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Molecular diagnosis of hemoplasma infection in cats and sheep with hematological studies

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ABSTRACT

Background: Diagnosis of hemoplasma infections is currently based on microscopical examination of blood smears, together with the results of specific polymerase chain reaction (PCR) assays.

Aim: In the present study, molecular detection using 16S rRNA was used as a diagnostic approach for detection and genetic characterization of the hemoplasma in cats and sheep. However, hematological studies were applied to sheep showing emaciation.

Methods: In the present study, blood smears examination, hemogram, blood cells count, and molecular detection using 16S rRNA were used as a diagnostic approach for the detection and genetic characterization of the hemoplasma in cats and sheep.

Results: We found that hemoplasma incidence in cats in the examined samples was 8%, and *Candidatus M. haemominutum* was detected at 202 bp band size by 16SrRNA and *Candidatus M. haemovis* in sheep. The infection rate in the examined sheep was 4% (2/50) by nested PCR giving specific bands at 1,060 and 506 bp. Giemsa-stained blood smear from an affected sheep with hemoplasma showed a small oval to ring-shaped organism noticed on the surface of erythrocytes. The organisms have other variable shape (cocci, small rods are arranged in short chains) with the presence of free organisms in the background of blood smear. Haemovis are epicellular parasites which attached in the indentation and invagination of the erythrocyte surface causing the direct injury of red blood cells. Poikilocytosis was very obvious. Abnormal features of red corpuscles were noticed as pointy or thorn-like projections. Destruction of erythrocytes causes anemia; these features led to a significant decrease in total erythrocyte count, and polychromasia was noticed. Moreover, neutrophils with foamy and opaque cytoplasm were observed. The leucogram showed leucopenia due to lymphocytopenia while, neutrophils rose, monocytes, and eosinophils showed an elevation level.

Conclusion: This is the first microscopical and molecular identification of haemovis in sheep in Giza, Egypt. Further investigation is recommended for epidemiology and control measures of haemotropic mycoplasma.

Keywords: Hemoplasma, Haemovis, CMhm, Hematological studies.

Introduction

Farm animals play an important role in maintaining global food (Ge *et al.*, 2024; Shalaby *et al.*, 2024; Al-Ebshahy *et al.*, 2025). They were subjected to different pathogens that affect their productivity (Ibrahiem *et al.*, 2023; Ata *et al.*, 2024; Kasem *et al.*, 2024; El-gbily *et al.*, 2025). Hemotropic mycoplasmas (HM) are small red blood cell (RBC) parasites that attach to the RBC resulting

in erythrocytic deformity and damage (Arendt *et al.*, 2024). In animals, disease manifestations due to HM infection are commonly reported in association with drug- or virus-induced immunosuppression, with stressors such as poor nutrition, pregnancy, or lactation, or with concurrent infection with other more virulent pathogens (Tanahara *et al.*, 2010). Because of the haemato-tropic nature, the infection results in anemia,

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weakness, paler mucous membranes, and tachypnea, in a wide range of animals (Tasker, 2020). However, infection in sheep causes anemia, fever, anorexia, and lymphadenopathy (Song *et al.*, 2014).

The pathogen was found to be transmitted mainly by blood-sucking insects, but other routes were also recorded either by blood transfusion or transplacental (de Souza Ferreira *et al.*, 2024).

Previously, the accurate laboratory diagnosis of hemoplasma infections was generally impaired by the insufficient sensitivity and specificity of the microscopic examination in blood-stained smears. All attempts to isolate and culture HM have failed, so the analysis of biological features and pathogenesis of the bacteria was limited (Kmetiuk *et al.*, 2025). The development of molecular methods shared in the accurate diagnosis of multiple pathogens (Ata *et al.*, 2021; Salman *et al.*, 2024), in particular polymerase chain reaction (PCR) amplification based on the 16SrDNA gene, has greatly facilitated the molecular characterization of these causative agents. This diagnostic approach has resulted in the discovery of several novel hemoplasmas species in different hosts (Willi *et al.*, 2006; Hoelzle *et al.*, 2011). The first molecular evidence of the occurrence of “*Candidatus M. haemovis*” in sheep and goats was reported in China (Wang *et al.*, 2017). At the national level first molecular evidence of the occurrence of hemoplasma in different animals as in cats *Candidatus M. haemominutum* (CMhm) and “*Candidatus M. haemovis*” in sheep and goats in Egypt was in 2024 (Eissa *et al.*, 2024).

In the present study, blood smears examination, hemogram, blood cells count, and molecular detection using 16S rRNA were used as a diagnostic approach for detection and genetic characterization of the hemoplasma in cats and sheep.

Material and Methods

Ethical approval

This research was done under the approval of the Institutional Animal Care and Use Committee (ARC-IACUC) of the Agricultural Research Center, with the IACUC Protocol Number: ARC AHRI 23 32

Animals

Studied animals were admitted from different localities of Giza governorate; Egypt, to the Animal Health Research Institute (AHRI) showing signs of anemia and emaciation or apparently healthy.

Blood sample collection

Hundred blood samples were collected on dipotassium Ethylene-Diamine Tetra Acetic Acid as an anticoagulant from the jugular veins of 50 sheep and from the caudal veins of 50 cats. The collected samples were stored in a cooler box and transported to the Mycoplasma department (AHRI main branch) for molecular diagnosis of hemoplasma and Pathology department (AHRI main branch) to determine parameters of hemogram which include erythrocyte count (RBCs),

total and differential leucocyte counts using Exigo Veterinary Hematology analyzer with serial No. 5169, and software version 2.7.6, manufactured by Boule Medical A B, Sweden.

Extraction of DNA

The total genomic DNA was extracted from blood samples using GF- 1 Blood DNA Extraction kit (VIVANTIS) Cod.no.GF-BD-100 (Malezia). The quality and concentration of the eluted DNA were assessed using spectrophotometer.

Molecular detection

The following primers were used for molecular identification of hemoplasma in cats. The following primers were used according to Tanahara *et al.* (2010) with modifications:

F-5' ATGCCCTCTGTGGGGGATAGCCG 3'

Mycoplasma haemofelis

Common R-5' ATGGTATTGCTCCATCAGACTTTCG

3' reverse primer CMhm

F-5' CTGGGAACTAGAGCTTCGCGAGC 3' specific forward (CMhm primer).

2-Primer used for *Candidatus M. turicensis* primer:

CMt F-5'AGAGGCGAAGGCGAAAACCT3'

R-5'CTACAACGCCGAAACACAAA 3'

The PCR thermal program included initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 45 seconds, annealing using a temperature gradient (56°C for 45 seconds), and polymerization (72°C for 45 seconds).

3-Nested PCR for hemoplasma of sheep according to Wang *et al.* (2017):

The following primers were used for the detection of hemoplasma in sheep, 1st PCR primer sets: A1 5'GGATAGCAGCCCCGAAAGG 3'

and A2 5' GCAGCCCCAAGGCATAAGG 3'

B1 5'CTACGGGAAGCAGCAGTG3' and

B2 5' CTCGACCTAACATCAAATAC CT3'

Amplification procedures: First round PCR was performed as follows, 94°C for 5 minutes, then 30 cycles of 94°C for 30 seconds, 61°C for 30 seconds, and 72°C for 1 minute, with a final step of extension at 72° for 7 minutes, followed by a second round of nested PCR at 94°C for 5 minutes, then 35 cycles of 94°C for 30 seconds, 59°C for 30 seconds, and 72°C for 45 seconds, with a final extension step at 72°C for 7 minutes.

Results

Hematological results

Table 1 illustrates changes in hemogram of sheep infected with haemovis. The erythrogram showed a significant decrease in total erythrocyte count and polychromasia was noticed. Also, the leucogram demonstrated the presence of leucopenia due to lymphocytopenia while neutrophils rose, also, monocytes, and eosinophils showed elevation.

Giemsa-stained blood smear from an affected sheep with hemoplasma showed a small oval to ring-shaped

organism noticed on the surface of erythrocytes (Fig. 1a and b). The organisms have other variable shape (cocci, small rods are arranged in short chains) blood smears showing erythrocytes injured by hemoplasma (Fig. 2b and c). Furthermore, the presence of free organisms in the background of blood smear (Fig. 3a) and abnormal features of red corpuscles was noticed as pointy or thorn-like projections (Fig. 3b and c). However, an activated neutrophil with foamy and opaque cytoplasm was recorded (Fig. 3d).

Molecular detection of hemoplasma in cats

In the present study, different primers were used to detect Feline hemoplasmas including *M. haemofelis*, *M. turicensis* and *M. haemominutum*, we found that only CMhm was detected with an incidence of 8% (4/50) at position 202 bp by 16SrRNA (Fig. 4).

Molecular detection of *Candidatus M. haemovis* in sheep

Blood samples from sheep from the Giza governorate were collected during October –December 2024. The infection rate in sheep was 4% (2/50) by nested PCR giving specific bands at 1,060 bp and 50 6bp (Figs. 5 and 6).

Discussion

Haemotropic mycoplasmas or hemoplasmas is a term including a group of small bacteria known as

Haemobartonella or *Eperythrozoon* spp. which lack a cell wall that infects the erythrocytes of laboratory, domestic, and wild animals (Alabi et al., 2020).

Infections caused by hemoplasmas can lead to a range of conditions, from severe hemolytic anemia that can be life-threatening to more subtle issues such as chronic anemia, poor growth, and infertility. Furthermore, these organisms may also contribute to the worsening of retroviral infections, tumors, and immune-mediated disorders. The close interaction between hemoplasma organisms and RBCs results in cellular damage through immune-mediated responses and other mechanisms that remain unclear. Even with a strong immune reaction and antibiotic treatment, infected animals likely become chronic carriers after their clinical symptoms have subsided (Messick, 2004). Our investigation revealed the presence of multiple stages of direct affected erythrocytes by hemoplasma (*haemovis*) beginning with the attachment of organism to RBC surfaces and then through residing in indentation and invagination, distortion of red blood corpuscle until reaches complete injury. More than 10% of erythrocytes become abnormally shaped (poikilocytosis) accompanied by intravascular

Table 1. Illustrated changes in hemogram of sheep infected with *haemovis*.

Groups	Apparent healthy sheep	Infected sheep
Total RBCs $\times 10^6/\text{ul}$	14.47 ± 0.45	10.76 ± 3.68
Total WBCs $\times 10^3/\text{ul}$	11.83 ± 0.153	10.55 ± 0.071
Neutrophils $\times 10^3/\text{ul}$	6.43 ± 0.495	4.3 ± 0.089
Lymphocytes $\times 10^3/\text{ul}$	6.943 ± 0.238	2.905 ± 0.205
Monocytes $\times 10^3/\text{ul}$	0.28 ± 0.069	0.657 ± 0.319
Eosinophils $\times 10^3/\text{ul}$	0.277 ± 0.064	0.757 ± 0.186
Basophils $\times 10^3/\text{ul}$	0.04 ± 0.069	0.055 ± 0.078

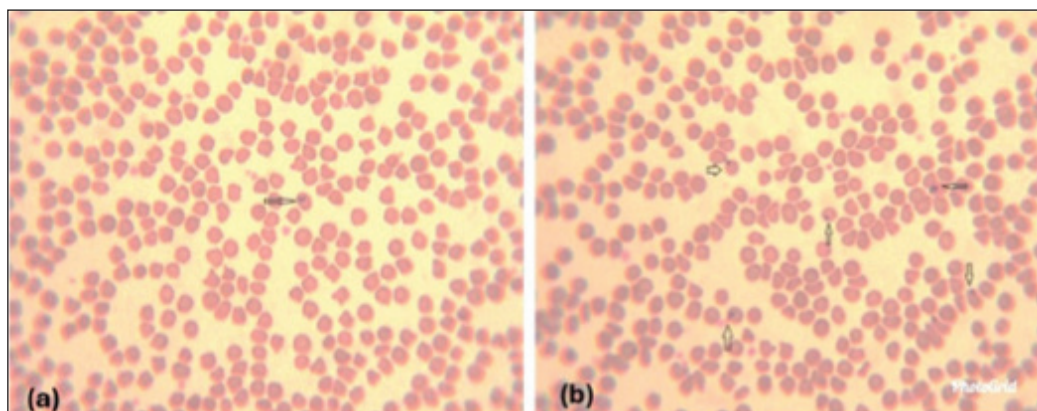


Fig. 1. (a, b): Blood smears showing different forms of hemoplasma attached to the surface of erythrocytes (arrows) (Wright, Giemsa stain).

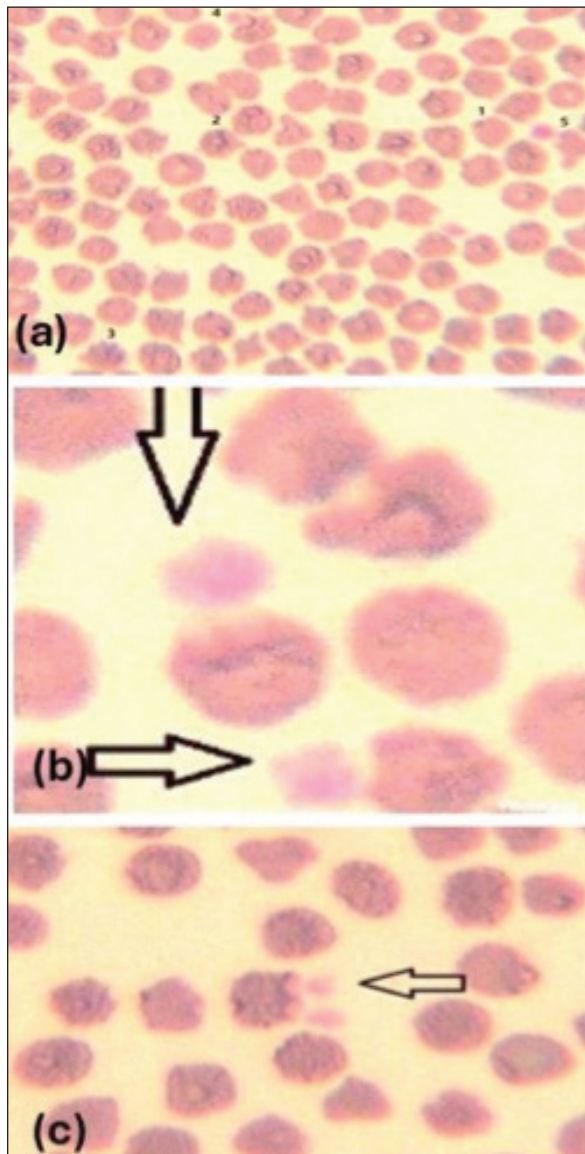


Fig. 2. (a): Blood smear showing stages of affected RBCs with hemoplasma, often, respectively (Wright, Giemsa stain). (b, c): blood smears showing erythrocytes injured by hemoplasma (Wright, Giemsa stain).

hemolysis and in addition to extravascular inducing hemolytic anemia.

In this study, polychromasia and immature RBCs were noticed in peripheral blood smear. Usually, it is a sign that erythrocytes are being destroyed quickly than body can replace them (hemolytic anemia) (Coles, 1986; Suzuki *et al.*, 2011). Leukogram in this study showed leucopenia due to lymphocytopenia while, neutrophils rose, also, monocytes and eosinophils showed elevation. In our study, the differential leucocytic count revealed a small number of activated neutrophils

with foamy opaque cytoplasm indicating the bacterial infection (Coles, 1986).

Suzuki *et al.* (2011) mentioned that variations in the hematological values might be attributed to infection stages rather than differences in the hemoplasma strains, which were almost identical in the 16S rRNA sequences as confirmed by Eissa *et al.* (2024).

Zachary (2017) reported that the effects of hemoplasma infection varied from subclinical to fatal anemia depending on host susceptibility, specific organism and dose. Intravascular hemolysis occurs, but anemia mainly is due to extravascular hemolysis. Although incomplete understanding of the pathogenic mechanisms but immune-mediated component is highly probable. Haemotropic mycoplasma induces unclear cold agglutination in infected individuals. These particular antibodies play an important role in the development of hemolytic anemia. According to the duration of hemolysis, there are varying degrees of erythroid hyperplasia in bone marrow. However, the red pulp of the spleen suffers from macrophage hyperplasia and erythrophagocytosis accompanied by increased plasma cell number as microscopical lesions during the acute hemoplasma infection. It is worth noting that although hemoplasma infection could occur in ovine species, clinical signs recorded in goats were milder than those of sheep (Stuen, 2016).

Multiple approaches were found to be used for the diagnosis of hemoplasma infection including microscopic examination. But, molecular detection was recorded to be the most sensitive tool for accurate diagnosis (Hampel *et al.*, 2014), especially this pathogen could not be cultivated and microscopic detection lacks specificity and sensitivity, and recommendation for only molecular studies should be considered (Arendt *et al.*, 2024).

Accordingly, the molecular approach was used for the diagnosis of the tested samples. Although felines were previously recorded by multiple hemoplasmas, based on the results obtained, only *M. haemominutum* was detected with a ratio of (8%). A variable range of infections from (9% to 16%) was recorded in European countries (Ravagnan *et al.*, 2017; Díaz-Regañón *et al.*, 2018). A higher rate was recorded in Thailand as the total infection of (38.05%) was positive for hemoplasma and 83.33% were infected with CMhm, while 13.33% were infected with *M. haemofelis*, and 3.33% with *Candidatus Mycoplasma turicensis* (Do *et al.*, 2020).

Interestingly, a recently published study about the infection rate in the same area was higher (20%) of CMhm but also, the *M. haemofelis* was not detected (Eissa *et al.*, 2024). It is worth to clear that fighting between cats was an important route for transmission of this pathogen between this animal species (Moore *et al.*, 2024).

A lower infection rate (4%) was determined in the examined blood samples of sheep. A similar rate

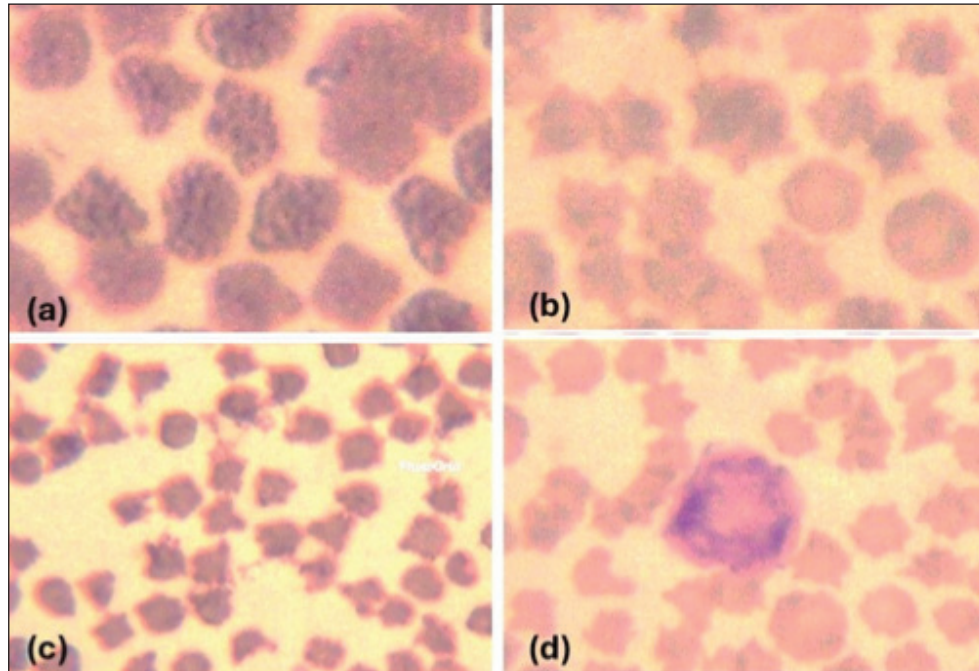


Fig. 3. (a): Blood smear showing deformation of many erythrocytes (Wright, Giemsa stain). (b, c): blood smears showing abnormal erythrocytes with features of poikilocytosis; pointy or thorn-like projections (destroyed erythrocytes, anemia) (Wright, Giemsa stain). (d): blood showing activated neutrophil with foamy and opaque cytoplasm (Wright, Giemsa stain).



Fig. 4. Lane 1: 100 bp DNA ladder; lanes 2 control positive (OQ565624) for CMhm; lane 3: control negative; lanes 4-7: positive samples for CMhm of cat at 202 bp.

(6%) was determined in Tunisia (Rjeibi *et al.*, 2015). However, a higher infection rate (39.3%) was recorded in Brazilian goats (Machado *et al.*, 2017). The highest extreme rate was found in 69% to 76% in the USA (Urie *et al.*, 2019) and in Brazil (79%) (Souza *et al.*, 2019). Infection in young age could lead to severe hemolytic

anemia (Paul *et al.*, 2020). While, in older ones, the infection rate is mostly chronic with less clinical signs but it greatly reflects on the animal productivity (Tagawa *et al.*, 2012). In conclusion, this is the first microscopical and molecular identification of haemovis in sheep in Giza, Egypt. Further investigation is

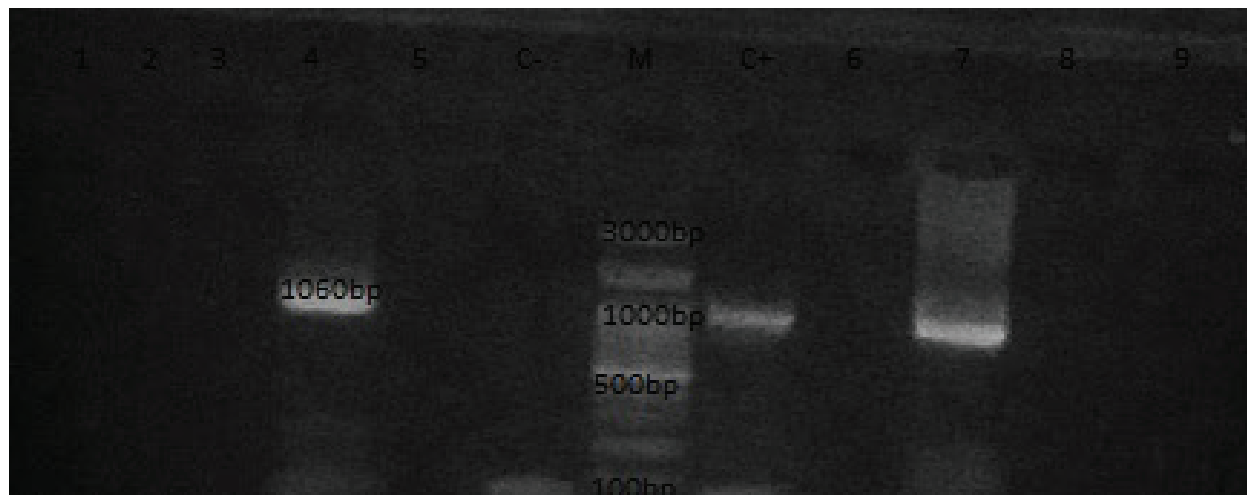


Fig. 5. PCR for sheep at 1,060 bp; Lane M: 100 bp ladder, lanes 1–3 and 5,6,8,9 negative samples for haemovis. Lane C-:control negative ; Lane C+: *M. ovis* positive strain (OQ310852); lane 4 &7: positive sheep samples at 1,060 bp.

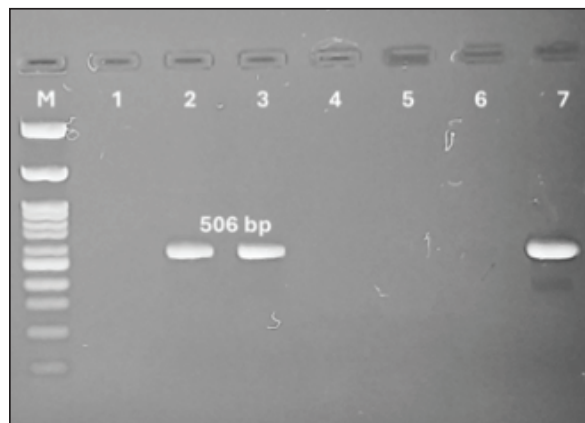


Fig. 6. Nested PCR for sheep samples at 506 bp; M: 100 bp ladder, lanes 1: Control negative. Lane 2: *M. Haemovis* positive strain OQ310852; lane 3 & 7: positive sheep samples at 506 bp; Lanes 4–6: Negative sheep samples.

recommended for epidemiology and control measures of haemotropic mycoplasma.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Authors' contributions

The authors contributed equally to this work.

Data availability

All data supporting the findings of this study are available within the manuscript.

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