



## A preliminary Study of Malaria infection (*Plasmodium* spp.) in Iraqi Livestock

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**M**ALARIA in livestock (cattle, buffalo, sheep, and goat) was detected and diagnosed in Mosul, Iraq. A total of forty infected animals of local breeds, from different ages and both sexes, showed *Plasmodium* parasites in their blood smears. Ten animals of each species were selected. All animals were clinically examined. The identification of *Plasmodium* parasites was done by microscopy and PCR assay. Fever, pale mucous membranes, lymphadenitis, jaundice, and recumbences are more common clinical signs were associated with malaria infection in livestock. Trophozoites, schizonts, and gametocytes appeared morphologically resemble those of *Plasmodium* spp. of humans. Sporozoites, Oocyte, and Ookinetes were observed in stained blood smears of some cows, sheep, and goats. A high degree of parasitemia and erythrocytes infected with two or more trophozoites were seen. Trophozoites were seen more visible in the peripheral blood smears stained with the direct Acridine Orange and wet mount preparations in comparison to the traditional Giemsa, and Leishman stains method. The presence of hemozoin crystals was diagnostic of malaria parasites. *Plasmodium* DNA was detected from all samples. We need further studies to genotype *Plasmodium* spp. in livestock and to understand the prevalence of malaria parasites in animals of Iraq.

**Keywords:** Malaria, *Plasmodium* spp., Livestock, Iraq.

### Introduction

Malaria is considered to be one of the most important infectious diseases in the world, and it will cause great morbidity and mortality in humans. It's caused by protozoan parasites of genus *Plasmodium*, including *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *P. knowlesi* belongs to phylum Apicomplexa and transmitted by blood-feeding of female mosquitoes of the genus *Anopheles*. Malaria (*Plasmodium* spp.) infection of animals, are more common than previously thought. *Plasmodium* exhibit a wide range of host preferences, such as humans, primates, ungulates, birds, rodents, chiroptera, and reptiles [1]. Malaria parasites of ungulates (hoofed mammals) were first observed in 1913 by Sir David Bruce during a survey of blood parasites

of grey duiker (*P. brucei*) and antelope (*P. cephalophi*) in Malawi [2]. In 1919, *Plasmodium bubalis* a malaria parasite of domestic water buffalo was identified in buffaloes blood film in India by Sheather [3]. *Plasmodium bubalis* has been reported again by molecular detection methods from water buffalo in southeast Asia [4,5]. *Plasmodium caprae* was identified recently in goat blood film by Kaewthamasorn et al. [6]. *Plasmodium caprae* DNA isolated from the domestic goat blood samples in Sudan and Kenya in Africa, Iran in west Asia, and Myanmar and Thailand in southeast Asia [6,7,8]. Also, ungulate malaria parasite was identified in blood smears of mouse deer (*P. traguli*), and a white-tailed deer (*P. odocoilei*) in North America [9]. *Plasmodium* parasites (presumptive *P. odocoilei*) was detected from white-tailed deer and *Anopheles* mosquitoes in several locations in the United States of

America [10]. Also, Plasmodium sequences from duiker antelope in Africa was reported in 2016 by Boundenga, et al. [11].

Malaria parasites in anopheline mosquitoes are maintained by humans and, in part, by non-human vertebrate hosts such as cows, pigs, goats, chicken, and dogs play an important role in their ecology blood-meal hosts [12]. Anopheles is happy to take a blood meal from beef, sheep and goats rather than from a human, which increase the use of livestock as bait to attract mosquitoes away from biting humans, and thus reduce malaria transmission, is an attractive proposition that has been mooted for many years [13]. The livestock play an important role in human malaria transmission, where a high percentage of families are involved in raising livestock also had the highest prevalence of clinical malaria because of keeping livestock like cattle, sheep, pigs, and goats in their house. [14]. Also, several studies have shown transfer and development of human Plasmodium in animals [15,16,17,18]. *Plasmodium knowlesi* is the only zoonotic malaria parasite of primates (macaques monkey) with a 24-hour erythrocytic cycle [19,20]. More recently, ungulates have been prioritized in many One Health initiatives because of close contact of humans and domestic ungulates, which creates a conduit for zoonotic transmission [21].

Although malaria parasites of the genus Plasmodium are known to infect a variety of vertebrate hosts, including ungulates and distributed globally, the clinical characterization of ungulate malaria infection has been limited [22]. Livestock such as cattle, buffalo, sheep, and goats are ecologically and economically important species, and the most widely distributed ungulates in Iraq. The microscopic and molecular-based study aimed to investigate malaria in Iraqi livestock, determine the characteristic morphology of different Plasmodium species, and evaluate the clinical effects of malaria on livestock health.

## **Materials and Methods**

### *Ethical approval*

The present study was approved by the College of Veterinary Medicine Committee.

### *Animal sampling and clinical examination*

During the examination of infected cattle, buffalo, sheep, and goats admitted to the Veterinary Teaching Hospital, the University of Mosul in Mosul, Iraq, we selected forty

animals (10 animals for each species) showed unidentified intra-erythrocyte and extra-erythrocyte structures in their blood smears. History taking and complete clinical examination were done including taking temperature, heart rate, respiratory rate, lymph node examination and mucosal observation for jaundice or pallor. One drop of blood was taken from the ear vein and subjected for blood smear microscopy. Blood samples were collected from all animals in four groups from jugular vein for molecular study.

### *Blood smear staining and microscopic analyses*

Thin and thick blood smears were prepared, air dried then stained with Giemsa Leishman's staining method for thin and thick smears was also used as a good alternative to Giemsa's stain for identifying Plasmodium parasites according to Sathpathi et al. [23]. Wet mounts were prepared by mixing a drop of blood with 10 to 15  $\mu$ l of a 10% solution of sodium dodecyl sulfate in water before examining under the Darkfield microscopy. Viewing was done at magnifications of x250 and x400 [25]. Giemsa or Leishman stain blood films were also examined under the Darkfield microscopy, but photography was done at x1,000 (under oil). The fluorescence microscopy (BX51 Olympus U-RFL-T-Japan) was used to look and enumeration for the malaria parasites by mixing a drop of infected blood with an acridine orange solution (0.1 mg/mL). Then, thin smears were made and examined under 100X [26]. The sizes of infected erythrocytes were measured relative to surrounding uninfected erythrocytes. The sizes of the parasites were measured using a measuring eyepiece micrometer at 1,000 $\times$  magnification. The Plasmodium parasite was identified and diagnosed by detailed morphological characteristics described for the diagnosis of human malaria [27, 28].

### *Determination of parasitaemia and parasite counts*

The parasitaemia of each infected animal in the study was calculated per  $\mu$ l of blood from thick blood films by counting the number of parasitized red blood cells per 200 white blood cells in over 100 fields and calculated using the actual white blood cell count of each animal. The percentage of each developmental stage of the parasite was determined based on the number of early trophozoites, late and mature trophozoites, schizonts, and gametocytes in thin blood films [19].

*DNA extraction and molecular assay*

Ten blood samples of each animal species (cattle, buffalo, sheep, and goats) were pooled into four groups. These pools contained DNA and RNA (spotted on FTA card) were extracted. Fifty  $\mu$ l pooled blood was used for DNA was extracted using the QiaAmp DNA mini kit (Qiagen, www.qiagen.com), immediately stored at  $-80^{\circ}\text{C}$ . To RNA extraction, 20  $\mu$ l pooled blood spotted on Whatman 3MM filter paper (FTA® Cards, QIAGEN GmbH), dried for 3 h at room temperature then stored at  $4^{\circ}\text{C}$  and subsequently confirmed by real-time PCR were sent to Provincial Laboratory for Public Health, Edmonton, Alberta, Canada using human DNA as a control positive to confirm the *Plasmodium* infection in these animals in our study and its relationship to major malaria species in humans.

*Statistical analysis*

The results of the present study were analyzed by SPSS software version 23.0 (SPSS Inc., Chicago, USA).

**Results***The clinical signs of malaria (*Plasmodium* spp.) infection in livestock*

The more common clinical signs were frequently recorded in all malaria infected animals in this study are fever, tachycardia, and

tachypnea. The lymph nodes enlargement were seen only in cattle and sheep, which determined in 100 % of cases. Mucosal pallor took place in 65 % of cases with a considerable difference was observed between cattle and buffalo were 70% and 80% respectively. Mucosal jaundice was recorded in 87.5% of all cases with significant differences in sheep and goat as 50% and 40%, respectively. Recumbences was also recorded in a terminal stage in some sheep and goats (Table 1).

*Morphological characteristics and differential counts of *Plasmodium* parasites*

We found intra-erythrocyte and extra-erythrocyte structures during the examination of stained blood smears of cattle, buffalo, sheep, and goats, consistent with malaria.

Different morphological stages of *Plasmodium* spp., were seen in peripheral blood films of different animal species in this study with a highly significant ( $P < 0.05$ ) parasitemia in cattle and sheep in comparison with buffalo and goats (Table 2).

In (Fig. 1 a-c) we have seen the rounded Oocysts with the characteristic “comma- shape,” sporozoites inside them, or seen free in the blood smear of a cow. Ookinete of *Plasmodium* parasite were also seen in Fig.1 d-f in thin blood smears of cow, sheep, and goats respectively.

**TABLE 1. The definite and relative frequencies of clinical signs of malaria (*Plasmodium* spp.) in Cattle, Buffalo, sheep and goats (n=10 for each species).**

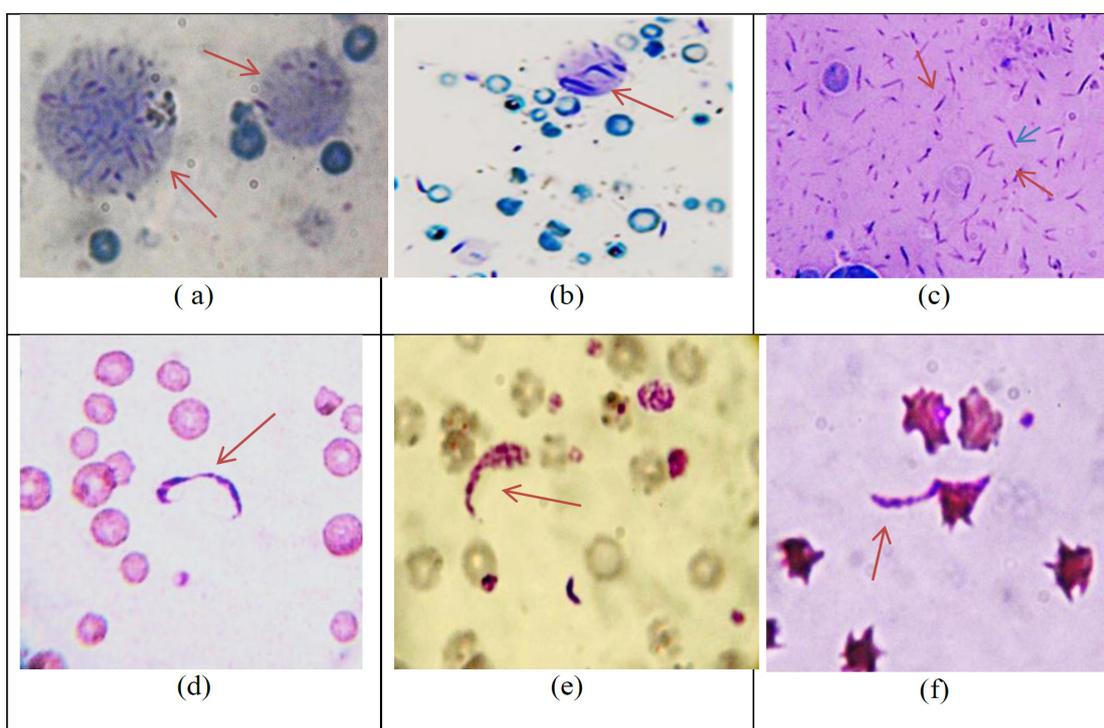
Clinical signs Fr. (%)	Cattle Fr. (%)	Buffalo Fr. (%)	Sheep Fr. (%)	Goats	Total Fr. (%)
<b>Fever</b>	10 (100)	10 (100)	10 (100)	10 (100)	40 (100)
<b>Tachycardia</b>	10 (100)	10 (100)	10 (100)	10 (100)	40 (100)
<b>Tachypnea</b>	10 (100)	10 (100)	10 (100)	10 (100)	40 (100)
<b>Lymphadenitis</b>	10 (100)	0 (0)	10 (100)	0 (0)	20 (50)
<b>Mucosal pallor</b>	7 (70) *	8 (80*)	5 (50*)	6 (60*)	26 (65)
<b>Mucosal jaundice</b>	*3 (30)	*2 (20)	5 (50)*	4 (40)*	35 (87.5)
<b>Recumbences</b>	0 (0)	0 (0)	2 (20) *	3 (30) *	5 ( 12.5)

Fr. (%) frequency, data were analyzed by  $\chi^2$  test \*  $P \leq 0.05$

**TABLE 2. Differential parasitaemia in animals naturally infected with malaria.**

Hosts	Parasitemia (parasite / $\mu$ l blood)	Percentages(%)			
		Early trophozoite	Mature trophozoites	Schizonts	Gametocytes
Cattle	11,390 $\pm$ 30.5*	28.0 $\pm$ 2.3	12.0 $\pm$ 2.6	8.0 $\pm$ 0.5	52.0 $\pm$ 2.7
Buffalo	6,502 $\pm$ 25.4	21.0 $\pm$ 1.8	34.0 $\pm$ 3.4	10.0 $\pm$ 1.2	35.0 $\pm$ 2.3
Sheep	9,821 $\pm$ 42.6*	27.0 $\pm$ 3.2	15.0 $\pm$ 3.8	23.0 $\pm$ 3.2	35.0 $\pm$ 1.8
Goats	7,500 $\pm$ 50.0	56.0 $\pm$ 2.0	19.0 $\pm$ 1.9	15.0 $\pm$ 1.6	10.0 $\pm$ 0.6

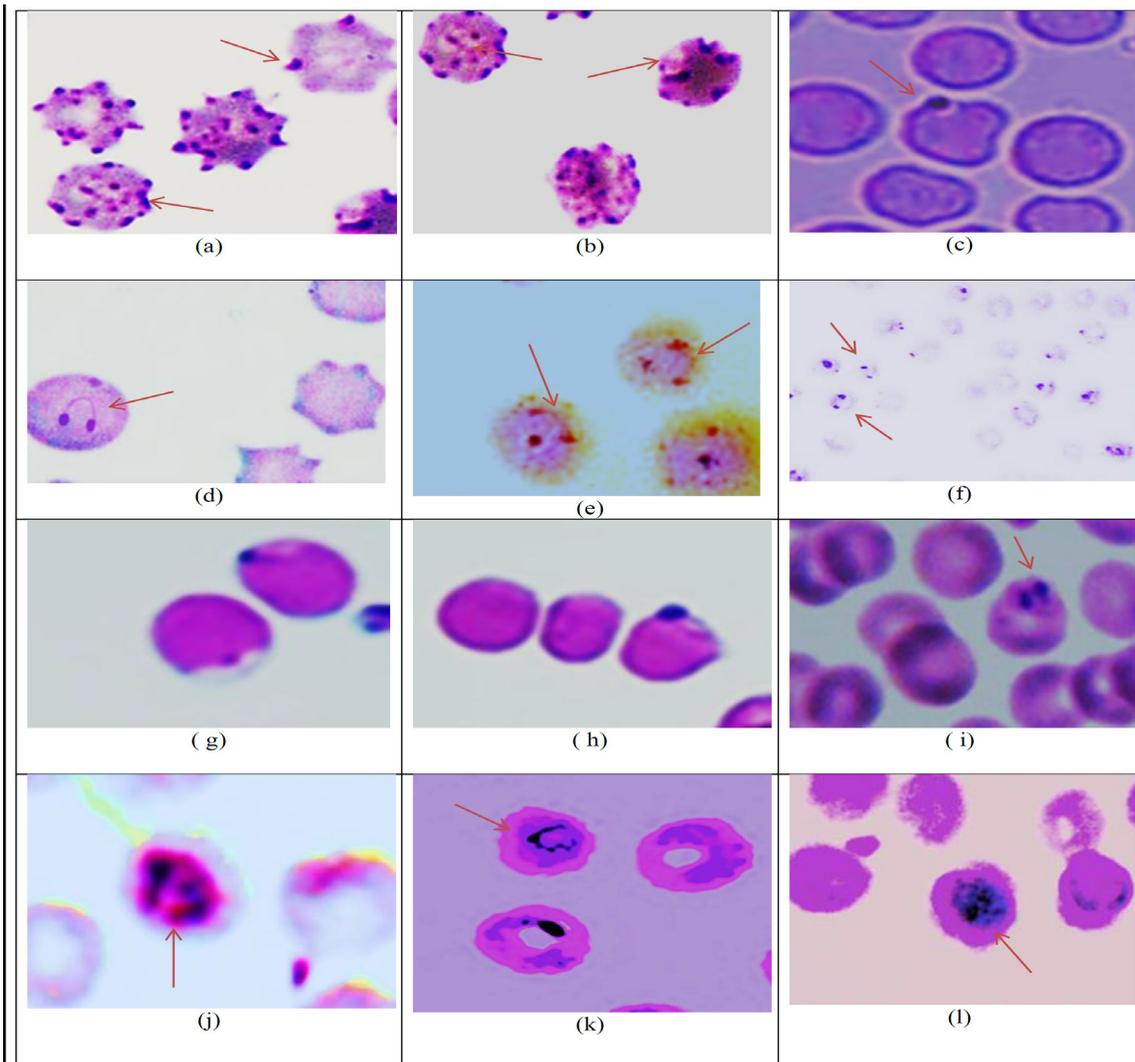
Values= (Mean  $\pm$  SD of 10/animal species) were statistically significant (\*)at  $P < 0.05$  in comparison between animal hosts.



**Fig. 1. Oocysts, sporozoite, and Ookinete of *Plasmodium* parasite in thin blood smears. (a-c) Round Oocysts showing “a coma shape” sporozoites inside them or free. (d-f) Ookinete in thin blood smears of cow (d), Sheep (e), and Goat (f) staining by Giemsa or Leishman.**

In ( Fig. 2 a-c), a unique accolé or appliqué form trophozoites founded on the periphery of the RBC, with one or multiple trophozoites in one cell in a thin blood smears of cows. Also, early ring-form trophozoites (rings) are similar to those of *P. knowlesi* and *P. falciparum*, as rings may show double chromatin dots. Headphone-form trophozoites were seen also in thin blood smear of sheep (Fig. 2d-f). Ring-form trophozoites have the characteristic signet ring shape, rings may possess double chromatin dots in cattle (Fig. 2g-

i). Some trophozoite stages appears as amoeboid in shape and measuring 1.2 to 1.8  $\mu$ m. Larger trophozoites appeared spherical and measured up to 5.0  $\mu$ m in thin blood smears of buffaloes. Infected erythrocytes appeared larger and spherical, as if swollen, and hyaline in appearance compared with normal erythrocytes (Fig.2 j). Also, in sheep and goats thin blood smears, we seen a trophozoite containing one small crystal and some trophozoites containing double rod-shaped crystals (Fig.2 k,l).



**Fig.2. Rings and Early trophozoites of *Plasmodium* spp. in thin blood smears (a,b) acollé or appliqué form in cow and buffalo (c), headphone-form in cow(d), sheep (e) and goat (f). (g-i) signet ring shape with double chromatin dot in cow. (j,k,l) amoeboid trophozoites in enlarged infected RBC and hemazoin crystals seen in Leishman-stained slides of buffalo, sheep and goat respectively, x 1,000 .**

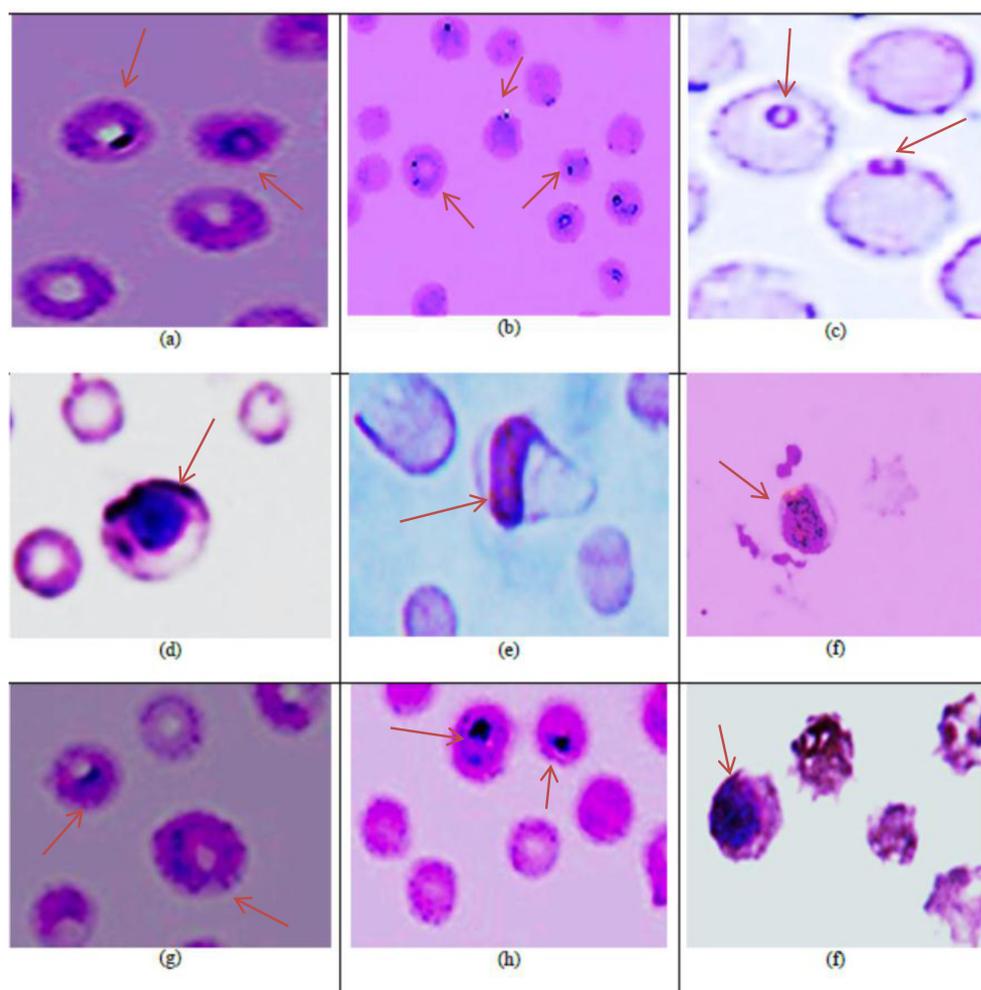
In Fig. 3, the late and mature trophozoites also appeared very similar to those of *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* in their characteristics.

The morphological characteristics of *Plasmodium* spp. in different naturally infected animal hosts in this study were summarized in the Table 3 and Table 4.

Careful examinations revealed that some minor morphological differences existed between *Plasmodium* spp. of cattle, buffalo and those parasites were seen in blood smears of sheep and goats. The schizonts of *Plasmodium* parasites

in blood smears of buffalo and sometimes in cows were elongated to an oval shape were seen firstly in Leishman stained blood smears. Mature schizonts of *Plasmodium* of cattle having 16 to 24 merozoites, compared with 4 to 16 for *Plasmodium* of buffalo, while in sheep and goats were approximately equal ( Fig. 4A & 4B).

Thin blood smears of cow stained with Giemsa and Leishman showed the characteristic sky-blue color of schizonts likes of *P. vivax* (Fig.4B:n-q). A large mass of golden brown pigment (hemozoin) was seen in the pre-schizont and schizont stage (Fig.4B:r).



**Fig. 3.** Late and Mature trophozoites of *Plasmodium* spp. in thin blood smears. (a-c) Rings trophozoites with one or double chromatin dot. (d, e, f) "Birds-eye" trophozoite in sheep and Band-form trophozoites like *P. malariae* in cow and buffalo. (g, h) mature trophozoites in an enlarged and distorted infected RBC. Schüffner's dots with hemazoin crystals are visible in goats. (i) Mature trophozoite, and Schüffner's dots seen in Giemsa-stained slide of cow, x 1,000.

**TABLE 3.** Morphological characteristics of *Plasmodium* spp. in animal hosts.

Animal Hosts	*Malaria pigment (%)		*Chromatin dots (%)			**Number of parasites in single erythrocyte (%)			Band trophozoite	Appliqué form
	Clumped	Scattered	Single	Double	Triple	1	2	3		
Cattle	80 ± 3.5	20 ± 0.9	70 ± 6.3	25 ± 1.5	5 ± 0.2	90 ± 3.2	7 ± 0.9	3 ± 0.2	Yes	Yes
Buffalo	75 ± 2.3	25 ± 1.6	95 ± 7.4	5 ± 0.3	0	72 ± 2.0	8 ± 0.5	0	No	No
Sheep	92 ± 3.5	8.0 ± 0.2	80 ± 3.6	20 ± 1.8	0	65 ± 1.5	5 ± 1.5	0	Yes	Yes
Goats	90 ± 2.5	10 ± 0.7	75 ± 4.6	25 ± 2.1	0	80 ± 2.3	20 ± 0.5	0	Yes	No

Value = Mean ± SD of 40 blood smears (10 / animal species). \*Based on early trophozoites or ring form stages, and these were absent in infected animal species. \*\*Based on early and late trophozoite stages. n = no pigment because no mature trophozoites were observed

TABLE 4. Comparison between *Plasmodium* spp. observed in blood smears of animal hosts.

Animal species	Presence of Schuffner's dots?	Size of infected cells	Number of merozoites in Schizont	Gametocyte shape	Presence of sporozoites and Oocytes	Exflagellation Microgamete
Cattle	Yes, large	Enlarged	16-24	Crescent, round, or oval	Yes	Yes
Buffalo	No	Enlarged	4-16	Crescent, Round	No	Yes
Sheep	Yes, fine	Smaller to normal	8-32	Crescent, Round	Yes	Yes
Goats	No	Smaller to normal	8-24	Crescent, Round	No	Yes

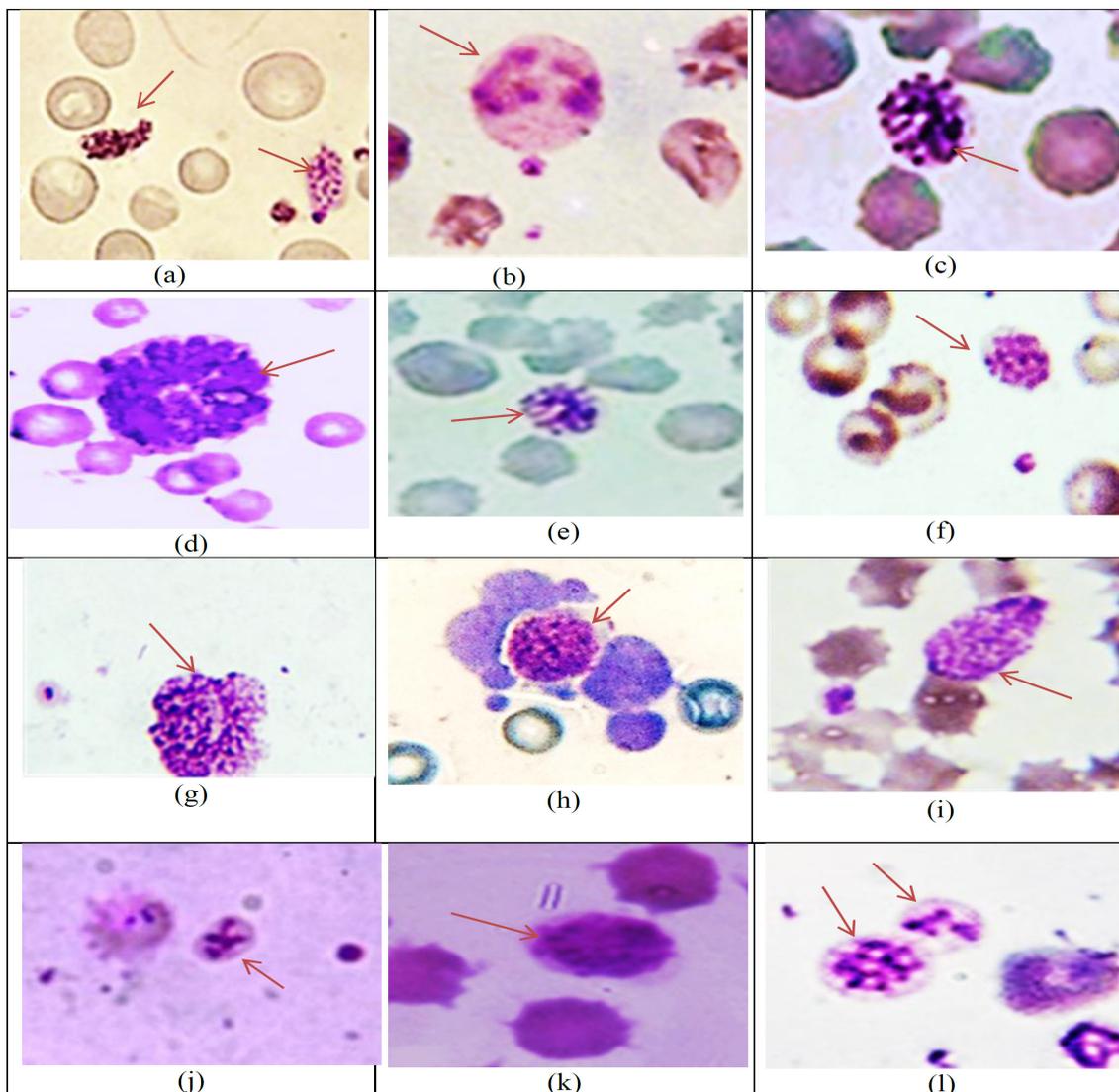
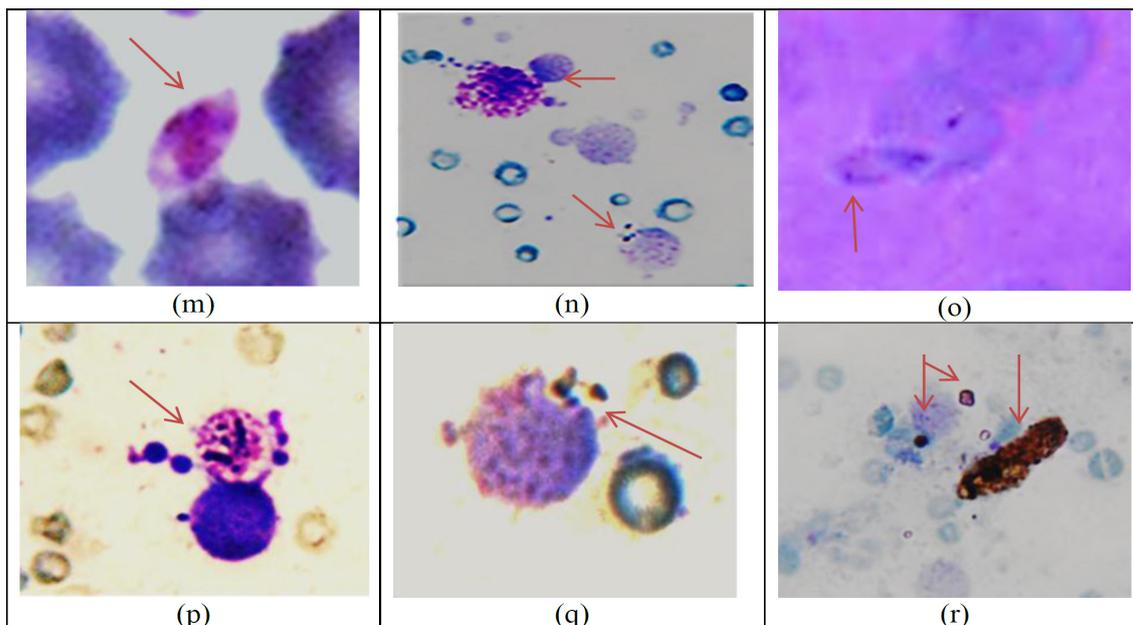


Fig. 4A. Developing schizonts of *Plasmodium* spp. in thin blood smears by Leishman stain (a) A large and amoeboid schizont, pigment is also usually arranged in more than one mass in a thin smear of buffalo. (b) Note the classic "rosette" appearance of the merozoites eight in number in a thin smear of a cow. (c-i) Mature schizonts containing (16 to 24) merozoites. (j-l) Mature schizonts containing merozoites (6 to 12) giving a coarse granular appearance.



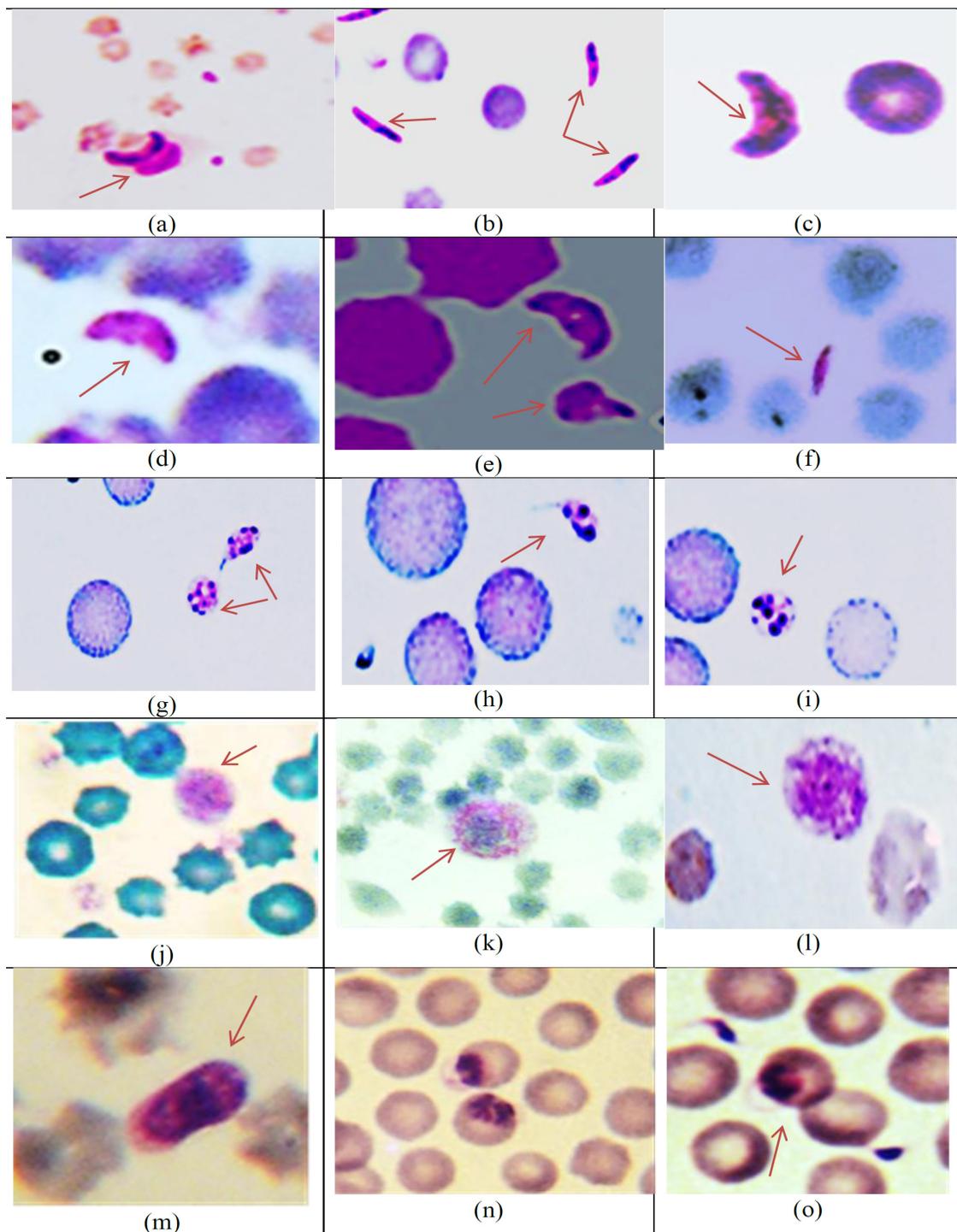
**Fig. 4B.** Merozoites, schizonts, and hemazoin pigment of *Plasmodium* parasites in blood smears (m) Merozoite escape from schizont in blood smear of sheep. (n-q) Rupture schizonts of *Plasmodium* parasite with the escape of merozoites, mature schizont appeared a characteristic sky-blue color. (r) Rupture schizont leave hemazoin of malaria parasite in cytoplasm or free in the blood smears of cows.

Infected erythrocytes, including male and female gametocytes, were readily seen by microscopy (Fig. 5A & 5B). Gametocytes of malaria parasites like *P. falciparum* appeared in stained blood smears of cattle, buffaloes, sheep, and goats with a typical crescent shape and are usually about 1.5 times the diameter of an erythrocyte in length. The female form, or macrogametocyte, was usually more slender and somewhat longer than the male, and the cytoplasm takes up a deeper blue color with Giemsa stain. The nucleus is small and compact, staining dark red, while the pigment granules are closely aggregated around it. The male form, or microgametocyte, was broader than the female and was more inclined to be sausage-shaped. The cytoplasm was either pale blue or tinted with pink and the nucleus, which stains dark pink, is large and less compact than in the female, while the pigment granules (hemazoin) are scattered in the cytoplasm around it (Fig. 5A: a-f). Gametocytes of some *Plasmodium* spp. were round, elongated, oval shape, and tend to fill the RBC in blood smears of all animals species especially buffaloes and goats (Fig. 5A: g-o).

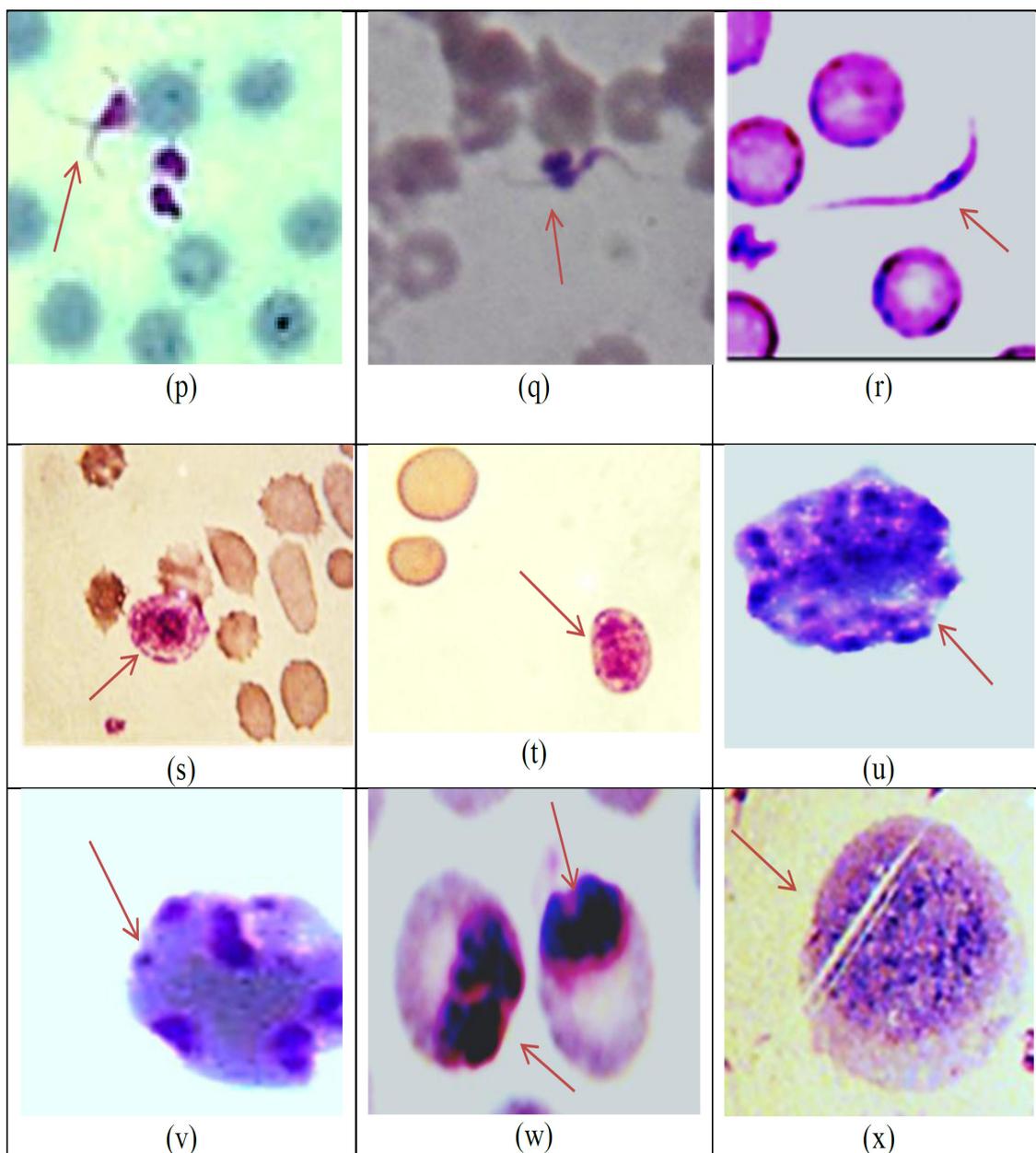
Although a source of initial confusion the structures in (Fig. 5 B: p-r) were eventually identified as ex-flagellating microgametes of

*Plasmodium* spp. We report different stages of ex-flagellation as seen in blood smear prepared from cattle and sheep infected with *Plasmodium* spp. Also, the round gametocytes occupy the entire red blood cell in blood smears of host, and measure 6 to 8  $\mu\text{m}$  in diameter with a granular appearance as well as hemazoin pigments (Fig. 5 B: s-x).

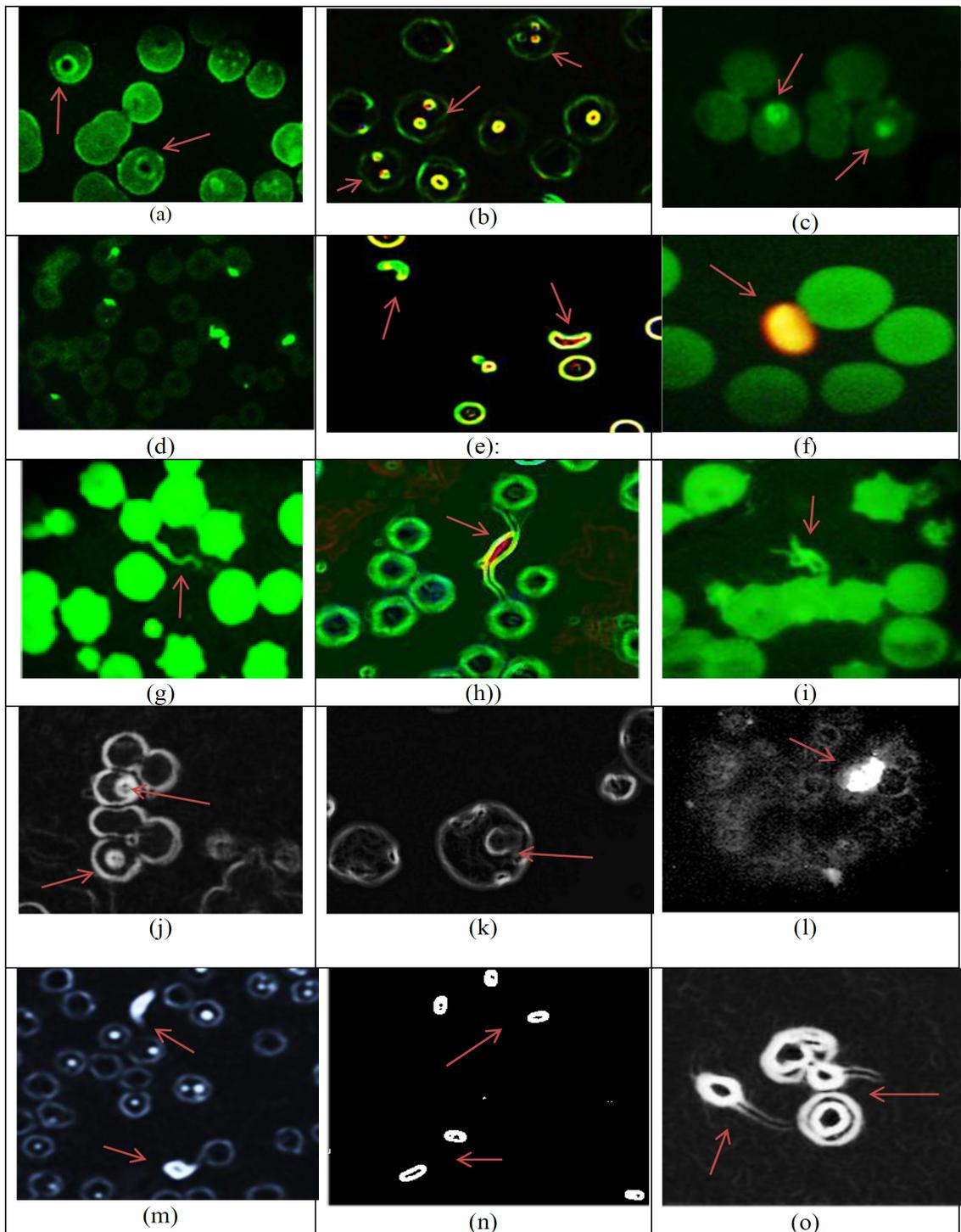
In Fig. 6, the classic ring-shaped trophozoites were seen more visible in the peripheral blood smears stained with the direct AO and wet mount preparations in comparison to the traditional Giemsa, and Leishman stains method. All ring forms observed in AO stain thin smears so far exhibited bright pigmentation and at times with two or three parasites per erythrocyte. (Fig. 6a-c). Also, The ex-flagellated microgamete was readily observed in acridine orange blood smears (Fig. 6h). The bright pigmentation in the crescent-shaped gametocytes is concentrated was seen very clearly in unstained thin blood smears under darkfield microscopy ( Fig. 6n). The Pigment present in different forms of *Plasmodium* species infecting animals exhibits light scattering when blood films are viewed by darkfield microscopy. In some specimens, ring forms of species like *P. falciparum* may exhibit less pigmentation but can be seen by this technique.



**Fig. 5A.** Gametocytes of *Plasmodium* spp. in staining blood smears with Giemsa or Leishman x 1,000. (a-c) Cattle blood smears, Note gametocytes are crescent - or sausage-shaped, (d) Gametocytes of *Plasmodium* spp. in thin blood smears of buffalo, the remnants of the host RBC can be seen. (e-g-i) The macrogametocytes and ex-flagellated microgametocytes in sheep. (j-l) In goats thin smears, a round gametocyte that is larger than normal red blood cells. Note the fimbriation of microgametocyte, it has a fine granular appearance as well as Schuffner's dots. (m-o) round, elongated, oval shape and the Schuffner's dots Gametocytes of *Plasmodium* parasite in thin blood smears of buffalo.



**Fig. 5B.** Gametocytes of *Plasmodium* parasite in thin blood smears. (p-r) Ex-flagellated microgametocytes in thin blood smears of sheep and cattle. (s and t) Mature macrogametocyte of Gametocytes like *P. malariae* was compact and tend to fill the RBC in blood smear of buffalo . The cytoplasm stains blue and the chromatin pink to red. An abundant dark pigment may be scattered throughout the cytoplasm. (u and v) Macrogametocytes of *P. spp.* in thin blood smears of cow, showing Schüffner's dots. (w) Male and female gametes with large granules in goat blood smear. (x) A round gametocyte that is larger than normal red blood cells. It has a granular appearance as well as Schuffner's dots.



**Fig. 6.** Various forms of malarial parasites in blood smears under fluorescent microscopy(a-i), and darkfield (j-o). (a-c) one or two trophozoites inside RBCs with red chromatin dot.(d) Bright forms of *Plasmodium* merozoites. (e) Male and female gametocytes (f) Bright orange Gametocyte. (g-i) Ex-flagellated microgametocytes. (j, k) *Plasmodium* trophozoites (wet mount); magnification, x400 under darkfield microscopy. (l) schizonts in thin smears stained routinely with Giemsa or Leishman stain can also be examined by darkfield. (m) *Plasmodium* ring forms with gametocytes (highly pigmented); magnification, x 1,000. (n and o) Male and female gametocytes.

### Real-time PCR

Of the dried blood spots on FTA® Cards, four samples (one for each animal species) tested positive for Plasmodium and of the purified DNA samples (one for each animal species), four were weakly positive for Plasmodium, but all samples were negative for the four major human species.

### Discussion

According to obtained results of the present study the clear clinical signs for clinical diagnosis of malaria in cattle, buffalo, sheep and, goats included fever, pale mucous membranes, lymph node enlargement with jaundice and recumbence of sheep and goat in the terminal stages should be differentiated from such disease due to its similar signs. The pattern of plasmodium infection differs between animal hosts according to the frequency of clinical signs. It is uncertain whether these differences reflect mainly the characteristics of host, parasite, pattern of exposure or other differences between animal populations. The presence of hemozoin crystals in blood smears were diagnostic for malaria parasites in these livestock [29], and were frequently rod-shaped for some *Plasmodium* spp. especially in buffaloes and goats as described in *Plasmodium bubalis* and *P. caprae* by Sheather ; Kaewthamasorn et al. [3,6]. The microscopic examination of a peripheral blood smear of some animals revealed the presence of Ookinetes of Plasmodium parasite. This unusual finding. Ookinetes are motile zygotes formation normally occurs in the mosquito gut by the combination of macrogametocytes and ex-flagellated microgametocytes. Ookinetes invade epithelial cells of the mosquito's mid-gut where an oocyst is formed, Ookinetes are very rarely found in blood smears, their presence on smears usually indicates a substantial delay occurred between the time the blood was collected and the time smear preparation [30]. Ex-flagellation of male microgametocytes in malarial parasites can occur spontaneously when the infected blood or blood smear is exposed to air occurring during the preparation, seeing the ex-flagellated microgametes in blood smears is quite unusual and it is likely to have occurred after the blood has been drawn but before the smear is made [31]. It is important to remember that the malaria parasites remain viable in the tube of blood collected using EDTA as anticoagulant. If the blood cap is removed and the blood became

aerated, then the parasites begin the life cycle seen in the mosquito [32]. Also, these cases showed that the confusion may arise during examination of blood smears from livestock can be reduced by paying attention to the fact that ex-flagellation may occur in *Plasmodium vivax* and *P. falciparum* infections in humans [31 & 32]. Schizonts were seen in the peripheral blood of cattle, buffaloes, sheep, and goats, and their presence may indicate a potentially serious parasitemia [19]. AO stained blood films by fluorescence microscopy, were a rapid diagnostic method leading to easily detection, enumeration, and identification of some important stages of Plasmodium in different animal species and comparison between *Plasmodium* spp. observed in blood smears of livestock. The duration to get results of malaria parasite diagnosis by Giemsa and Leishman stains were longer than AO methods [26 & 33]. Wet mount preparation under darkfield microscopy was described long ago as a good and rapid technique for the detection of all forms of Plasmodium parasites certain highly pigmented forms, including ring forms, schizonts, and gametocytes, in unstained blood smears in the comparison of stained blood smears [25]. However, the drawback is Acridine Orange stained and wet mounts cannot be preserved, unlike the Giemsa or Leishman stained smears [34,35]. Acridine Orange and wet mount techniques offer the distinct advantages of rapid diagnosis, increased sensitivity, and adaptability to fieldwork. Our experience with *Plasmodium* spp. in animals is still minimal, but parasites in a suspected infection can be done under the darkfield microscope. From the results that we obtained by microscopic examination of the blood smears of livestock in the study, it was found that the morphological forms of *plasmodium* spp. have great similarity between them. it is never possible to diagnose species based on morphology of trophozoites or other stages of Plasmodium parasite. Now the general detection of malaria parasites, which is based on the amplification of Plasmodium nuclear or mitochondrial DNA, the specific detection of gametocytes depends on mRNA [36 & 37]. We used FTA cards in the study which filter papers were most suitable for storage of gametocyte mRNA in dried whole blood samples in the comparison to the DNA extracted sample of whole blood [38]. According to the molecular study results, the livestock infected

with malaria of genes *Plasmodium*, but all samples were negative for the four major human species. The previous studies on the molecular characterization of malaria parasites of domestic water buffalo (*Plasmodium bubalis*) and African goats (*Plasmodium caprae*) revealed that two ungulate malaria species, are phylogenetically distinct from all other mammalian *Plasmodium* spp. and most closely with hemosporidian species that infect birds, bats, and lizards, rather than grouping with primate-infecting *Plasmodium* spp. [6,8].

### Conclusion

We present the first microscopic and molecular-based study on livestock malaria parasites (*Plasmodium* spp.) in Iraq. Further studies are required to molecular identification of the different *Plasmodium* species in these livestock to understand the prevalence of ungulate malaria parasites in Iraq.

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### Competing interests

The authors declare that they have no competing interests.

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### دراسة أولية لمرض الملاريا (*Plasmodium* spp.) في الماشية العراقية

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تم اكتشاف وتشخيص مرض الملاريا الذي يصيب المواشي (الأبقار والجاموس والأغنام والماعز) في الموصل - العراق. أظهر إجمالي 40 حيواناً من السلالات المحلية، من مختلف الأعمار ومن كلا الجنسين طفيليات المتصورة في لطخات الدم. اختير عشرة حيوانات من كل نوع من الماشية. واخضعت جميع الحيوانات لفحوصات سريرية كاملة. تم التعرف على طفيليات المتصورة عن طريق الفحص المجهرى ومقايسة تفاعل البلمرة المتسلسل. كانت الحمى، وشحوب الأغشية المخاطية، والتهاب العقد اللمفية، واليرقان، والاستلقاء في كثير من الأحيان علامات سريرية مرتبطة بعدوى الملاريا في الماشية. من خلال الفحص المجهرى للطحاط الدموية ظهرت النشاطات والاقسومات والامشاج من الناحية الشكلية مشابهة لتلك الموجودة في انواع المتصورة في الإنسان. كما لوحظت الاكياس البوغية والبيوغيات والبيوضة المتحركة في لطخات الدم لبعض الأبقار والأغنام والماعز. شوهدت درجة عالية من التطفل وكانت بعض كرات الدم الحمر مصابة باتنين أو أكثر من النشاطات. شوهد الشكل الحلقي الكلاسيكي أكثر وضوحاً في لطخات الدم المحيطي المصبوغة بصبغة برتقال الاكردين المباشر والمستحضرات الرطبة مقارنةً بطريقة صبغة جيمسا وليشمان التقليدية. كان وجود بلورات الهيموزين تشخيصياً لطفيليات الملاريا في هذه الدراسة. تم الكشف عن الحمض النووي للمتصورة في جميع العينات. ويعد هذا التسجل الأول لطفيليات الملاريا في الماشية العراقية. نحتاج الى دراسات أخرى لمعرفة التركيب الجيني للانواع المتصورة وفهم مدى انتشارها في حيوانات العراق.