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A NEW RECORD, *TRYPANOSOMA (SCHIZOTRYPANUM) VESPERTILIONIS* BATTAGLIA, 1904, FROM *TAPHOZOUS NUDIVENTRIS* GRETZSCHMAR, 1830 (MAMMALIA: CHIROPTERA) IN QENA, EGYPT

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Abstract

The present study characterized morphologically *Trypanosome (Schizotrypanum) vespertilionis* Battaglia, 1904 isolated from insectivorous bats captured in Dandara caves was appeared for the first time record in Qena province this parasite was had a zoonotic importance so that the present work aims to describe the different stages by light microscopy.

Of twenty-five insectivorous bats examined, three bats were found infected with *T. (S) vespertilionis* with an average infection rate of 8.5%. Also, eleven bats (48%) were infested by ticks Ixodida sp. Most stages of *T. (S.) vespertilionis* in the blood were trypomastigote stages, at the same time spheromastigots, epimastigote and amastigotes stages with two shapes slender and broad. Small size and peculiar morphological features of the blood stream trypomastigotes, the more remarkable one being the large round kinetoplast. A posterior body end o appeared as a bulge or small stretch. The free flagellum was fairly long, the undulating membrane was well developed, tightly surrounding the body and had only 2-4 shallow undulation.

Key words: Trypanosomes, Schizotrypanum, Ixodoidea, Bat, Chiroptera, Microchiroptera.

Introduction

Genus *Trypanosom*a Gruby, 1843 infects all vertebrates by haematophagous vectors, multiply as epimastigotes that in general differentiate to trypomastigotes infective to vertebrate hosts. All members of this genus are characterized by the unique presence of trypomastigotes, which are forms absent in other genera of Trypanosomatidae.

Hoare (1972) stated that trypanosomes are identified by the eccentric kinetoplast position and the commonest hosts were among Atriodactyla and Chiroptera. Bats' trypanosomes are represented by the small *cruzi*-like forms of subgenus *Schizotrypanum* and large ones of subgenus *Megatrypanum*. Subgenus *Schizotrypanum* Chagas, 1909 comprised species zoonotic parasitosis only, mainly bats, where they multiply as amastigotes to trypomastigotes, released by cells and invade new cells, and are infective to a new mammalian host. The intracellular development in vertebrate cells is unique of this subgenus.

Schizotrypanum in bats: *Trypanosoma vespertilionis* in Europe, the Americas, Africa and Asia; *T. dionisii* and *T. pipistrelli* in the Old World, *T. pteropi* and *T .hipposideri* in Australia; *T .hedricki* and *T. myotis* in North America; *T. phyllostomae* and *T. marinkellei* in Central and South America (Molyneux, 1991).

Several trypanosome species infect mammals in Africa. Three most important belong to *T. brucei* complex that causes Nagana Disease in cattle. *T. gambiense* causes the chronic sleeping sickness in man and *T. rhodesiense* causes the acute form. *Trypanosoma Schizotrypanum* species were restricted to bats worldwide, with the exception of *T.* (*S.) cruzi* that also infects other mammals. More than 70 Trypanosomes were recorded in bats and transmitted by haemathophagous arthropods (Stevens *et al*. 2001). Marinkle (1982) in Columbia recorded *T. magadermae*. Woo and Hawkins (1975) in East-Africa reported *T. vespertilionis* and *T. hypergi*. Bower and Woo 1982 in Canada isolated *T. vespertilionis, T. hydereicki, T. dionissi* and *T. myoti* from different bats' species. Gardener and Molyneux (1988) in Britain described *T. megatrypanum incertum* in bat (*P. pipistrellus*). In Egypt, Morsy *et al.* (1986) in Cairo, Fahmy *et al*. (1978) in Assiut and Abdel-Rahman *et al.* (2001) in Sohag identified four different species of *T.*

megatrypanium and *Schizotrypanum* in in the bat *P. kuhli*.

All trypanosomes species were identified morphologically in hosts' blood (Loftis *et al*, 2005). Bats trypanosomes are relatively little known, but, pathogenic trypanosomes were intensively studied. Livestock became intermediate or amplifier hosts that spill over into humans, or directly via wildlife and/or vectors (Childs, 2007). So, the bats trypanosomes zoonosis affected human health and welfare (Garnham, 1973).

Chagas disease is zoonosis in the Americas, with triatomine insect (winged bug) *as* a vector (Cimicidae and Triatominae) where development is restricted to digestive tracts as epimastigotes then to infective metacyclic trypomastigotes (Hoare, 1972).

The insectivorous bats are the more commonly the trypanosomes infected hosts (Gardner and Molyneux1988). Streblids ser-ved as mechanical or biological vectors of pathogens and maintained pathogens within bat colonies and bites humans in the bat roosts (Lloyd 2002).

On the other hand, ticks are blood-suckers ectoparasites (Schofield and Dolling, 1993). Heise (1988) reported that arthropods may travel on their hosts' body for dispersal, not just because they did not escape when the bat emerged from the roost while they were feeding.

This study reported *Trypanosoma* (*Schizotrypanum*) *vespertilionis* Battaglia, 1904, from *Taphozous nudiventris* Gretzschmar, 1830 as a new record in Qena Governorate.

Material and methods

A total of 25 bats *Taphozous nudiventris* (the nacked–bellied bat) were captured from Dandara caves, Qena Governorate, Upper Egypt identified and kept at 28-35^{o}C separately in cages. They belong to family Microchiroptera and genera Chiroptera.

For morphological description by light microscopy, thick and thin blood smears were made from the blood and these were later stained in Giemsa's stain.

Trypanosomes were drawn using a Camera Lucida. Also, different stages were photographed by Olympus BH2 light microscope. Tissue smears were made (spleen, liver), on clean slides, as thick and thin films. Dried thick and thin smears of liver and spleen at post mortem and impression smears of organs were stained in Giemsa stain after fixation in methanol.

Ticks were collected from bats after they sprayed with a light insecticide before exposure to anesthesia, preserved in 70% ethanol. Ticks were subsequently crushed (squeezing technique) and the gut contents were separately examined by light microscope. The preparations were washed with saline, air dried, fixed with methanol collected ticks cleared with by put them in 10% Na OH (sodium hydroxide) and stained with Giemsa stain. All measurements were in micrometer and identified as posterior extremity to middle of nucleus (P.N.), kinetoplast to middle of nucleus (K.N.), and middle of nucleus to anterior extremity (N.A.).

Results

Out of 25 bats examined only 3 were infected (8.5%) with this parasite**.** The parasite's biological cycle includes three fundamental forms characterized by the relative positions of the flagellum kinetoplast, and nucleus. Trypomastigotes: 22μm long found in mammalian blood and the hindgut of ixodide they does not multiply. Epimastigotes: Also 20 μm long, kinetoplast anterior to the nucleus, fusiform. They represent the parasite's multiplicative form in the tick intestine, and are the predominant form, the amastigotes: Approximately 2μm in diameter, round without an emergent flagellum.

The nucleus and the kinetoplast are clearly stained. There are almost as many trypanosomes as there are red blood cells, the peripheral smear showed a large number of trypanosomes. The present specimen is a *cruzi*-like trypanosome with the typical crescentric shape in stained specimens. It is a very small organism with a large prominent kinetoplast at its pointed posterior tip. The nucleus is in the posterior half of the body

and its free flagellum is more than two-third its body length. No dividing trypomastigote forms were in blood smears, all forms are detected in blood smears, spleen and liver squeezing. The free flagellum was fairly long. The undulating membrane was well developed, tightly surrounding the body and had only 2-3 shallow undulation as shown in Figs.1, general morphology is similar in all species of this subgenus Schizotrypanum. Most of stages of *T. vespertilionis* which appeared in the blood bat were amastigotes stages and were measured (5-7.14×4.8-7µm) in diameter (Fig. 1). Spheromastigots also were appeared (Fig. 2). A heavy infection of a trypanosome was observed in wet preparations of blood of a *Taphozous*. The body was slightly slender, small in size and with appearing for the amastigote stages in a heavy in the blood cells. The cytoplasm was granular and the kinetoplast nearly was a half size of the nucleus. The nucleus was oval or rounded in shape and measured (1.68-2.76µm x1.23-2µm. Epimastigotes are characterized by the following features: cytostome, disk-shaped and compacted kinetoplast.

The impression smear of liver and spleen contained large number of sphaeromastigotes. These forms showed no sign of division nor did these forms or the trypomastigotes were found in the impression smear. The spheromastigotes were round or oval in shape with a centrally poisoned vacuolated region that remained unstained. The diminutions of the spheromastigotes were 6µ if round or 7µx4µ if oval. The nucleus was always elongated and situated peripherally and was 4µx1.4µ. A round or occasionally oval kinetoplast was located peripherally opposite the nucleus. The diameter of the kinetoplast was approximately 1.1-1.3µ. The flagellum of the spheromastigotes originated from close to the kinetoplast and continued around the periphery of the organism approximately seven eights of the way before becoming a free flagellum; the undulating membrane was very narrow and not readily apparent or undulating. The free flagellum varies in length between 6 and 10 µ. In the trypomastigote (Fig.1) the large kinetoplast is postnuclear and is at the most posterior part of the body; the flagellum emerges from the flagellar pocket, and runs along the entire length of the body as an undulating membrane; trypomastigotes, found both in the blood and in the tick may be pleomorphic ranging in length from 12-40 µm, and the posterior end is more pointed than that of *T. cruzi*.

In the epimastigote the kinetoplast was anterior to the nucleus, with a short undulating membrane running about half the length of the body.

Trypanosomes range in size from 17-22µm with the average length being 20µm. Trypomastigotes, perhaps represented by the intermediate forms, which would develop inside cells; the large kinetoplast is postnuclear and is at the most posterior part of the body; the flagellum emerges from the flagellar pocket, and runs along the entire length of the body as an undulating membrane; trypomastigotes, may be pleomorphic ranging in length from 12-34 µm. Epimastigote was found in salivary glands of tick and blood stream; kinetoplast anterior to nucleus, with a short undulating membrane running about half the length of the body.

Spheromastigotes were round or oval in shape with a centrally positioned vacuolated region that remained unstained (Figs.1&3) the dimentions of the sphaeromastigotes were 6µ if round or 7µx4µ if oval. Nucleus was always elongated and situated peripherally 4µx4µ. A round or occasionally oval kinetoplast was located peripherally opposite nucleus. Diameter of kinetoplast was 1.1-1.3µ. The flagellum of the sphaermastigoates original from close to the kinetoplast and continued around the periphery of the organism, the undulating membrane was very narrow and not really apparent. Multiplies in the blood as trypomastigote because we did see one trypomastigote with 2 kinetoplasts and 2 nuclei (Figs.1&3).

Morphological features distinguished Ixodida from other ticks, such as the straight rostrum ad pressed to the gula, and the ability of the third rostral segment to flex upwards, seem to have been derived in association with adaptations for feeding on vertebrate hosts and more stringent differences between the haematophagous and predatory forms of Ixodida are apparent (figs. 2 & 6).

Discussion

This parasite was seen for the first time in Qena Governorate. The description of *T. (S) vespertilionis* conformed closely to that found in the literature. But, there were some differences; in the position of the nucleus and prominent posterior end of trypomastigotes form.

The present species morphologically resembling *T. rangeli* infecting bats have only been described in Colombia (Marinkelle, 1976) *T. heybergi* was previously described by Rodhain (1923) in *Nycteris hispida*, *T. Schisotrypanum vespertilionis* by Fahmy *et al.* (1978) in Assiut and Morsy *et al*. (1986) in Cairo. The present forms different from others in being longer, a nucleus far from middle of body, and in having longer free flagellum (Figs.1 & 2). Hoare (1972) considered the difference in body size and length of free flagellum as possible individual variations. The present specimens were most closely similar to Dean and Sugay (1963) specimens; but differed in larger size and a longer free flagellum.

In the present study, the nucleus was in the posterior region of the body while in most descriptions of the trypanosome, the nucleus was in the body anterior region. Hoare (1972) in his review concluded that undue emphasis had been placed on the position of the nucleus in this trypanosome and that the variation was too great for it to have taxonomic value.

In the present study, measurements were closely resembled those given by Reichenow (1940) in Tanzania. One small difference was in the free flagellum length. The present trypanosomes showed a slightly longer free flagellum.

The present specimen was recorded from Coleuraafra, *Rhinolophuse loquens*, *Taphozous perforatus*, *Pipistrellus nanus* and *T. (M.) heybergi* as a relatively large trypanosome with kinetoplast nearer to the nucleus than to posterior tip; nucleus occupied a marginal position near the middle of body. Length of free flagellum was quite variable but undulating membrane well developed.

Bat trypanosomes (as *T. dionisii* and *T. vespertilionis*) are morphologically indistinguishable from *T. cruzi*. Therefore, bat trypanosomes could only be assigned to *T. cruzi* subgenus *T.* (*Schizotrypanum*) sp. based on morphology. *Schizotrypanum* as subgenus, *T. (S.) cruzi* was the type species (Hoare, 1964, 1972). Moreover, an interesting aspect of *T. cruzi* biology was described by Deane *et al*. (1984) who identified stages typically found in triatomines in the lumen of anal glands of the opossum *Didelphis marsupialis*.

T. (M.) mpapuense like *T. (M.) heybergi* is a relatively large trypanosome with a pointed posterior tip. However, its kinetoplast is closer to its nucleus than that of *T. (M.) heybergi.* It has a short free flagellum, and the undulating membrane well developed.

The free flagellum varied in length between 6 and 10µ similar forms were found in *T. cruzi* by Correa and Barrette (1964); Wood (1953) and Patena (1969) however, these authors did not exhibit vacuoles. The transformation process from sphaeromastigote to trypomastigote was similar to that described by Rodriguez and Marinkelle (1970) in the Tulahuen strain of *T. cruzi* with the opening up of the region between the kinetoplast and the point where the flagellum became free flagellum.

Vianna (1911) evidencing the parasite multiplication in several tissues, mainly myocardium and skeletal muscles also who discovered the intracellular multiplication of *T. cruzi,* and correctly interpreted it as occurring by binary fission, They multiply inside

host cells, producing their rupture, and liberating trypomastigotes into the blood stream that can once again invade any nucleated cell (Lisboa *et al*, 2008). They can be grown in culture in muscle cells, fibroblasts, and macrophages among others (Fahmy *et al*, 1978). In the present work premature stages were discovered in spleen and liver but slender stages are present in blood.

The morphological variability of trypomastigotes was early reported by Chagas (1909) who described the slender and broad forms. However, other types, as short, very broad and intermediate forms were found. The rate of these forms varied according to the parasite strain; the phase of infection, as well as the host species. Recent studies have suggested that the different trypanosome forms may have also different biological roles. Schmatz *et al*. (1983) provided evidences that the slender forms would be more fitted for cellular invasion, whereas the broad ones would more promptly develop in arthropods. However, Deane (1979) emphasized the possible occurrence of undifferentiated or ambivalent blood stream. trypomastigotes, perhaps represented by the intermediate forms that develop either inside cells or in axenic cultures .

In the present study, the *T. vespertilionis* like trypanosomes was identical with those of Abdel- Rahman *et al*. (2001) which they named *Trypanosome* (*Schizotrypanum*) *assiutis* sp. nov., which measured 19.4µm in maximum body length, one nucleus 1.16µm encountered in local Egyptian bat *Vesperugo kuhli*. In the present study, *Trypanosoma* measured 22µm in maximum body length, nucleus measured 1.68-2.7µm.and encountered in *Taphozous nudiventris* bat.

All *T. vansi* isolates described (Pinto *et al*, 2012) have maximum of body length of 34µm, and mean length of *T. vivax* ranged between 18.73-25.4µm. But, the present parasite measured 22µm in maximum length.

The present specimens morphologically resembling *T. rangeli* infecting bats have only been described in Colombia Marinkelle (1976). Similar forms were found in *T. cruzi* by Petana (1969); the former described by them however, did not exhibit vacuoles. But, the transformation process from spheromastigotes to trypomastigote agreed with Rodriguez and Marinklle (1970) in the Tulahuen strain of *T. cruzi* with the opening up of the region between the kinetoplast and the point where the flagellum leaves the body surface.

T. (M.) mpapuense like *T. (M.) heybergi* is a relatively large trypanosome with a pointed posterior tip. However, its kinetoplast is closer to its nucleus than that of *T. (M.) heybergi*. It has a short free flagellum, and the undulating membrane well developed. It probably multiplies in the blood as trypomastigote as one trypomastigote with 2 kinetoplasts and 2 nuclei was detected in the present study.

The body of the epimastigotes owns seems to be less stumpy than those of *T.cruzi* and also the aspect of the flagella is perhaps more suggestive of a free than undulating membrane. The morphological similarity between the blood forms of *T. vespertilionis* and *T. cruzi* has been recognized for some time but in addition to this the pattern of development from amastigotes present.

Anonyme (1991) mentioned that mammals could be infected by conventional direct transmission by insect-vector or by ingestion of infective stages present in these insects or in their excreta, or in the consumption of food and contaminated with opossum feces. Tejera (1920) reported that *T. rangeli* is a non-pathogenic trypanosome and mammals were infected by feces inoculation some triatomine-vectors that shared hosts with *T. cruzi* (D'Alessandro and Saravia, 1992). Mouthparts of ticks are adapted for piercing skin and sucking blood. Like most blood-sucking arthropods, they injected anticoagulant saliva during feeding to prevent blood coagulation (Schofield and Dolling 1993).

D'Alessandro (1976) found the mean total *T. vespertilionis* in *C. Iretrrlarirrs* differed markedly from *T. hrdrick*i and *T. myti*. Nei-

ther promastigotes nor multiple fission stages, epimastigotes changed into metatrypanosomes in all *C. Iretrrlarirrs.*

Rodhain (1939) reported metatrypanosomes after 8 days in *C. Iretrrlarirrs*. The stages of *T. vespertilionis* in present study were similar to those described from cultures (Bower and Woo, 1981). Brack (1968) reported both promastigote like forms and multiple fission forms for *T. cruzi* in the vector. *T. myoti* and *T. hedricki*, however, do not develop sphaeromastigotes in *Cimex*, a stage believed was the initial stage in the formation of metacyclic trypanosomes.

The morphological similarity between *T. vespertilionis* and *T. cruzi* was recognized for some time but in addition to this the pattern of development from amastigote to trypomastigote via the sphaeromastigote was found in *T. vespertilionis* a process (Petana, 1969; Rodriguez and Marin-Kelle, 1970) and sphaeromastigote stages in *T. cruzi* was found epimastigotes as well. Chatton and Courrier (1921) described epimastigoate from tissues of infected bats. Rodriguez and Marinkelle (1970) did not find epimastigote forms in the developmental cycle amastigote to trypomastigote. Shape and size of the different stages were identical with those of *T. c. cruzi*. The frequencies in the various stages of flagellates in different parts of intestine as those of *T. c. cruzi* in *Triatoma dimidiata* were reported by Petana (1971).

In the present study, low levels of parasitaemia and low counts of tissue forms in bat agreed with Marinkelle (1976) who considered natural resistance of bats to infection was due to high environmental temperature of bats reflected in body temperature.

Trypanosoma lewisi is a cosmopolitan species originally found in *Rattus* spp., nonpathogenic, host-restricted, and trans-mitted by rat fleas and was reported in Upper Egypt (Sakla and Monib, 1984). This species has been recorded as an opportunist blood parasite of human beings mainly in Asia, with a case in Africa. In Brazil, this species was recorded in captive monkeys. As *T. lewisi* can share vertebrate hosts both with *T. rangeli* and *T. cruzi*, some markers for the differential diagnosis of these species were demonstrated (de Sousa, 2014). In Egypt, *Trypanosoma evansi* is the cause of trypanosomiasis (Surra) which multiples in blood and body fluids and transmitted by tabanids flies were reported in camels and as zoonotic trypanosomiasis in a camels' keeper man (Haridy *et al*, 2011).

Conclusion

The Egyptian bat *Taphozous nudiventris* is recorded as a new host for *Trypanosoma vespertilionis* in Qena Governorate. The ixodids ticks are ectopartasites on this bat species infected with *T. vespertilionis*.

Now is *T. vespertilionis of bats* zoonoses that originate in wildlife?

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Explanation of figures

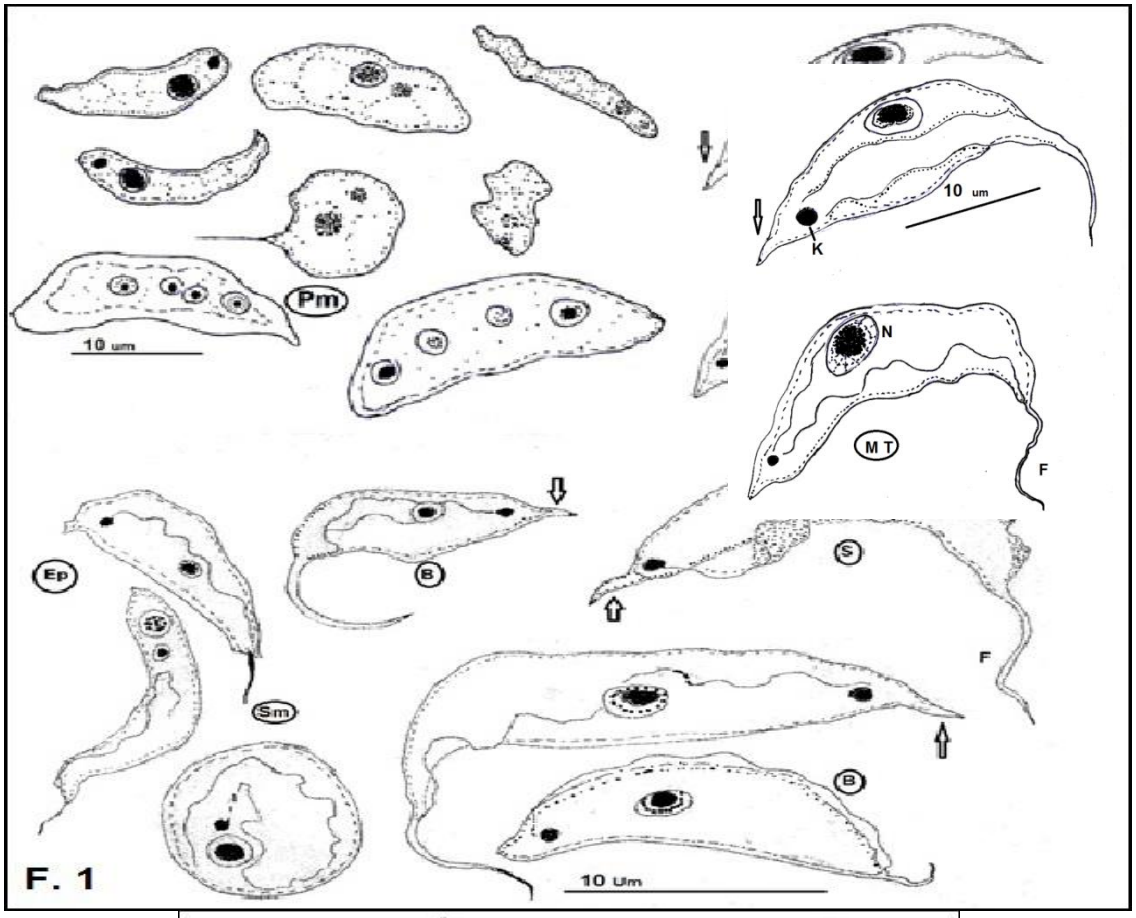
Fig. 1: Camera Lucida drawings. All stages of *Trypanosoma(S) vespertilionis* in bats note prominent kinetoplast (K) and broad (B) and slender(S) trypomastigoat, Short sausage-shape of triangular trypomastigotes, Rounded trypomastigotes,. Amastigotes, Sphaeromastigotes (Sm), Pear-shaped epimastigotes(Em), Large elongated epimastigotes. Transitional form (T), Short spindle-shaped epimastigotes(Se), metacyclic trypomastigotes(MT),premature(PM) stages in natural infection of liver and spleen.

Fig. 2: Camera Lucida drawing female Ixodida sp. with magnification of mouth parts. Metacyclic trypomastigoat stage found in gut. Note long flagellum more remarkable one being the large round kinetoplast., large nucleus(N) a posterior body end as a bulge or small stretch. Free flagellum fairly long, the undulating membrane was well developed, tightly surrounding body with 2-4 shallow (um) undulation, broad (B) and slender(S) metatrypomastigotes note prominent posterior end.

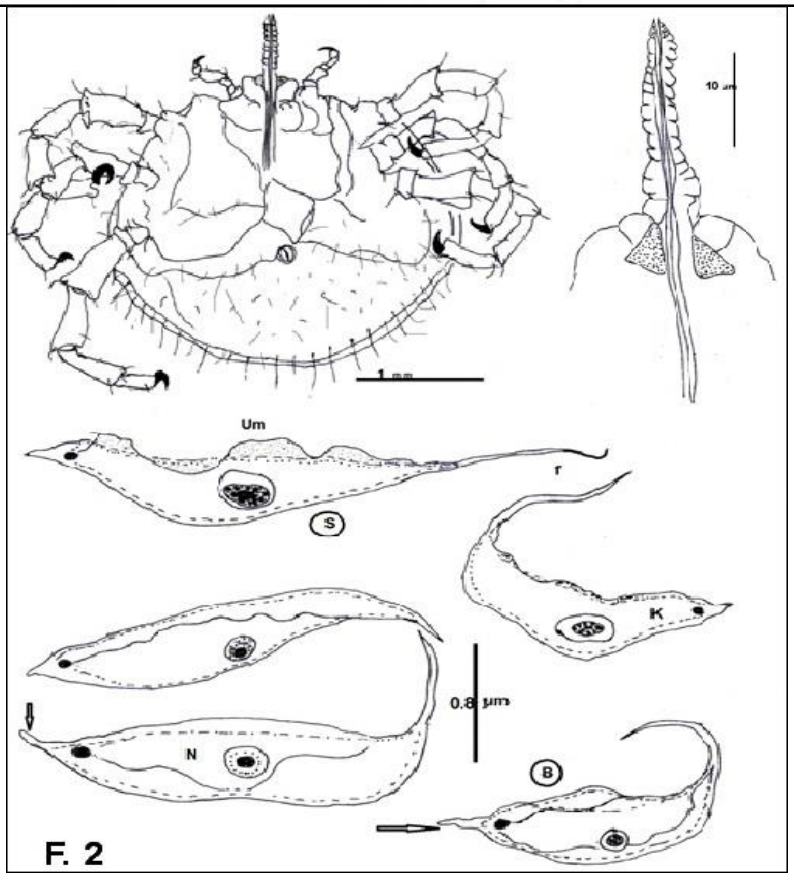
Fig.3: Photomicrographs metacyclic trypomastigoat (MT) and trypomastigoat (TM) stages of *Trypanosoma vespertilionis* in blood stream of bat; note prominent kinetoplast, large nucleus and long Geimsa stained free flagellum.

Fig. 4: Photomicrographs Epimastigote stages of *T. vespertilionis* in blood stream of insectivorous bat; note the prominent kinetoplast, large nucleus, small Geimsa stained free flagellum.

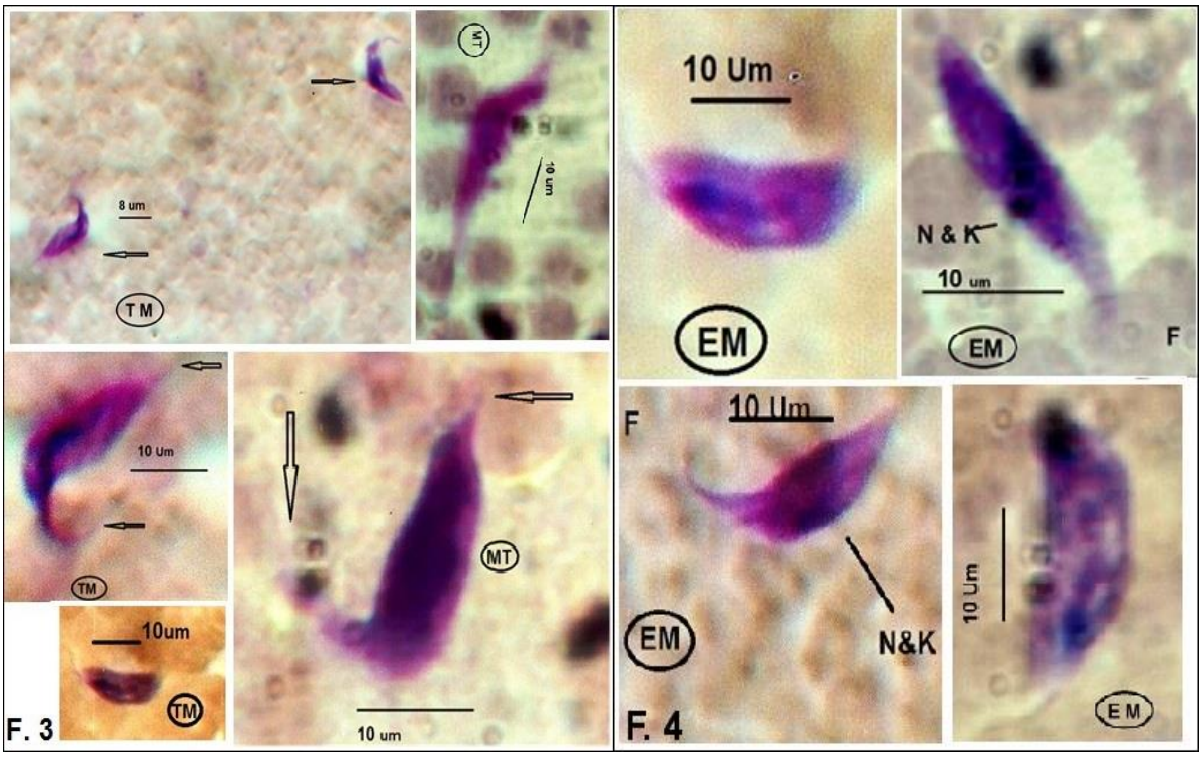
Fig. 5: Photomicrograph showing male (m) and female (F) ixodida sp., and infective stage metacyclic trypomatigoat(MT) free flagellum fairly long, undulating membrane well developed, tightly surrounding body with 2-4 shallow undulation, body posterior end as a bulge or small stretch. Mouthparts of ticks especially adapted for piercing skin and sucking blood.



F. 1



F. 2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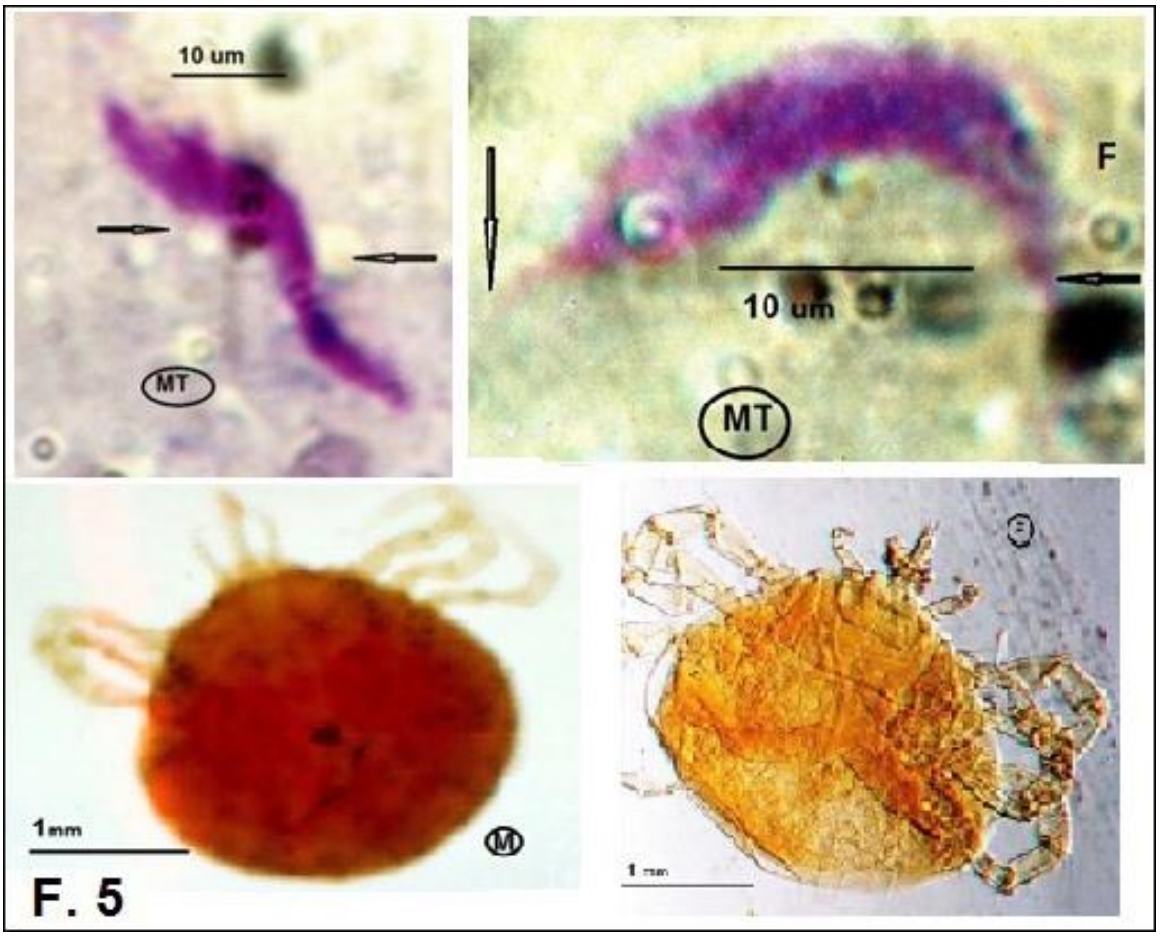
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