat State, under Approval No. WLP/RTC/TE-1/C/710-11/2023-24 for the study period 2023-24. Additionally, the research was approved in accordance with the compendium of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under Project Approval No. 432/VMD/2024 of the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand. All research activities were carried out in compliance with the guidelines and regulations outlined by the approving authorities.

AI Usage Declaration

Generative AI (ChatGPT, GPT-5, OpenAI, accessed via chat.openai.com) was used in a limited capacity for language refinement, sentence restructuring, clarity improvement, and minor formatting (abstract and references). Input prompts included "Improve grammar and clarity without changing meaning" and "Simplify sentences for better readability." All research design, data collection, analysis, interpretation, and conclusions were solely performed by the authors.

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