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Detection of Trypanosoma in sheep and goat in Mosul City

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Abstract
In this study, 385 blood samples were collected from sheep and goats of different ages, sex, in order to investigate the presence of trypanosomes in the Mosul city .
Blood was examined using routine diagnostic methods (wet blood smear, thin blood smear, thick blood smear, lymphocyte layer method) and stained with a (*Gimsa ,Leishman, acridine orangedye) .*
The study showed that the total infection rate in (sheep and goats) was 21%, and the study also proved that the highest infection rate appeared using the lymphocyte separation method, 21%, without significance differences with thin smears, with significant differences with thick and wet blood smears. There were significant differences between thick and wet blood smears, the results of blood smears stained with Gimsastain indicated the highest infection rate of 21% without a significant difference with acridine orange stain, while blood smears stain with Leishman recorded the lowest infection rate of 16.1%. The results of the study showed that the infection rate In sheep and goats was21.9% and 19.4% respectively, , with no significant differences. , the results showed high percentage of infection in animals greater than one to two years in each of the sheep and goats 40% and 38.5 %, respectively. The highest infection rate in females was recorded in each of the sheep and goats 28.3%, 30.5 % .

Key words: *Trypanosoma , sheep,goat ,Mosul*

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I. Introduction

African animal trypanosomiasis is one of the most important tropical parasitic diseases and is considered a major gift to ruminant production in Africa, Asia and South America (CarlosGtierrez et al.,2006) trypanosoma is one of the protozoa. Single-celled, spindle-shaped, lives outside blood cells in the bloodstream and other body fluids (OIE, 2013) it is belongs to the family Trypanosomatidae The name of the parasite genus is derived from the Greek word trypano meaning digger and soma meaning body due to its key-like movement (Eoly and Lucheis 2009)

The parasite has a wide range of hosts as well as its ability to infect mammals fish, birds and reptiles T.congolense. T, vivaxT.brucei and Tsimuli.is the main species of the genus Trypanosomawhich infects domestic and wild animals, which constitutes an important reservoir of the parasite (Abhishek,2016), the most important species that infects sheep ,goats and cattle is the T.Congolese T. Vivax, T. brucei, causes a disease called Nagana, (Taylor 2016). The two species are T.congolens. T.vivax are two major pathogens of sheep . (Baral, 2010) the parasite is transmitted in nature cyclically by tsetse flies The parasite is also transmitted mechanically by biting flies such as horse fly Tabanus, .(Cherry et al.,2021)

the parasite directly affects the production of milk and meat and cause reduce birth rates, in addition to that it increases abortion rates and affects the size and composition of the herd.Thus, the parasite affects the health status by causing anemia, weakness, weight loss, neurological symptoms, abortion and infertility, and thus causes huge economic losses due to poor production and all feeding and treatment cost (Esther,2021)

diagnosed of the parasite is attempt by several methods such as direct blood Smears and tissue fluids .the thinand thick smear which staining with special dyes to detect the presence of parasite is widely used ,serological tests such as indirect agglutination test, enzyme-linked assay test and immunofluorescence is also used to diagnosis of parasite (Ahmed et al.,2020).

The Objectives of the study Examine the blood by routin methods to detect the presence of the parasite by thin, wet blood smears and thick smears as well as the method of concentration and then staining with different stain (Gimsa, Leishman and acridine orange) to determine the total parasite infection rate in sheep and goats and comparison between the efficiency of the stains used in the diagnosis of parasite

II. Materials And Methods

Animals of study :

The study included 385 sheep and goats of different age,sex

some of these animals suffer from avarious clinical signs and the some of the animals were apparently healthy. .

Sample collection

55 ml of blood was collected from a jugular vein and the blood was placed in EDTA anticoagulant tubes for the purpose of making the following blood smears

Routine methods for parasite detection (microscopic examination) smear were prepared .

a - Thin Blood smears

Several smears were prepared for each Sample , one drop of blood put in slide and then spreading it at the angle of another glass slide

The slide was left to dry and then fixed with absolute methanol alcohol.

Then the slide was stained using Gimsa, Leishman and acridinorange stain

and then examined with a light microscope (Rosendahlet al 2009).

b-Thick blood smear

a large drop of blood was placed in the middle of the slide and then spread with a wooden stick ,.

Then the slide was stained using Gimsa, Leishman and acridinorange stain

and then examined with a light microscope (Rosendahl et al 2009)

c-Wet blood smear A drop of blood was placed on the center of a slide , then examined directly under a light microscope (Sirigireddy et al.,2014) .

d-Buffy coat layer of lymphocytes, take 3 ml of blood and put it in tube .

Aequal amount of ficoll was added to the blood slowly . then placed in a centrifuge 3,000 rpm for 10 minutes .

The blood layers consisting of the red blood cells layer, the plasma layer, the lymphocyte layer, and the ficoll layer were observed .

the lymphocyte cell layer aspirated using a Pasteur pipette and then spread on a slide. It was left to dry and then stained using Gimsa, Leishman and acridinorange stain and then examined under a light microscope Musa et al., 2005) .(

- Statistical Analysis

Statistical analysis was carried out using Chi-Square test using the statistical program - IBM SPSS version 19 (Leech et al. 2007

Results

The results of the study for the detection of Trypanosoma parasites in 385 blood samples collected from both sheep and goats,using the routine diagnostic methods adopted in the study,showed atotal infection rate was 21%. the highest infection rate was recordedin the lymphocyte separation method without significant differences with thin smears and with asignificant difference with thick and wet blood smears.while the wet blood smear method recorded the lowest infection rate 13.5% Table-1 –

Table-1 comparison between methods for detection of Trypanosoma

Methods	Number of samples	Number of positive samples	Percentage of infection %
Wet blood smear	385	52	13.5a
Thin blood smear		74	19.2a
Thick blood smear		67	17.4b
Buffy coat		81	21a
Total		81	21

in order to reach ahigh accurate diagnostic level,blood smears were stained with three stains , it was found that the highest infection rate was obtained using Gimsastain 21% while the lowest infection rate was using Leishmanstain 16.1% and there are statistically significant differences with the Gimsa and acridinorange.while there was no significant difference between the Gimsa and the acridinorange stain.Table 2. Figure 1,2 and 3

Table-2 comparison between the efficacy of stains for detection of trypanosome

Stain	Number of samples	Number of positive samples	Percentage of infection %
Gimsa	385	81	21a
Leishman		62	16.1b
acridin orange		70	18.2a

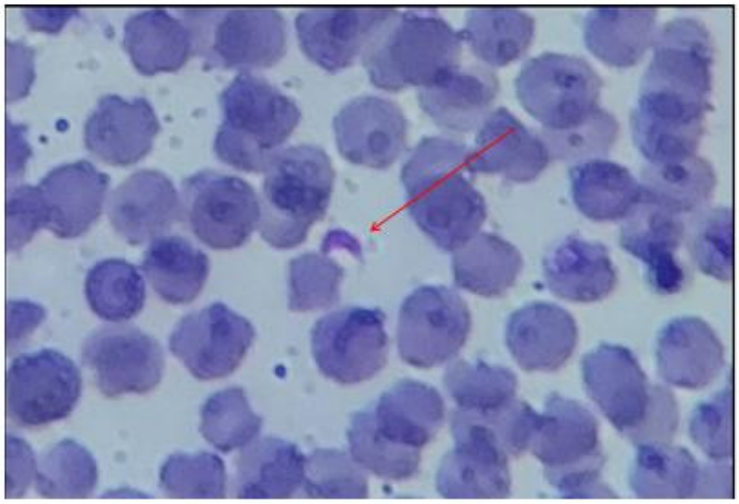

Figure -1- thick blood smear stained with giemsa showed the trypanosome parasite

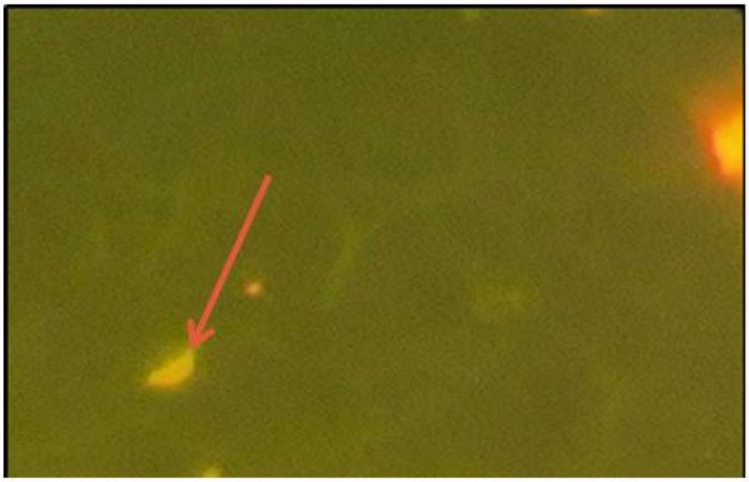

Figure -2- buffy coat smear stained with acridine orange showed trypansoma parasite

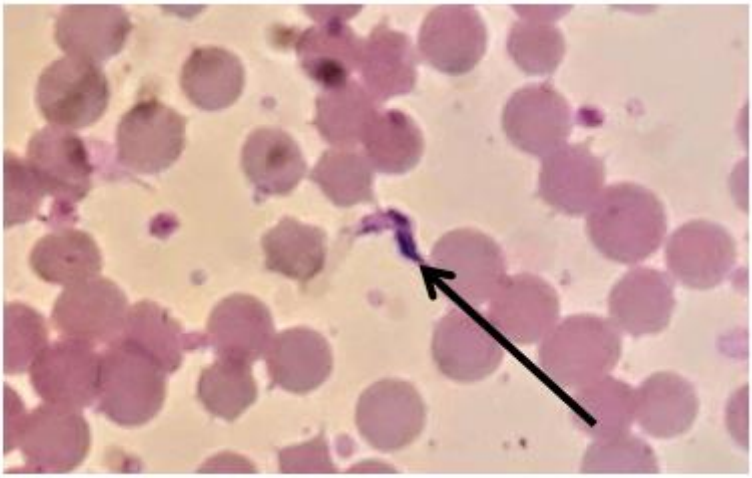

Figure -3-thick blood smear stain with leishman stain showed trypanosomaparsite

The study showed that the infection rate of Trypanosomes using routine diagnostic methods was higher in sheep than goats,and there were no significant differences between them.table-3-

Table-3 prevalence of trypanosma in sheep and goat

Animals	Number of samples	Number of positive samples	Percentage of infection %
Sheep	251	55	21.9a
Goats	134	26	19.4a

Highest infection rate of trypanosome was recorded in sheep 38.5% older than one to two years with significant variance with other aged groups .in goats it was found that the highest infection rate was in the age groups of more than one to two years40%.Table 4

Table-4 prevalence of trypanosma in sheep and goat according to age

Age	Animals	Number of samples	Number of positive samples	Percentage of infection %
Less than one year	Sheep	83	13	15.7a
	Goats	39	7	17.9a
One year –two years	Sheep	96	37	38.5b
	goats	40	16	40b
More than two years	Sheep	72	5	6.9a
	goats	55	3	5.4c

The relationships between the infection rate of trypanosome with sex in of sheep.showed the highest infection rate was in female while the lowest infection rate was recorded in males with significant variance ,while the relationship of infection rate of trypanosome parasite in goats showed high prevelance of infection in female than male with significant differences (table 5).

Table-5 prevalence of trypanosma in sheep and goat according to gender

Gender	Animal	Number of samples	Number of positive samples	Percentage of infection %
Male	sheep	133	19	14.2a
	goats	74	9	12.2a
Female	sheep	118	36	30.5b
	goats	60	17	28.3b

III. Discussion

African animal trypanosomiasis is one of the diseases that affects all domestic animals and severely affects the livestock industry in many countries of the world and causes great economic losses .

The diagnosis of the disease is difficult Because the clinical diagnosis of trypanosomiasis in host mammals is difficult due to the symptoms that overlap with many parasites of the blood.

The results showed that the trypanosoma was detected using different techniques, and there were different rates of infection, the highest once when used the concentration method while they were low using wet smears with a significant difference between them and the methods of thick and wet smears. When comparing these results with the other studies, it was found that there are differences In the technique of diagnosis, the researcher, Daniel et al. 1994, mentioned that the concentration methods, including the lymphocyte layer method, are more sensitive than the standard methods for detecting trypanosomes, including the thin and wet smears .

While McNamara et al. 1995, Bailey and Smith 1992. Carolinaet al., 2020 showed that the lymphoid layer method is one of the most sensitive methods for detecting the parasite, while two studies conducted by researchers (Desquesnes,1997) indicated that the lymphocyte method using ficoll is still one of the most sensitive, fast and inexpensive methods, while the researcher, Marc Desquesnes (2004) stated that the sensitivity of the techniques depends on stage of infection , acute or chronic, in cases of chronic infection, the sensitivity of the techniques is somewhat low, so it is recommended to use more than one method and technique to reach the results of detection , especially in cases of outbreaks of epidemics or in new diseases emerging in a region (Gloria et al. 2021, between researchers Grab &BWayo 1982. The method of isolating the parasite from the blood using ficoll is the most sensitive method for the diagnosis of trypanosome infection, and it is one of the most common methods of diagnosis Betancourt & Julio 1979 while Kralzer&Ondisk1989 indicated that this technique is capable of Detection of 100 parasites in 1 ml of body fluids. Marc Desquesnes 2004 mentioned that the routine methods of parasite diagnosis represented by blood and lymphatic smears are among the traditional methods used to detect the parasite, but it lacks sensitivity as its sensitivity is 10 parasites per 1 ml of Body fluids .

The results of smears were shown using three stains specialized for this purpose, There are different percentages in the infection rates, where the highest percentage was recorded in the acridinorange stain. It was not possible to find a study showing the extent of variation in the efficiency of these stains to detect trypanosome parasite,so several studies showing the efficiency of these dyes for the detection of many blood parasites .

In a study conducted by Sanghamitra et al., 2014, for the detection of malaria parasites in blood smears using gimsa and Leishman dyes, no significant differences were found in the examination ratios, but the researchers mention and said that the Leishman formula gave a clearer pattern to the structures. .

The researchers, Reghuet al. 2007, pointed out that the gold standard for detecting blood parasites is the use of the Gimsa stain The researchers also pointed out that the acridine orange quick-application and efficient techniques for the investigation of blood protozoa. Htut et al. 2002, al. showed the efficiency of the acridine orange compared to the Gimsa stain. and in similarly of its results, it was found that there are no differences between the efficiency of the two stains .

And when comparing the two stain with orange acridine and Leishman stain by researchers Neeta and Ashwini2016 to detect the parasite, the efficiency of acridine stain was shown compared to Leishman stain. As for researchers Peter et al. 1995, HUSSEIN and AGHWAN, it was not found that they had significant differences in the efficiency of the Gimsa and orange acridin stains for the detection of some blood parasites, while the researchers, , Enock et al, 2019indicated that the acridineorange stained is not less efficient than other traditional methods of detection.

The results showed that the infection rate of the trypanosoma parasite in sheep was 21.9% and in goats was 19.4% and there were no significant differences between them, and these results agree according to the researcher (Abera, 2016), where the results showed the percentage of infection of trypanosome parasite infection in the sheep , while the infection rate in goats was 0.4 and there were no significant differences, and this convergence in the rates of infection between was explained by the nature of the breeding as both of them They were raised together and shared in the same barns and pastures. In addition, the variation in the number of samples has a role in the variation of infection rates .

Several studies dealt with the variation in infection rates between sheep and goats, as researchers indicated (Ng'ayoet al1996.). and, Snow et al. 2005 However, the infection caused by the parasites of trypanosomes is high in goats, and attribute the goats to the fact that the goats have some resistance, in addition to the researchers Ng'ayo et al (2005). indicated that goats have the property of immune tolerance, these results confirmed Also by researchers (Gael et al., 2020), as their results showed a difference in the rate of infection in sheep compared to goats, and the reason was attributed to the impact of trypanosome infection with external environmental conditions as well as sensitivity in sheep to trypanosome infection, while researchers (Kiran and idris 2017,) indicated that A high rate of infection with trypanosoma parasites in sheep compared to goats, whichrecorded lower rates of infection, and this was explained by the fact that sheep are more sensitive to infection with parasite as well as their lack of immunity to infection and their rapid impact on it.

While the researchers (Onditi et al., 2007) pointed out that , it may be due to behavioral reasons, including kicking, leg movements, and the gnawing movement of the skin of goat. The movement of the guilt as well as which prevents the biological transfer of feeding on them, while the researchers (Olatele and Adelegan 1998) indicated that the clinical symptoms appearing on goats are subclinical or show slight clinical symptoms, and contrary to what was mentioned, It was also found during this study that the highest rate of infection with the parasite was for age groups aged between one and two years, 38.5%, and there are clear significant differences.

Other studies indicated the discrepancy the difference between infection rate (Zelalem et al 2015). stated that the parasite prevalence rate increases with ages greater than one year. He mentioned that the reason may be due to the fact that this may cause an increase in the chance of exposure to biological transmission compared to younger ages, in addition to that the period Its survival in the herd has a greater chance, while Geoffrey , et al 2017) mentioned that large animals (greater than two years) gave a low rate of infection and this matches the results of our study, and this harmed the animal's immune response as a result of previous exposure to parasite infection, another study It agreed with the results of our study (Idehen et al. 2018,). The infection rate was high in ages between one and two years and decreased in ages greater than two years. The reason for this was explained by several reasons, including the increased chance of exposure to the vector with age, (Idlehen et al. 2018,). It was pointed out that young animals whose immune system is not fully qualified to resist infection are sensitive to infection as well as the relationship between the rate of infection with the parasite by sex in sheep and goats by using routine methods in the diagnosis, was found that the highest percentage of infection was in females compared to males and that there were significant statistical differences

.Several studies indicated the relationship of the sex of small ruminants with trypanosome infection. Ezehuira et al. 2009, indicated that there was no significant difference between males and females infected with trypanosomes, (Idiehenet al., 2018). The infection rate for males is higher than that of females, while (Sam–

Wobo et al.,2010) to the rates of infection with grouse that are twice as high as the infection rates for males, and there are many explanations that indicated the possibility of females being infected more than males, including the difference between the numbers between males and females in the herd, which increases the chance of female exposure to infection due to the large number of them as well as the purpose of breeding The animal, which is often productive and childbirth, milk and yearning, which requires environmental considerations for many years, and this benefit differs from males, which are raised high for meat production, not to mention the few age for reproductive purposes and other reasons, including the exposure of females to stress factors, including pregnancy and childbirth, which may play a role in Increased susceptibility to infection

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