RESEARCH ARTICLE

Molecular Diagnosis and Synergistic Therapeutic Strategies for Pigeon Malaria

Tamileniyan Elangovan[1], Jeevitha Murugesan[1], Karthika Koperumselvan[1]*, Veeraselvam Muthusamy[1], Latchumikanthan Annamalai[2], Saravanan Mani[1], Velusamy Rangasamy[2]

Abstract

A flock of 12 domestic pigeons were presented to Veterinary Clinical Complex of the Institute at Orathanadu (India) with the history of complete inappetence, greenish diarrhoea and regurgitation. Clinical examination of the birds showed signs of dullness and soiled vent. On blood smear examination a characteristic halter shaped gamont of *Haemoproteus columbae* encircling the nucleated red blood cells were observed and confirmed molecularly as *Haemoproteus* sp. with genus-specific primer. The blood picture revealed increased white blood cells count suggesting acute parasitemia. The flies *Pseudolynchia canariensis* were recovered from the wings and body surface of the pigeons. The birds were treated with Chloroquine phosphate @ 5 mg/kg b.wt., orally for six days and two doses of Buparvaquone @ 5 mg/kg b.wt., i/m at 72 h interval with multivitamin supplementation. After six days of synergistic therapy the clinical condition of the pigeons showed complete recovery without any complication.

Key words: Buparvaquone, Chloroquine, Domestic pigeon, *Haemoproteus* sp., Pigeon malaria.

Ind J Vet Sci and Biotech (2024): 10.48165/ijvsbt.20.6.29

Introduction

Pigeon rearing is a profitable sector that includes both large-scale aviaries and households. Pigeons may be found in almost every town and city in the globe, where they can live with humans as a source of food, hobby, and experimentation. Additionally, pigeons play a crucial role in scientific research, serving as a model organism in studies of behaviour, genetics, and navigation. This multifaceted interaction highlights the importance and versatility of pigeons in human society. Parasitic infections are of significant health concerns in pigeons, affecting growth rate, egg production, immunosuppression and in severe cases, mortality. Pigeons are susceptible to protozoan diseases, including *Haemoproteus columbae* an apicomplexan parasite of pigeons, is often known as 'pseudomalaria' or 'pigeon malaria' due to its close relationship to *Plasmodium* species (Friend and Franson, 1999) transmitted by hematophagous, hippoboscid fly *Pseudolynchia canariensis*. This infection affects both domestic and wild pigeons and it previously thought to be non-pathogenic (Benett *et al.,* 1988), these infections recently have been linked to decreased fertility in wild birds. The condition is lethal for young squabs and distressed pigeons, but non-pathogenic in adult birds (Rosyadi *et al.,* 2021). Clinical symptoms of *H. columbae* infection include anorexia, lethargy, depression, dyspnea, torticollis and diarrhoea (Soulsby, 1982; Masharana and Kumar, 2016). The present study was directed towards the synergistic effect of buparvaquone and chloroquine against *H. columbae* in domestic pigeons of Cauvery Delta region of Tamil Nadu.

[1]Department of Veterinary Medicine, Veterinary College and Research Institute, Orathanadu-614625 TANUVAS, Tamil Nadu, India

[2]Department of Veterinary Parasitology, Veterinary College and Research Institute, Orathanadu-614625 TANUVAS, Tamil Nadu, India

Corresponding Author: Karthika Koperumselvan, Department of Veterinary Medicine, Veterinary College and Research Institute, Orathanadu-614625 TANUVAS, Tamil Nadu, India. e-mail: karthika1988.6@gmail.com

How to cite this article: Elangovan, T., Murugesan, J., Koperumselvan, K., Muthusamy, V., Annamalai, L., Mani, S., & Rangasamy, V. (2024). Molecular Diagnosis and Synergistic Therapeutic Strategies for Pigeon Malaria. Ind J Vet Sci and Biotech. 20(6), 151-154.

Source of support: Nil

Conflict of interest: None

Submitted 28/06/2024 **Accepted** 02/08/2024 **Published** 10/11/2024

Materials and Methods

A flock of 12 pigeons was reported to the Exotic and Special Species Medicine Referral Clinic, Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, TANUVAS (India) with the history of complete inappetence, diarrhoea and regurgitation. Among 12 birds, 2 pigeons showed the presence of torticollis (Fig. 1). Twelve pigeons in the flock had already died with the same symptoms. On clinical examination lethargy, ruffled feather, ocular discharge from both the eyes, and soiled vent were observed.

Sample Collection and Processing

Ectoparasites noticed over the feather region were collected in 70 % ethanol for morphological identification. Blood samples

from wing vein of all pigeons were collected in heparin vacutainers and clot activators. Thin blood smears were made and subjected to Giemsa's staining (9:1 dilution) for screening haemoparasites. The haematological parameters were determined as per standard protocol (Coles, 1986), and biochemical analysis was done using automatic analyzer (Spectra®).

DNA Isolation and Polymerase Chain Reaction

DNA was extracted from 200 µL of heparinized blood using the Qiagen DNeasy® blood & tissue kit and stored at -20°C for future use. The rRNA gene of *Haemoproteus* sp. was subjected to PCR using genus-specific primers under the following cyclic conditions with initial denaturation of 94°C for 5 min, denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, extension at 72°C for 30 sec for 36 cycles and final extension at 72°C for 5 min. The PCR amplicons were electrophoresed on 1.5% agarose gel and visualised under UV transilluminator.

Treatment Protocol

The pigeons were treated with oral Chloroquine phosphate (Lariago®) @ 5 mg/kg b.wt. with two doses of Buparvaquone (Butalex®) @ 5 mg/kg b.wt. were given intramuscularly at three days intervals (Raval *et al.,* 2016; Joshi *et al.,* 2017). In addition, multivitamin syrup (Birdsplus®) @ 0.5 mL was also provided daily for one week in drinking water orally. In addition, deltamethrin spray was recommended for vector control.

Results and Discussion

The blood smear examination revealed intra-cytoplasmic gametocytes of *Haemoproteus columabe* encircling the nucleated RBC of the pigeon (Fig. 2). Haemogram suggested slight elevation of leucocytes, heterophils and eosinophils suggestive of acute parasitemia, with the normal level of other blood cells and serum parameters (Table 1). The ectoparasites recovered from the wing were identified as *Pseudolynchia canariensis* (Fig. 3) based on the morphological characters like short antennae, piercing-sucking mouthparts for blood-feeding and strong claws for clinging. The isolated DNA amplified using genus-specific primers yielded 523 bp amplicons, confirming the rRNA gene of *Haemoproteus* sp. (Fig. 4). Regular monitoring revealed complete recovery of the pigeons in one week with the absence of *Haemoproteus* sp. infection in blood smear examination.

Pigeon malaria is caused by *Haemoproteus columbae*, a haematozoan that is genetically identical to the malaria parasite *Plasmodium*. The clinical signs observed were anorexia, lethargy, diarrhoea, torticollis and regurgitation, which was in accordance with previous researchers (Selvaraj *et al.,* 2013; Varshney *et al.,* 2014; Masharana and Kumar, 2016; Joshi *et al.,* 2017). *Haemoproteus columbae* infection was also previously reported in pigeons from Chennai (Selvaraj *et al.,* 2013) and Thanjavur (Tamileniyan *et al.,* 2023) in Tamil Nadu. Most *H. columbae* infections in pigeons are asymptomatic and non-pathogenic. Nevertheless, young and immunocompromised pigeons may suffer from the disease. Schizogony in the lung and liver endothelium results in the expulsion of merozoites followed by their entry into the red blood cells and development into gametocytes (Soulsby, 1982). In blood smears, gametocytes are evident and partially surround the nuclei of red blood cells. In the natural avian hosts, *Haemoproteus* sp. are generally considered to cause minimal pathogenicity, especially in comparison to the genera *Plasmodium* and *Leucocytozoon* in avian hosts (Rosyadi *et al.,* 2021). However, in accidental hosts, such as captive birds in zoos and aviaries, clinical and fatal *Haemoproteus* infections have been documented. In these accidental hosts, severe haemoproteosis and death have been reported due to the rupture of megalomeronts in the muscle and liver, leading to significant pathological changes (Atkinson, 2009; Rosyadi *et al.,* 2021). During the infection in pigeons, significant increase was observed in total WBC count, heterophils and eosinophil levels. After recovery, the leucocytes count and eosinophil levels were reduced indicating the reduction of parasitic load. Mehmood *et al.* (2019) also reported a significant eosinophila in domestic pigeons infected with *Haemoproteus columabe*.

Table. 1: Haemotology and biochemical profile of pigeons affected with malarial parasites

Parameters	Infective Phase (n=12)	After Recovery (n=12)
Hemoglobin (g/dL)	11.40 ± 2.01	11.32 ± 2.55
RBC (10^6/µL)	2.92 ± 1.03	2.45 ± 1.40
WBC (10^3/µL)	29.46 ± 7.91	25.77 ± 6.88
Heterophils (%)	42.9 ± 3.13	39.9 ± 3.11
Lymphocytes (%)	41.30 ± 6.52	40.50 ± 6.33
Monocytes (%)	11.22 ± 4.12	11.01 ± 4.21
Basophils (%)	1.39 ± 1.21	1.01 ± 0.99
Eosinophils (%)	11.66 ± 2.11	7.3 ± 2.52
AST (U/L)	310.1 ± 155	299 ± 156
ALT (U/L)	71.9 ± 61.9	75.9 ± 58.3
Glucose (mg/dL)	270 ± 96.3	291 ± 85.5
Urea (mg/dL)	19.49 ± 3.6	20.66 ± 3.5
Creatinine (mg/dL)	0.21 ± 0.11	0.2 ± 0.19
Total protein (g/dL)	9.1 ± 0.9	9.8 ± 0.7

Treatment is frequently administered during a deadly epidemic in a flock when morbidity percentages are high. Chloroquine is a well-known antimalarial medication that works against *Plasmodium* sp. and *Haemoproteus* sp. by inhibiting haeme polymerase and resulting in accumulation of toxic haeme derivatives, which damage the protozoal cellular structures (Zhou *et al.,* 2020). Buparvaquone, a hydroxynaphthoquinone, destroys protozoan parasites by blocking mitochondrial electron transport at the cytochrome complex. This impairment in mitochondrial activity shuts off the energy production and metabolic functions, resulting in its eradication (Saralaya

Fig. 1: Pigeon showing torticollis

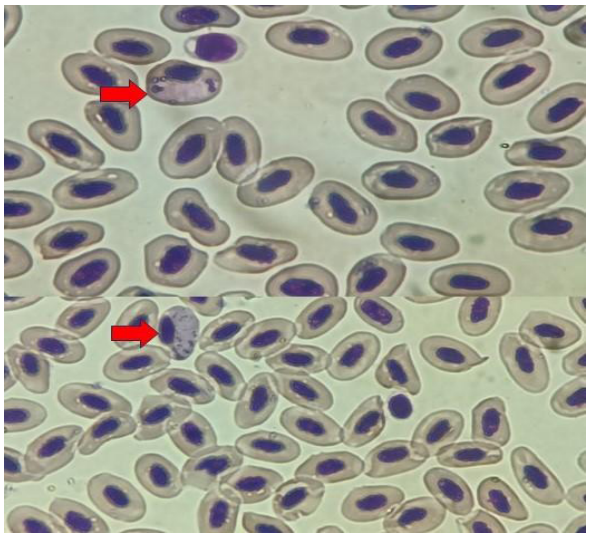

Fig. 2: Halter shaped gametocytes of *Haemoproteus* sp.

Fig. 3: *Pseudolynchia canariensis* recovered from the wings and body of the pigeons

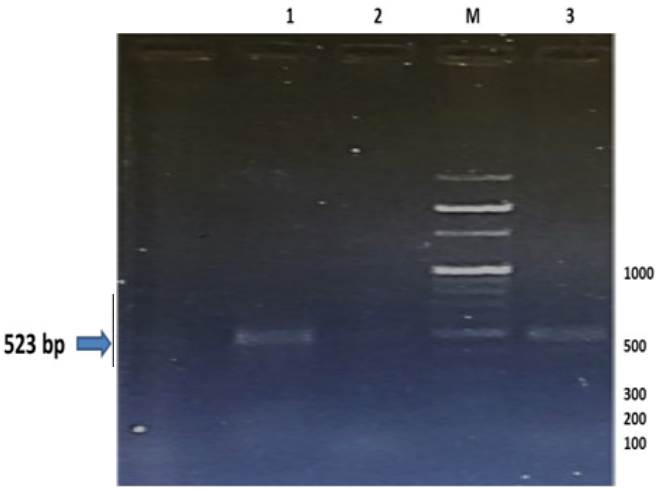

Fig. 4: PCR amplification of 523 bp rRNA gene of *Haemoproteus* sp.; M- 100 bp DNA Ladder; L_1 ,L_2 & L_3- *Haemoproteus* sp.

et al., 2023). Successful therapeutic regimen attained using a single therapy of buparvaquone against *Haemoproteus columbae* by previous researchers (El-Metenawy. 1999; Raval *et al.,* 2016; Joshi *et al.,* 2017) corroborates our findings.

Earlier researchers (Selvaraj *et al.,* 2013; Chand *et al.,* 2018; Manohar *et al.,* 2020) have demonstrated that chloroquine therapy has a positive effect on treating *Haemoproteus* infections in pigeons. Chloroquine and buparvaquone act on different biochemical pathways within *Haemoproteus* sp. making them potentially complementary in combined therapy. Understanding these mechanisms is crucial for developing effective treatment protocols against pigeon malaria, potentially reducing morbidity and mortality in affected pigeon populations. Our study successfully establishes a chemotherapeutic protocol utilizing chloroquine and buparvaquone for the treatment of *Haemoproteus columbae* in domestic pigeons. This research represents the first documented instance of effectively employing these drugs in combination to combat this parasitic infection in pigeons with molecular confirmation, offering a promising approach to managing *Haemoproteus* infections in avian species.

Further research is needed to optimize dosages and administration strategies for these drugs to maximize their efficacy and minimize resistance development in *Haemoproteus* infection in pigeons.

Acknowledgement

The authors express their sincere thanks to the Dean and the Professor and Head,Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, TANUVAS, for

the provision of research facilities and unwavering support to carry out the study.

References

Atkinson, C.T. (2009). *Haemoproteus*. In: *Parasitic Diseases of Wild Birds*. Wiley: Hoboken, NJ, USA, pp. 11-34.

Benett, G.F., Caines, J.R., & Bishop, M.A. (1988). The influenza of blood parasites on the body mass of passeriform birds. *Journal of Wildlife Diseases, 24,* 339-343.

Chand, N., Singh, H., & Kumar, A. (2018). Successful therapeutic management of pigeon (*Columba livia domestica*) malaria with Chloroquine. *Indian Journal of Animal Research*, *52,* 326-327.

Coles, E. H. (1986). *Veterinary Clinical Pathology.* 4th ed., WB Saunders.

El-Metenawy, T.M. (1999). Therapeutic effects of some antihaematozoal drugs against *Haemoproteuscolumbae* in domestic pigeons. *DTW. Deutsche Tierarztliche Wochenschrift*, *106*(2), 72.

Friend, M., & Franson, J. (1999). Parasitic diseases. In Ciganovich, E. A. (Ed.), *Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds*. US Geological Survey, pp. 193-262.

Joshi, V., Dimri, U., Alam, S., & Gopalakrishnan, A. (2017). Buparvaquone therapy in a rock pigeon infected with *Haemoproteus columbae* showing torticollis. *Journal of Parasitic Diseases*, *41*(2), 514-516.

Maharana, B.R., & Kumar, B. (2016). Pseudomalaria in a domestic pigeon: A case report. *Journal of Parasitic Diseases*, *16,* 1-3.

Manohar, M., Justin Davis, K., Sali, S., & Vijayakumar, K. (2020). Therapeutic management of pigeon malaria with chloroquine: A case report. *Journal of Food and Animal Sciences*, *1*(1), 63-65.

Mehmood, S., Nashiruddullah, N., Ahmed, J.A., & Borkataki, S. (2019). Parasitic affections of domesticated pigeons (*Columba livia*) in Jammu, India. *Annals of Parasitology*, *65*(1), 53-64.

Raval, S.H., Singh, R.D., & Joshi, D. V. (2016). Management of *Haemoproteuscolumbae* infestation in White Fantail Pigeons. *Intas Polivet*, *17,* 214-215.

Rosyadi, I., Salasia, S.I.O., Argamjav, B., & Sato, H. (2021). Impact of subclinical *Haemoproteuscolumbae* infection on farmed domestic pigeons from Central Java (Yogyakarta), Indonesia, with special reference to changes in the hemogram. *Pathogens*, *10*(4), 440.

Saralaya, S.S., Shashiprabha, & Kanakamajalu, S. (2023). A comprehensive review on the therapeutic applications and synthetic approaches of Buparvaquone. *Mapana Journal of Sciences*, *22*(2), 141-168.

Selvaraj, P., Umesh, C.G., Preethi, U., Sumathi, D., Nambi, A.P., & Prathaban, S. (2013). *Haemoproteus columbae* infection in a flock of pigeons. *Indian Journal of Field Veterinarians*, *9,* 61-62.

Soulsby, E.J.L. (1982). *Helminths, Arthropods and Protozoa of Domesticated Animals*. 7th edn., ELBS, London, pp. 1-772

Tamileniyan, E., Jeevitha, M., Karthika, K., Veeraselvam, M., Latchumikanthan, A., Velusamy, R., Saravanan, M., Yogeshpriya, S., & Jayalakshmi, K. (2023). Therapeutic management of concurrent infection of *Haemoproteus columbae* and *Trichomonas* spp. in domestic rock pigeons. *Indian Journal of Veterinary Medicine*, *43,* 68-70.

Varshney, J.P., Deshmukh, V.V., & Chaudhary, P.S. (2014). Pseudomalaria (*Haemoproteus columbae*) in pigeon shelter. *Intas Polivet*, 15, 176-177.

Zhou, W., Wang, H., Yang, Y., Chen, Z. S., Zou, C., & Zhang, J. (2020). Chloroquine against malaria, cancers and viral diseases. *Drug Discovery Today*, *25*(11), 2012-2022.