Introduction
Trypanosomiasis is considered as one of the most common and important diseases that affect human and many animals including cats (Panigrahi et al., 2015). Flies such as Stomaxys, Tabanum, and Triatomids bugs spread the disease, and some recent resources showed that the disease can be transmitted by ingestion of dead animals infected recently with trypanosomosis (Nwoha, 2013). Clinical signs start to appear on affected cats after 2–3 weeks of flies’ bite, initially with a local skin inflammatory reaction (Chancre) appearing at the site of biting. The size of Chancre is depending on several factors such as the immune status of the affected cat, virulence of Trypanosoma spp., and inoculation dose of Trypanosoma species. The parasites are dividing and multiplying inside Chancre and then enter the adjacent lymph nodes then the lymph vessels and finally the bloodstream (parasitemia) (Aloba et al., 2022). Clinical signs are varies depending on the severity of the disease and Trypanosoma spp, which include anorexia, anemia, fever, enlarged superficial lymph nodes, conjunctivitis as well as edema of limbs (Zenad and Radhy, 2020). The disease has three forms; acute, subacute, and chronic, where the acute form is highly fatal. The disease is common in cats as they are more exposed to vectors (Solikhah, 2021).

There are many techniques used for the diagnosis of Trypanosoma spp. including wet smear. The ideal way to diagnose Trypanosoma parasite in cats is using a thin blood smear stained with one of Romanowsky stains as Giemsa stain and examined under microscope (magnification of 1,000×) (Solikhah et al., 2019). In addition, for accurate diagnosis, polymerase chain reaction (PCR) technique is considered as a very good procedure for the diagnosis of trypanosomiasis (Njiru et al., 2005).

The current study was aimed to provide information about the trypanosomiasis of cats in Mosul city of Iraq using microscopical examination and molecular methods.

Material and Methods
Animals and samples collection
A total number of 120 blood samples were collected from the cephalic vein of young cats (≤1 year) and old cats (>1 year), 53 males and 67 females, from different regions in Mosul city of Iraq. All blood sample were microscopically examined using blood smears stained with Giemsa (Soulsby, 1986; Konnai et al., 2009; Prasad et al., 2015; Salvioni et al., 2021). Positive samples were stored at −20°C until PCR tested. Clinical signs were recorded for each cat.
Molecular examination
Genomic DNAs from 35 positive blood samples were extracted by using column pure blood genomics DNA mini kits (Alanazi, 2018). Applied biological materials by using conventional PCR technique was performed for the detection of *Trypanosoma* spp. and *Trypanosoma evansi* (Table 1).

Hematological examination
Hematological examination was used for hemoglobin concentration (HB), packed cell volume (PCV), total red blood cells (TRBC), MCV, MCHC, and total leukocyte count (TLC). Parasitemia were calculated according to Da Silva *et al.* (2010) and Al-thuwaini (2021).

Statistical analysis
Statistical analysis done by using chi-square test in IBM-SPSS statistics version 19 program (Leech *et al.*, 2007).

Ethical approval
The ethical approval was issued by the Institutional Animal Care and Use Committee (UM.VET.2021.16) of the Faculty of Veterinary Medicine, University of Mosul on the 2nd of September 2021.

Results and Discussion
The microscopic examination of blood smears stained with Giemsa in this study showed that the infection rate of *Trypanosoma* spp. was 34.2% (41 out of 120 cats). The infection rate was significantly \( p < 0.05 \) higher in males (49.1%) compared with that in females (22.4%) (Table 2). The highest rate of infection was reported in younger cats (≤1 years). Statistical analysis showed significant differences between ages (Table 3). All 35 microscopically positive samples were examined by PCR technique and showed that the infection rate of *Trypanosoma* spp. was 31.4% (11 out of 35 cats), and with *T. evansi* was 20% (7 out of 35 cats). Positive bands were at 480 bp for *Trypanosoma* spp. and 151 bp for *T. evansi* (Figs. 1 and 2). The infected cats with *Trypanosoma* spp. were suffering from pale mucous membranes, fever, dullness, anorexia, emaciation, muco-purulent ocular discharge, incoordination and anemia (Fig. 3). In addition, there was decrease in TRBC, HB, PCV, with a macrocytic hypochromic anemia type and increase in TLC due to increase of lymphocytes, eosinophils, basophils, monocytes and increase in parasitemia (Table 4).

The infectious rate of *Trypanosoma* infection in the current study was higher than that reported in other studies such as in Southern Louisiana and US with infection rates of 7.3% and 11.4%, respectively (Zecca *et al.*, 2020; Eric *et al.*, 2021). The difference of the infection rate of *Trypanosoma* spp. might be due to different efficacy of the control programs and sensitivity of diagnostic tests (Alanazi, 2018). The results of molecular methods are more sensitive and many researchers revealed *Trypanosoma* spp. using the PCR technique (Faraj *et al.*, 2015; Eric *et al.*, 2021).

Table 1. PCR protocol used to detect *Trypanosoma* spp. and *T. evansi* in DNA samples.

| Target gene | Primer sequences | Initial denaturation | Amplification (40 cycle) | Denaturation | Annealing | Extension | Product Co. | Final extension |
|-------------|------------------|---------------------|-------------------------|-------------|-----------|-----------|-------------|----------------|
| ITS-1 Gene  | F (5'CCGGAAGTTCACCGATATTG-3') | 95/2 | 95/30 | 58/30 | 72/60 | 72/5 | Bioingenetech | 95/30 |
| VSG Gene    | F (5' CTGAAGAGGTTGGAAATGGAGAAG-3') | 95/2 | 95/30 | 58/30 | 72/60 | 72/5 | Bioingenetech | 95/30 |

(ITS gene): specific for genus *Trypanosoma* (Njiru *et al.*, 2005); (Rotat VSG gene): specific for *Trypanosoma evansi* (Konnai *et al.*, 2009).
Table 2. The infections rate of *Trypanosome* spp. according to the sex.

| Sex    | No. examined cats | No. infected cats | Infectious rate% |
|--------|-------------------|-------------------|------------------|
| Male   | 53                | 26                | 49.1*            |
| Female | 67                | 15                | 22.4*            |
| Total  | 120               | 41                | 34.2             |

(*): Significant value (*p* < 0.05).

Table 3. Infections rate with *Trypanosoma* spp according to the age.

| Age    | No. examined cats | No. infected cats | Infection rate% |
|--------|-------------------|-------------------|------------------|
| ≤1 year| 75                | 34                | 45.3*            |
| >1 year| 45                | 7                 | 15.6*            |
| Total  | 120               | 41                | 34.2             |

*Significant value (*p* < 0.05).

Fig. 1. Gel electrophoresis by using (ITS1) showing M: molecular size marker 100–1,200 base pair. Lane C+: *Trypanosoma* sp. DNA positive control. Lane C−: negative PCR control. Lanes 1–4: template DNA of *Trypanosoma* sp. at 480 bp isolated from cats.

Fig. 2. Gel electrophoresis using RoTat VSG of *T. evansi*. M: molecular size marker 100–1,200 base pair. Lane C+: *T. evansi* DNA positive control. Lane C−: negative PCR control. Lanes 2,4,7,8: template DNA of *T. evansi* isolated from cats at 151 bp. Lanes 1,3,5,6,9: negative DNA samples.
On the other hand, the infection rate was lower in older ages as that could be due to the older animals may produce better immunity against the parasite infection (Eric et al., 2021). The current study showed that there was an increase in the infection rate in males than females. These results are similar to the results of Faraj et al. (2015) and in contrast with Lauricella et al. (2010) who reported that both males and females were affected in similar rates. The clinical signs observed in infected cats were similar to other studies (Gurtler et al., 2007). Change in blood values were observed in affected cats compared to control cats. There was a decrease in TRBC, HB concentration and PCV that causes anemia, similar to others studies done on cats (Da silva et al., 2009). The increase in WBCs was due to increase in lymphocytes, eosinophils, basophils, and monocytes, and decrease of neutrophils was reported, as reported in other studies (Al-Badrani, 2012; Marwa and Alobaidii, 2022). The reason for this decrease could be a result of secondary bacterial infection (Da silva et al., 2009). However, Chaudhary and Iqbal (2000) found increase in the levels of neutrophils in camels infected with T. evansi.

**Author contributions**

NHM and MAA: designed, photographed, and supervised this study. NHM and DAM: collected laboratory samples, conducted the practical part of the study and assisted in data analysis. NHM and MAA:

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**Table 4.** Hematologic parameters of control and infected cats with trypanosomiasis in 10 cats.

| Parameters                  | Control cats (mean ± SE) | Infected cats (mean ± SE) |
|-----------------------------|--------------------------|----------------------------|
| TRBC (10⁹/microliter)       | 6.0 ± 0.46               | 4.20 ± 0.40                |
| HB (g/dl)                   | 10.07 ± 0.77             | 8.55 ± 0.35                |
| PCV (%)                     | 34.80 ± 1.00             | 27.40 ± 0.69               |
| MCV/fl                      | 57.35 ± 2.11             | 54.78 ± 2.40               |
| MCHC (g/dl)                 | 34.44 ± 1.56             | 30.09 ± 1.36               |
| Parasitemia (%)             | 13.57 ± 5.91             |                            |
| TLC (10³/microliter)        | 9.10 ± 0.55              | 7.85 ± 0.85                |
| Neutrophils (%)             | 65.78 ± 0.18             | 58.50 ± 0.26               |
| Lymphocyte (%)              | 25.35 ± 0.13             | 30.15 ± 0.33               |
| Eosinophil (%)              | 5.11 ± 0.05              | 7.70 ± 0.03                |
| Basophils (%)               | 0.06 ± 0.03              | 0.09 ± 0.02                |
| Monocytes (%)               | 4.45 ± 0.35              | 4.68 ± 1.85                |

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**Fig. 3.** Cat suffers from some clinical trypanosomiasis such as pale mucous membranes, fever, dullness, anorexia, emaciation, muco-purulent ocular discharge, incoordination, and anemia.
contributed to the drafting of the manuscript. All authors contributed to the conduct of the study and discussed the results to a satisfactory scientific conclusion.

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Conflict of interest
The authors declare that there is no conflict of interest.

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