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The Genus *Rhipicephalus* in Egypt: Morphological Description of Species and Molecular Detection of Pathogens Threatening Animal Health

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Abstract

Ticks are vectors of several pathogenic agents, causing illnesses that range from mild to severe in humans, domestic animals, and wildlife. Accurate morphological identification of ticks is crucial for assessing the epidemiological status of tick-borne diseases. In Egypt, the contribution of ticks in the transmission of zoonotic diseases remains unclear because of the scarce data on tick diversity. This study aims to investigate the morphological characteristics and prevalence of *Rhipicephalus* tick species infesting domestic animals in Egypt, and to detect the associated tick-borne pathogens using molecular techniques. From October 2021 to March 2024, ticks were gathered from cattle, dogs, and camels across ten Egyptian governorates: Cairo, Kalyoubia, Alexandria, Kafr El-Sheikh, Beheira, Gharbia, Menoufia, Giza, Ismailia, and Sharkia. The collected ticks were morphologically described and tested for the presence of *Babesia*, *Ehrlichia/Anaplasma*, and *Borrelia* pathogens using Polymerase Chain Reaction (PCR). A total of 4,488 ticks were collected from domesticated animals, representing eight species of the genus *Rhipicephalus*: *Rhipicephalus annulatus, R. microplus, R. decoloratus, R. evertsi, R. pulchellus, R. sanguineus, R. simus, and R. turanicus. Babesia* was detected in *R. annulatus, R. sanguineus*, and *R. evertsi*. *Borrelia burgdorferi* was identified in *R. annulatus, R. microplus, R. sanguineus,* and *R. simus. Ehrlichia/Anaplasma* were detected in *R. sanguineus, R. decoloratus,* and *R. turanicus.* In the present study, we demonstrated the introduction of several *Rhipicephalus* tick species that had not been previously reported in Egypt, including *Rhipicephalus microplus, R. simus, R. evertsi*, and *R. turanicus* and confirmed the presence of pathogens using PCR techniques.

Keywords: *Rhipicephalus*, Tick-borne diseases, PCR, Egypt.

Introduction

Ticks are a primary group of obligatory hematophagous ectoparasites that infest animals worldwide. They pose significant risks to animal health, hinder growth and reproduction, and transmit infections that lead to substantial economic losses [1,2,3]. Since the turn of the 19th century, when Smith and Kilborne first described tick-transmitted illness [4], multiple tick species have been recognized as reservoirs and transmitters of a wide range of infections that can cause serious illness and death in both humans and animals. Due to their increasing prevalence, virulence, and socioeconomic impact, several of the illnesses that have been reported since then, such as East Coast fever and Crimean-Congo haemorrhagic fever, present significant public health, veterinary, and socioeconomic challenges [5]**.** A variety of microorganisms are transmitted by *Rhipicephalus spp*., including viruses, bacteria, rickettsia, protozoa, and even certain helminths. *Theileria, Anaplasma, Ehrlichia*, and *Babesia* species, along with other *Rhipicephalus*-borne infections, have been identified

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as being relevant to the region in question. Symptoms in host animals frequently include anaemia, jaundice, leukopenia, thrombocytopenia, and other clinical indicators of haemolytic alterations. Infestations of *Rhipicephalus* ticks also cause ectoparasitic discomfort in both humans and animals [6,7,8].

Rhipicephalus species have successfully coexisted with humans and are known to be active throughout the year, being most prevalent in tropical and subtropical climates [9]. Their occurrence records are concentrated in southern Africa, Europe, North and South America, as well as Asia and Australia. This genus is distributed globally across nearly all continents, except for the extremely low temperatures found at the North and South Poles and in some specific countries. Many species distributions are expected to expand or contract due to global warming and climate change [9]. In Egypt, the majority of this genus is found in the northern and central regions of Egypt. Egypt, situated on the southern shore of the Mediterranean Sea, serves as a crucial migration route for birds traveling from Eurasia to Africa for breeding and wintering [10].

Despite the significant economic burden caused by tick-borne diseases, reliable data remain limited, as statistics on the incidence of these diseases and the global distribution of various tick vectors are often either unavailable or outdated in many African countries. Therefore, it is essential to accurately identify and update tick distribution to predict the likelihood of the development or re-emergence of tick-borne diseases in the sub-region [11].

Accurate taxonomic identification of tick species is crucial for the effective control and surveillance of tick-borne diseases. Traditional identification methods have relied on the morphological examination of adult specimens. Although molecular techniques and phylogenetic analysis are now widely used in tick systematics, conventional morphological characterization remains the keystone of tick identification. This work aims to employ conventional morphological identification techniques to support comprehensive surveillance of *Rhipicephalus* tick species and the pathogens they harbour in Egypt.

Material and Methods

Tick Collection and Morphological Identification

Ticks were collected from camels, cattle, and dogs during a survey conducted from October 2021 to March 2024. The survey encompassed several governorates in northern and central Egypt, including Cairo (30°02′N, 31°13′E), Alexandria (31°12'N, 29°57'E), Giza (30°00'N, 31°12'E), Kalyoubia (30°18'N, 31°15'E), Sharkia)30°42′N, 31°37′E(, Kafr El-Sheikh (31°06'N, 30°56'E), Gharbia (30°52′N, 31°03′E), Menoufia (30°35'N, 30°59'E), Ismailia (30°35'N, 32°16') and Beheira (30°37′N, 30°26′E) Governorates (Fig. 1).

Animals (n = 200; cattle = 50, camels = 60, and dogs = 90) were thoroughly examined for tick infestation, focusing on areas such as inner thighs, udder, scrotum, neck, dewlap, and axilla. Ticks were collected manually using sterile forceps and subsequently transferred to the Entomology Department laboratory at the Faculty of Science, Ain Shams University. The collected ticks were categorized by host species and sex. Any dermal remnants adhering to the ticks' mouthparts were carefully removed. The ticks were stored in a solution of (70%) ethanol and (30%) glycerol to ensure their suitability for subsequent identification and study.

Morphological identification of tick species was performed using diagnostic keys [12,13,14,15,16] and Digital Microscope Magnifying Glass (Andonstar Digital Microscope, 2000x)

DNA Extraction from Ticks

Tick pools were prepared from 2 to 3 individuals of the same species of collected ticks from each governorate and each host to identify the exact infected species associated with each specific host and the precise locality affected by the pathogen. For *Rhipicephalus annulatus* and *Rhipicephalus sanguineus*, larger pools comprising more than 15 individuals were formed due to their high abundance. Approximately 100 pools of the selected species, originating from different hosts, were chosen for PCR analysis.

DNA was extracted from the collected tick species following the manufacturer's instructions provided in EasyPure® Genomic DNA Extraction kit. Tick bodies were homogenized by grinding in liquid nitrogen, and the resulting material was placed in labelled tubes. After adding the buffer and Proteinase K solution, the mixture was thoroughly combined with the ground tick bodies and incubated at 56 ºC until complete lysis occurred. Subsequently, ethanol was added, and the tube was vortexed to ensure thorough mixing. The mixture was then transferred to a spin column, centrifuged, and the flow-through was discarded. After a series of washing steps, the mixture was centrifuged again. Finally, Elution Buffer was added, incubated at ambient temperature before being centrifuged to collect the purified DNA. The extracted DNA was stored at -20 ºC for further processing [17].

Molecular Detection of Pathogens in Ticks

Conventional PCR was conducted to detect pathogen DNA in the collected tick species (Table 1), specifically targeting rRNA genes for *Babesia*, *Borrelia burgdorferi*, and *Ehrlichia/Anaplasma*. For *Babesia* DNA detection, the 18S rRNA gene was

amplified using forward primer 3.1 (5′-CTCCTTCCTTTAAGTGATAAG) and reverse primer 5.1 (5′-CCTGGTTGATCCTGCCAGTAGT) [18]. The thermal cycling conditions included an initial denaturation phase carried out at 94°C for 1 minute, followed by 30 cycles of 94°C for 1 minute, 48°C for 1 minute, and 72°C for 2 minutes, with a final extension at 72°C for 5 minutes. Regarding *Borrelia burgdorferi* DNA detection, the 23S rRNA gene was targeted using forward primer Bb23Sf (5′-CGAGTCTTAAAAGGGCGATTTAGT) and reverse primer Bb23Sr (5′-GCTTCAGCCTGGCCATAAATAG) [19]. The thermal cycling conditions included an initial denaturation at 95°C for 15 minutes, followed by 46 cycles of 95°C for 20 seconds, 58°C for 30 seconds, and 68°C for 30 seconds, with a final extension at 68°C for 10 minutes. For *Ehrlichia/Anaplasma* DNA detection, the 18S rRNA gene was targeted using forward primer (5′-AGAACGAACGCTGGCGGCAAGCC) and reverse primer (5′-CGTATTACCGCGGCTGCTGGCA) [18]. The thermal cycling conditions included an initial denaturation at 94°C for 3 minutes, followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 2 minutes. PCR was conducted using 5 µl of genomic DNA ,1 µl of each primer (0.5 µM), distilled water and Taq 2X Master Mix (New England Biolabs, UK). Products (10 µl) were mixed with 2 µl of loading dye (Qiagen, Germany) and electrophoresed on (1.5%) agarose gel stained with ethidium bromide. Amplicons were stored at -20 °C before further analysis.

Serological and Molecular Detection of Babesia in Canine Blood

Blood samples were collected from dogs, and thin fixed blood smears were prepared using traditional methods and stained with the Hemacolor Rapid Staining kit. This kit offers the convenience of a quick stain while maintaining the clarity of Pappenheim staining. The staining solutions, red eosin, and blue Azur were applied separately to ensure stability. The blood samples were analysed under a light microscope with a magnification of 100x.

The total DNA from the canine blood sample was isolated using the EasyPure Blood Genomic DNA Kit (Beijing TransGen Biotech Co., Ltd., China). In brief, the blood sample was resuspended in sterile water and combined with a lysis solution comprising proteinase K and Binding Buffer. The lysis mixture was incubated at room temperature. The lysates were then subsequently added to a centrifugal column to bind the DNA. The bound DNA underwent a series of washing and then subsequently eluted from the centrifugal column. Spectrophotometry was employed to quantify the extracted genomic DNA. The DNA samples were kept at −20 °C until they were analysed.

Results

Tick Identification

A total of 4,488 ticks were collected from cattle, dogs, and camels. Among the collected ticks, 2,382 females and 2,106 males were identified as belonging to the genus *Rhipicephalus*. Eight species were identified: *Rhipicephalus annulatus, R. decoloratus, R. evertsi, R. microplus, R. pulchellus, R. sanguineus, R. simus,* and *R. turanicus. Rhipicephalus sanguineus* was the most prevalent species, accounting for (54.8%) and was the most widely distributed species in Egypt. It mostly lives on dogs and was collected from various locations: Cairo (40%), Alexandria (20%), Kalyoubia (20%), Giza (10%), Gharbia (5%), Beheira (3%), and Ismailia (2%). This was followed by *Rhipicephalus annulatus*, which represents (44.5%) of the collected specimens. The collections were from Cairo (50%), Kalyoubia (30%), Sharkia (9%), Kafr El-Sheikh (6%) and Gharbia (5%). *Rhipicephalus microplus* (n=2, 0.04%) and *R. simus* (n=3, 0.07%) represent the least number collected and were collected from cattle and camel. Both *R. turanicus* and *R. evertsi* have the same number of species (n=5, 0.11%) specimens for each. *R. turanicus* was collected from cattle, while *R. evertsi* was collected from cattle and camels. *R. pulchellus* was collected from camels, number of specimens were 6 (0.13%). Only 10 (0.22%) specimens of *R. decoloratus* were collected from cattle in Cairo and Giza governorates (Table 2).

Rhipicephalus sanguineus (Fig.2)

This species is widely distributed in Egypt, infecting all body parts of dogs, including the head, eyelids, ears, legs, tail, and other areas.

Male (dorsal view)

Male *R. sanguineus* is reddish-brown with an elongated body, measuring 4-5 mm in length (Fig. 2A). It is medium to large in size, with distinct punctuations and dark legs (Fig. 2A & B). The festoons are prominent, and a bulged caudal appendage is visible at the posterior centre of the scutum (Fig. 2A & C). The anterior spur of the first coxa is short and not easily visible from dorsal (Fig. 2B & D). Three deep posterior grooves- oval medially and early circular laterally (Fig. 2A). The basis capituli are sharply defined laterally (Fig. 2A & D). The scutum is fully covered with long lateral grooves reaching the eyes, which are pale and convex (Fig. 2A & C). Distinct punctations present on the scutum and the festoons (Fig. 2A).

Male (ventral view)

Ventrally, the tick displays a distinct posterior anal groove beneath the anus (Fig. 2F). The anus is bordered by dark, comma-shaped adanal plates that are broad at the base and slightly curved, with prominent accessory adanal plates adjacent to them

(Fig. 2B & F). The spiracle plates have a wide head and a very narrow tail, measuring about half the width of the adjacent festoon (Fig. 2E). The legs are dark brown with fine yellow hairs posteriorly (Fig. 2B).

Rhipicephalus annulatus (Fig. 3)

Cattle are the primary hosts of *Rhipicephalus annulatus*, a species that is widely distributed throughout Egypt. Specimens have been collected from Cairo, Kalyoubia, Sharkia, Kafr el-Sheikh, and Gharbia. Of the 4,488 specimens examined, 1995 (44.5%) were identified as *R. annulatus.*

Male (dorsal view)

Males measure 1 to 1.3 mm in length and have an oval, pale brown body, darker than the legs, (Fig. 3A). Coxa 1 spurs are visible dorsally, while the plates' spurs are not observable (Fig. 3A & F). Cornua are present (Fig. 3A). Legs terminate in claws and pulvilli (Fig. 3E), the scutum has four longitudinal hair lines, with the inner two intersecting centrally (Fig. 3A). Dense yellow hairs cover both the scutum and legs (Fig. 3A & B).

Male (ventral view)

The caudal appendage is absent in males (Fig. 3A). The hypostome has four columns of teeth arranged in a 4 by 4 pattern (Fig. 3D). The ventral plates are large, and their spurs are indistinct, exhibiting blunt ends on both adanal plates and accessory adanal plates (Fig. 3B &F). Coxa 1 has two distinct spurs located posteriorly (Fig. 3C). Coxa 2 has a smooth edge with no spurs (Fig. 3C).

Rhipicephalus microplus (Fig. 4)

Male (dorsal view)

The body measures 1.5 mm to 2.3 mm and is oval to rectangular, with four lateral circular grooves; two mid-body and two posteriors on the scutum (Fig. 4A). The short palp has a concave internal margin without a protuberance (Fig. 4D). Ventral plate spurs are not visible dorsally (Fig. 4F & G). The scutum is darker than the outer lateral margins (Fig. 4A & E) and features a short hexagonal capitulum (Fig. 4D & B). Pale cream legs and distinct cornua are observed (Fig. 4A). The spurs of coxa 1 are long, with the anterior spur visible dorsally (Fig. 4A & B).

Male (ventral view)

Ventrally, the hypostomal teeth are aligned in a standard 4x4 columns (Fig. 4D), and the spiracle is circular (Fig. 4C). The ventral plate and adanal plate spurs, as well as the accessory adanal spurs, are indistinct (Fig. 4B & F). The caudal appendage is very narrow and small (Fig. 4E & F), and ventral plate spurs are not visible dorsally (Fig. 4E & F).

Rhipicephalus decoloratus (Fig. 5)

Male (dorsal view)

This species is darker than *R. annulatus* and *R. microplus* (Fig. 5A). The body size ranges from 2 mm to 2.4 mm, and it is oval-shaped, being widest in the middle (Fig. 5A). Cornua are present (Fig. 5A). The posterior view of coxa 1 is divided into two sharp chitinous spurs, with a sharp external edge on the lateral view of this coxa (Fig. 5D). The scutum is covered with dense yellow to white hairs arranged randomly (Fig. 5D).

Male (ventral view)

The only species of the subgenus *Boophilus* that possesses three columns of teeth on the hypostome is *Rhipicephalus decoloratus*; this characteristic is specific for this species (Fig. 5C). The adanal plates and accessory adanal plates are notably large, with terminal spurs extending beyond the body line and visible dorsally (Fig. 5A & B). A distinctive bristle-bearing protuberance on the internal ventral surface of the basal palpal segment (Fig. 5F). This characteristic plays a crucial role in accurately identifying this tick species. The spiracles are spherical.

Rhipicephalus simus (Fig. 6)

In this study, this species was collected from cattle in Kalyoubia Governorate.

Male (dorsal view)

The body size measures 3.6 mm to 4 mm, with a large, dark brown to blackish scutum exhibiting a smooth, shiny appearance (Fig. 6A). Minute interstitial and setiferous punctuations are present, while posterior grooves are absent (Fig. 6A & G). The scapular grooves are not deep, and the cervical fields are smooth with no wrinkles (Fig. 6A). The anterior spur of the first coxa is not visible dorsally and the eyes are flat (Fig. 6A). Lateral grooves are distinct and exhibit a punctuate texture and the basis capituli is hexagonal (Fig. 6A & C). There are four columns of setiferous punctuations in the posterior region of the scutum (Fig. 6A).

Male (ventral view)

Ventrally, both the accessory adanal plates and adanal plates are large, with adanal plates sharply curved (Fig. 6B & D). Fed males possess a prominent caudal appendage that extends beyond the outline of the body (Fig. 6D & G). The spiracles are large, strongly curved, and terminate at half the width of the adjacent festoon; spiracle area lacks dense hairs (Fig. 6E).

Rhipicephalus evertsi (Fig. 7)

Rhipicephalus evertsi is a medium to large, robust tick that ranges from 3.5 mm to 4 mm in size.

Commonly known as the red-legged tick. It was collected from cattle in this study.

Male (dorsal view)

The scutum has a rough, uneven texture due to pronounced wrinkling texture in the cervical field (Fig. 7A). Anterior spurs of coxa 1 are visible dorsally (Fig. 7D). Interstitial punctation is widely distributed and varies in size, while setiferous punctations are absent (Fig. 7A & E). The eyes are beady and convex, and the conscutum is dark in color (Fig. 7A & E). Three flat, wrinkled posterior grooves, and distinct wrinkled lateral grooves are present in the posterior region (Fig. 7A & E).

Male (ventral view)

Accessory adanal plates are small, while anal plates are broad and have straighter views, resembling a triangular shape (Fig. 7C). No caudal appendage is present on the posterior view of the body (Fig. 7B). The spiracle plate areas are broad and covered with dense setae (Fig. 7F). The legs exhibit a uniform orange to red color ring on each leg segment (Fig. 7A & E).

Rhipicephalus turanicus (Fig. 8)

Male (dorsal view)

The length of this specimen ranges from 3.3 to 4.5 mm. Male *R. turanicus* shows shallow cervical fields and a prominent, dark-coloured caudal appendage projecting beyond the body when fed (Fig. 8A). Interstitial punctations range from small to medium with setiferous punctations present (Fig. 8A). The anterior spur of coxae 1 is not visible from a dorsal perspective (Fig. 8A & F). The cervical fields are depressed and smooth and the eyes are flat (Fig. 8A). There are distinct posterior grooves with deep wrinkled depressions while, the posterolateral grooves are nearly spherical (Fig 8A & D). Lateral grooves are textured but not punctate (Fig. 8A & D).

Male (ventral view)

The spiral plate tails are broad, matching the width of the adjacent festoon (Fig. 8E). The three dorsal grooves are clearly visible (Fig. 8D). The accessory adanal plates are significant in size and the adanal plates are large and trapezoid in shape and prominent (Fig. 8B). The caudal appendage is wide and protrudes as a prominent bulge (Fig. 8B).

Rhipicephalus pulchellus (Fig. 9)

The Zebra tick, commonly known as *R. pulchellus*, is the only ornate *Rhipicephalus* species, easily recognized by distinctive white stripes on a black background, which resemble a zebra. It infests various hosts, including humans. In this study, six specimens (0.13%) out of 4,488 collected from camels in Cairo were identified as *R. pulchellus* (Fig. 9).

Male (dorsal view)

Male *R. pulchellus* measures from 4 mm to 5 mm and displays distinctive white enamel stripes on a dark brown conscutum (Fig. 9). The scutum has widely distributed minute interstitial puncta and separate setiferous structures. The basis capituli has blunt lateral angles, and the eyes are flat. Males have anterior spurs on coxae 1 (Fig. 9). The conscutum's ivory-white pattern on a dark background is a key identifying feature (Fig. 9).

Molecular Detection and Distribution of Tick-Borne Pathogens

Comparative analysis of the PCR results across the eight *Rhipicephalus* species revealed notable variation in both the number of ticks tested and the infection prevalence (Fig. 10). *R. sanguineus* had the largest tested sample size (n=765) and the greatest number of positive cases (n=60, 7.84%). Similarly, *R. annulatus* demonstrated a high infection rate, with 45(6.52%) out of 690 tested samples. In contrast, *R. microplus*, *R. evertsi*, *R. simus*, and *R. turanicus* were tested in much lower numbers (ranging from 2 to 6 specimens each), with one to three positive cases recorded. Conversely, *R. pulchellus* tested negatives for all screened pathogens (Fig. 10).

PCR analysis detected *Babesia* DNA in *R. annulatus* (n=30, 4.53%) collected from cattle in Sharkia, *R. evertsi* (n=2, 50%) collected from cattle in Cairo, and *R. sanguineus* (n= 3, 3.92%) collected from dogs in Cairo (Fig. 10 & 11 and Table 3). Additionally, one blood sample from a dog tested positive for canine babesiosis, as confirmed by PCR analysis, clinical signs, and microscopic examination (Fig. 12). PCR amplification produced a 450 bp DNA fragment specific to the *Babesia* genus, confirming the presence of *Babesia* in both the blood sample and the three tick species (Fig. 13).

Borrelia burgdorferi DNA was detected in *R. annulatus* (n=15, 2.17%) collected from cattle in Sharkia, *R. microplus* (n=1, 50%) collected from cattle in Kalyoubia, *R. sanguineus* (n=15, 1.96%) collected from dogs in Ismailia, and *R. simus* (n=3, 100%) collected from cattle in Kalyoubia (Fig. 10 & 11 and Table 3). Amplification using *Borrelia burgdorferi*-specific primers yielded a 75 bp DNA fragment in all positive samples, indicating the presence of *Borrelia burgdorferi* (Fig. 14).

Ehrlichia/Anaplasma was detected in R. decoloratus (n=3, 33.33%) collected from cattle in Giza, R. *sanguineus* (n=15, 1.96%) collected from dogs in Alexandria, and *R. turanicus* (n=3, 100%) collected from cattle in Giza (Fig. 10 & 11 and Table 3). PCR reactions that produced a 450 bp band were considered positive for the *Ehrlichia/Anaplasma* infection (Fig. 15).

Discussion

The burden of tick-borne diseases (TBDs) on public health is significantly underappreciated and has the potential to overwhelm health systems and economic conditions in countries like Egypt [20]. Several governorates in Egypt have reported cases of babesiosis, theileriosis, anaplasmosis, rickettsiosis, ehrlichiosis, and borreliosis [10]. One of the primary challenges in preventing and managing TBDs is disrupting the transmission chain that involves ticks and their vertebrate hosts [21]. Although modern molecular techniques serve as powerful tools for confirming tick species, morphological identification remains the fundamental and most widely practiced method in tick taxonomy. In the present study, the collected *Rhipicephalus* tick species were identified based on morphological characteristics despite the limited availability of detailed reference images. This represents a notable effort, as most previous studies in the region have focused on only two to three *Rhipicephalus* tick species [22,23,24]. Interestingly, the current study provides the morphological identification of a broader range of *Rhipicephalus* tick species, contributing valuable reference data and supporting the need for more detailed morphological documentation of tick fauna in Egypt.

Egypt shares seasonal climate phases, hot summer and mild winter [21]. It is reasonable to infer that *Rhipicephalus* species in Egypt may exhibit analogous seasonal acceleration in development, reproduction and pathogen transmission during warmer months, thereby increasing risk of pathogen transmission during late spring to autumn [21,22]. *Rhipicephalus* ticks were locally collected from various animals across multiple governorates. Recent studies have shown that *Rhipicephalus* was collected from Beni-Suef, Ismailia, Kalyoubia, Giza, Dakahlia, Beheira and Sharkia [22,23,24,25,26]. These findings align with our observations regarding the distribution of *Rhipicephalus* species.

In Egypt, ticks of the *Rhipicephalus* genus play a significant role in transmitting pathogens to both animals and humans. Among these ticks, *Rhipicephalus annulatus, R. decoloratus, R. sanguineus, R. evertsi, and R. simus* are recognized as vectors. These ticks have been found to harbour pathogenic microorganisms, including species such as *Ehrlichia/Anaplasma, Babesia, and Theileria* [27, 28]. In this study, *Rhipicephalus spp*. ticks were morphologically and clinically examined to detect the presence of three different types of parasites transmitted by tick vectors, specifically *Babesia, Borrelia burgdorferi, and Ehrlichia/Anaplasma*.

Additionally, historical evidence indicates that ticks from the *R. sanguineus* group have infested dogs in the Mediterranean region since ancient times, underscoring their long-standing significance as vectors. The introduction of new tick species to Egypt, likely through the trade of animals, highlights the ongoing relevance of tick-borne diseases in the region [6,29]. *Rhipicephalus sanguineus,* the most prevalent species in this genus, is considered the primary dog-infesting tick in Egypt [6,29,30]. This accounts for the high number of *R. sanguineus* collected in our study around 54.8%.

While *Rhipicephalus annulatus* is the primary tick infesting cattle in Egypt [22,31], this study found that *R. annulatus* accounted for 1,995 all of which were collected from cattle. Cattle infestation by *R. annulatus* often increases during the summer months [32]. *Rhipicephalus turanicus* was not previously detected in Egypt; however, this species is endemic to neighbouring countries, including the southern region of Sudan [33,34], Palestine, and Tunisia [35]. Additionally, both *R. microplus* and *R. decoloratus* have not been identified in Egypt. As a result, this is the first documented evidence of the morphological features of *R. microplus* in Egypt, based on adult specimens collected during our survey. Historically, *R. microplus* was introduced to the eastern and southern regions of Africa from Southern Asia via Madagascar following the outbreak of rinderpest in 1896 [33]. In West Africa, it was first discovered in Ivory Coast through the importation of live cattle from Brazil in 2007 [36], and more recently in Benin, Burkina Faso, and Mali [37,38]. This may explain the potential introduction of this species to Egypt. Previous studies reported that *R. pulchellus* was collected from camels, with (2%) of their collection being *R. pulchellus* [39]. This percentage is like our findings, where *R. pulchellus* constituted (0.13%) of the collected specimens. This tick was also collected from the same host species (camels). Locally, *R. simus* has not been identified, but many previous studies have indicated that this species is established in central and southern Africa [40], and Sudan [41].

Our findings indicate that domestic animals in the area are infested with various species of the genus *Rhipicephalus*, which serve as vectors for numerous diseases. Furthermore, at least three of the tested pathogens were found to be positive in certain tick species.

Research on *Babesia sp*. has primarily been driven by efforts to manage the agents responsible for the disease in both humans and animals. However, given the wide range of mammals and birds identified as potential carriers of *Babesia* species, it is reasonable to conclude that nearly all vertebrates can be infected as long as they act as suitable hosts for the various *Babesia*-carrying ticks [42].

In PCR test for *Babesia* spp., this pathogen was detected in the blood samples collected from dogs, in addition to its positive detection within *R. microplus. R. sanguineus,* and *R. evertsi, which* were also found to carry *Babesia* as part of their normal parasitic load. These findings are broadly consistent with prior

local observations [39,43,44,45], as well as global studies [11,46,47,48]. This pathogen was naturally isolated from ticks, which aligns with the results of various studies involving *R. microplus* [49,50], *R. evertsi* [46,53], *R. sanguineus* [54,55,56]. In our surveillance, we observed a case of a dog infected with a tick carrying *Babesia*, which exhibited several symptoms, including weakness, fever, red urine, and lack of appetite, as *Babesia* destroys red blood cells, leading to anaemia.

According to previous studies, *Borrelia burgdorferi*, the spirochete responsible for Lyme borreliosis (LB), is detected in ticks worldwide. Notable regions with the presence of *B. burgdorferi* include Central Europe, Eastern Asia, and Western Europe [57]. In Egypt, tick infestation rates are significant, affecting animals such as camels, cattle, and dogs. A local study found *B. burgdorferi* in one dog (1.67%), with *Rhipicephalus sanguineus* as the vector [27]. This finding matches our study regarding the presence of *B. burgdorferi* in *R. sanguineus* collected from dogs.

For *Ehrlichia/Anaplasma*, our results are consistent with previous studies that detected the presence of these pathogens in *R. sanguineus* [58,59]. Globally, they have been identified in Iran [48,60] and Brazil [61]. This pathogen has been found in various tick species, consistent with findings from different studies, including those that detected it in *Rhipicephalus sanguineus* ticks [57,59], and in *Rhipicephalus decoloratus* [62]. These studies likely provide valuable insights into our understanding of these pathogens. Furthermore, these findings underscore the importance of tick surveillance and the potential risks they pose to both animal and human health. *Rhipicephalus microplus* harbours the largest numbers of different pathogens within this genus [7]. The most frequently transmitted microorganisms by *R. microplus* and *R. sanguineus* are *Babesia* [21,63]. In the present study, despite the limited sample sizes for *R. microplus, R. evertsi, R. simus* and *R. turanicus* which are considered as the less common species, the presence of pathogens was still confirmed, indicating their potential-though less prominent-epidemiological importance. The variation in infection rates among tick species may be attributed to differences in vector competence, host preference, feeding duration, co-evolution, ecological factors and geographic distribution [64]. Notably the two most abundant species, *R. sanguineus and R. annulatus*, showed both high testing numbers and higher positivity rates, underscoring their epidemiological significance in pathogen transmission cycles within the studied regions [22,23,44]. As a result, the detection of multiple *Rhipicephalus* species carrying tick-borne pathogens in this study raises important epidemiological concerns. Some of these species, such as *R. microplus, R. simus, R. evertsi, R. turanicus* and *R. decoloratus* have not been widely reported in Egypt before, and their ability to harbour pathogens such as *Babesia*, *Ehrlichia/Anaplasma* and *Borrelia* suggests a potential expansion of diseases risk. This emerging pattern may reflect ecological changes, host movements, or climate-related shifts that favour the introduction and the establishment of new tick populations. Consequently, the presence of these species should be considered an early warning signal for the possible emergence or re-emergence of vector-borne diseases in Egypt. Continuous surveillance and molecular monitoring are essential to assess their distribution, infection dynamics and potential impact on animal and public health [63,64]. To mitigate the risks posed by tick-borne-pathogens effective control must be prioritized. Surveillance programs are essential to monitor their distribution, abundance and infection status, as well as integrated tick control measures including the strategic use of acaricides. Additionally, raising awareness among veterinarians, farmers, and public health workers about tick prevention and early disease detection is critical. [63,64].

Conclusion

This study confirms the presence of multiple *Rhipicephalus* tick species in Egypt and the detection of tick-borne pathogens known to threaten livestock health. The identification of tick species and the detection of their pathogens emphasize the critical need for enhanced surveillance and integrated vector control strategies in the region.

Declarations statement

Ethics approval and consent to participate

This research was approved by the research ethics committee from the Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2025/6/1).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interests

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Author contributions

All authors have read and approved the manuscript. **Abozeid, S., Elsayed, A.K., Samy, A.M., Allayeh, A.K., and Yousery, A.,** designed the in experiments **Abozeid, S., Elsayed, A.K., Samy, A.M., Allayeh, A.K., and Yousery, A.,** reviewed the

manuscript. **Abozeid, S., Yousery, A., and Elsayed, A.K.,** wrote the manuscript. **Abozeid, S., and Yousery, A.,** performed the data analysis.

Consent for publication

Not Applicable.

Consent to participate

Not applicable.

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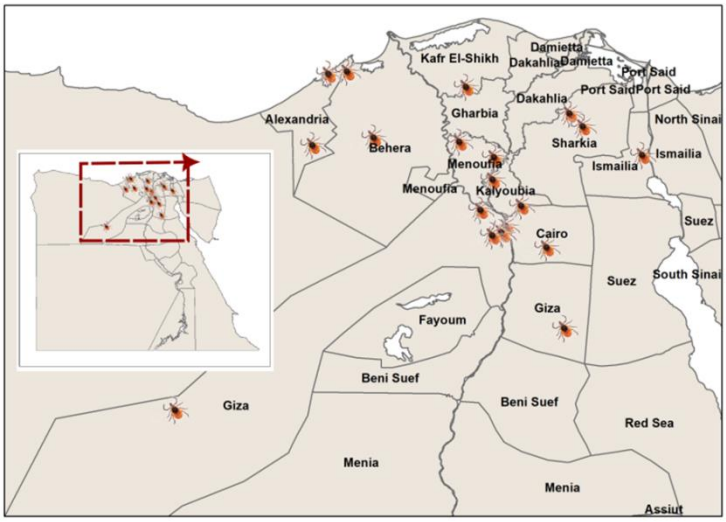


Fig. 1. Zoomed-in map of Egypt illustrating the selected study areas. Tick symbols indicate the locations where tick specimens were collected from their hosts

TABLE 1. Oligonucleotide Primers used in Polymerase Chain Reaction Assays

Target organism	Target Gene	Primer	Primer sequence (5'-3')	Product size (bp)	Annealing temp. (°C)	Ref.
***Babesia* spp.**	18S rRNA	3.1 5.1	CTCCTTCCTTTAAGTGATAAG CCTGGTTGATCCTGCCAGTAGT	450	48	[18]
Borrelia burgdorferi	23S rRNA	Bb23Sf Bb23Sr	CGAGTCTTAAAAGGGCGATTTAGT GCTTCAGCCTGGCCATAAATAG	75	58	[19]
***Ehrlichia /Anaplasma* genera.**	16S rRNA	ECC ECB	AGAACGAACGCTGGCGGCAAGCC CGTATTACCGCGGCTGCTGGCA	450	55	[18]

TABLE 2. Prevalence of *Rhipicephalus* Tick Species Collected from Different Hosts and Governorates in Egypt.

Species name	No. of species* (%)	♂	♀	Host	Locality (%)
Rhipicephalus annulatus	1995 (44.5%)	795	1200	Cattle	Cairo (50%), Kalyoubia (30%), Sharkia (9%), Kafr El-Shikh (6%) and Gharbia (5%).
Rhipicephalus decoloratus	10 (0.22%)	7	3	Cattle	Cairo (80%) and Giza (20%).
Rhipicephalus evertsi	5 (0.11%)	5	-	Cattle & camels	Cairo

Rhipicephalus microplus	2 (0.04%)	2	-	Cattle	Kalyoubia
Rhipicephalus pulchellus	6 (0.13%)	6	-	Camels	Cairo
Rhipicephalus sanguineus	2460 (54.8%)	900	1560	Dogs	Cairo (40%), Alexandria (20%), Kalyoubia (20%), Giza (10%), Gharbia (5%), Behera (2%), Menoufia (2%) and Ismailia (1%)
Rhipicephalus simus	3 (0.07%)	3	-	Cattle & camels	Kalyoubia
Rhipicephalus turanicus	5 (0.11%)	3	2	Cattle	Giza
Total	**4,488**	**2128**	**2360**		

*Number of species of ♂ males and ♀ females based on gender, collected from host in different localities.

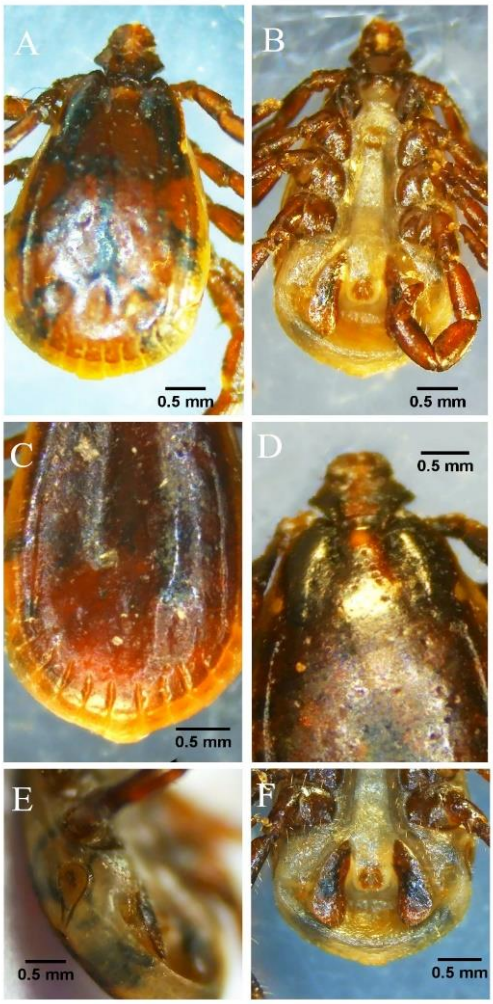


Fig. 2. *Rhipicephalus sanguineus* male: A) Dorsal view; B) Ventral view; C) Postero-dorsal region displaying the festoons and caudal appendages; D) Antero-dorsal region illustrating the eyes and lateral grooves; E) Spiracle with a narrow tail; F) Adanal and accessory adanal plates.

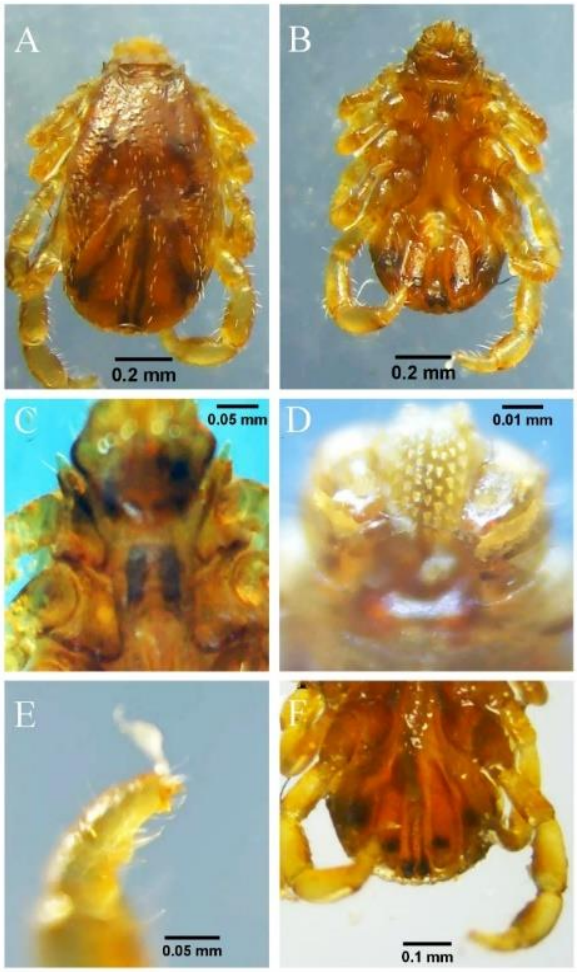


Fig. 3. *Rhipicephalus annulatus* male: A) Dorsal view; B) Ventral view; C) Antero-ventral region showing the anterior coxal spur; D) Hypostome with four columns of teeth E) claws and the pulvilli without terminal spurs; F) Posterio-ventral region displaying the adanal and accessory adanal plates

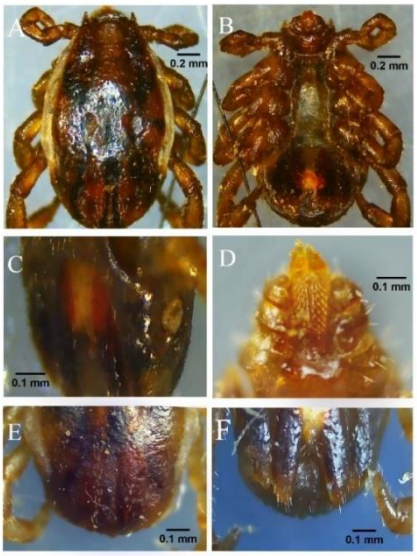


Fig. 4. ***Rhipicephalus microplus*** **male: A) Dorsal view; B) Ventral view; C) Spiracle and the spiracular area; D) Hypostome with four columns of teeth; E) Postero-dorsal region with reduced caudal appendages; F) Postero-ventral region displaying adanal and accessory adanal plates**

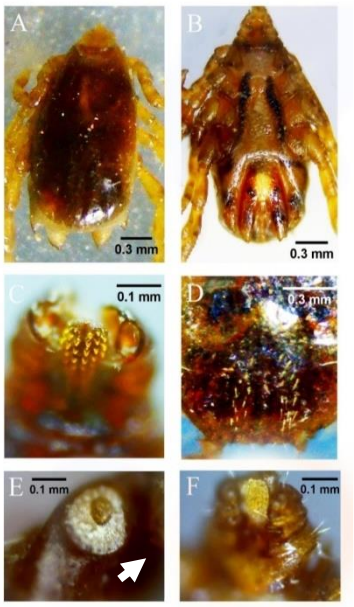


Fig. 5. ***Rhipicephalus decoloratus*** **male: A) Dorsal view; B) Ventral view; C) Hypostome with three columns of teeth; D) postero-dorsal view of the scutum with yellow hairs; E) Spiracle; F) Small bristle-bearing protuberance on the internal ventral surface of the basal palpal segment**

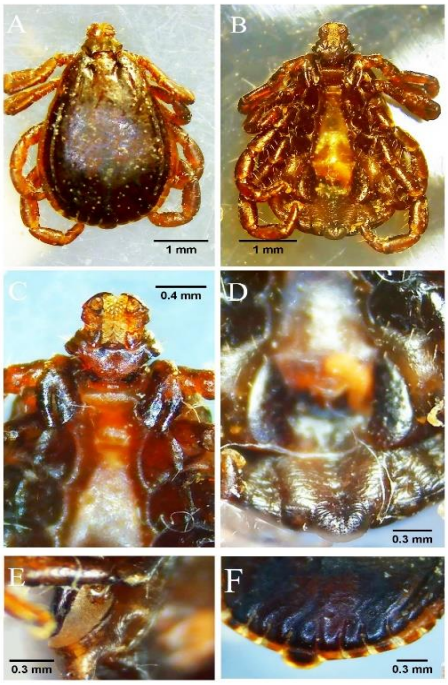


Fig. 6. ***Rhipicephalus simus*** **male: A) Dorsal view displaying the glossy, shiny scutum; B) Ventral view; C) Antero-ventral region showing that coxa 1 is divided into two uneven spurs, with the outer spur being conical and narrower; D) Curved adanal plates accompanied by large accessory adanal plates; E) Large, curved spiracle; F) Shiny festoons and caudal appendage protruding beyond the body outline.**

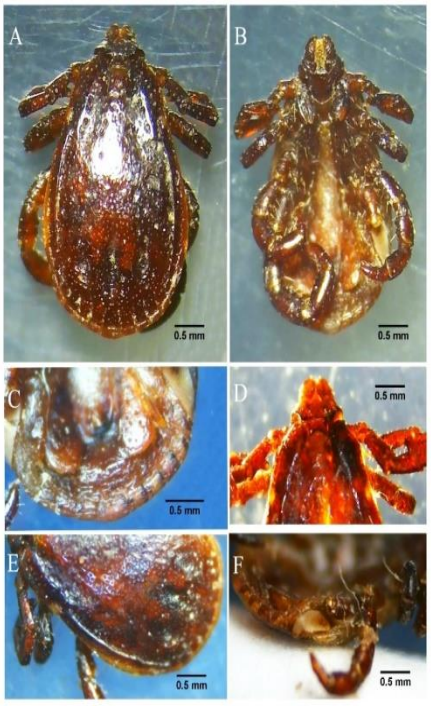


Fig. 7. ***Rhipicephalus evertsi*** **male: A) Dorsal view showing the rough surface of the scutum; B) Ventral view; C) Postro-ventral region displaying the anal and accessory adanal plates; D) Dorsal view of coxa 1 spur; E) Posterior grooves, with middle groove being oval and the lateral two grooves being circular; F) Large, curved spiracle**

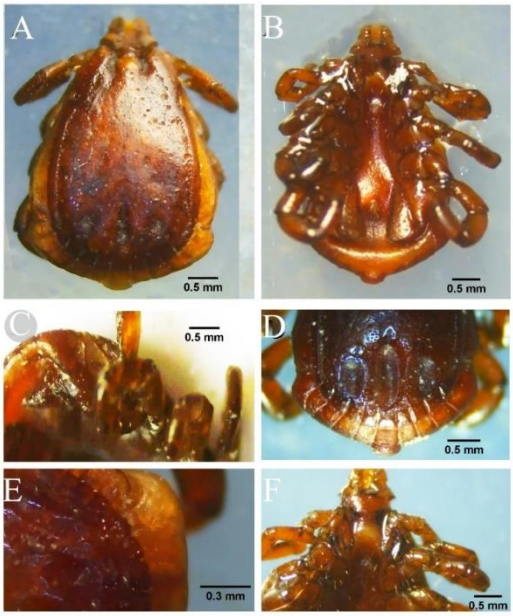
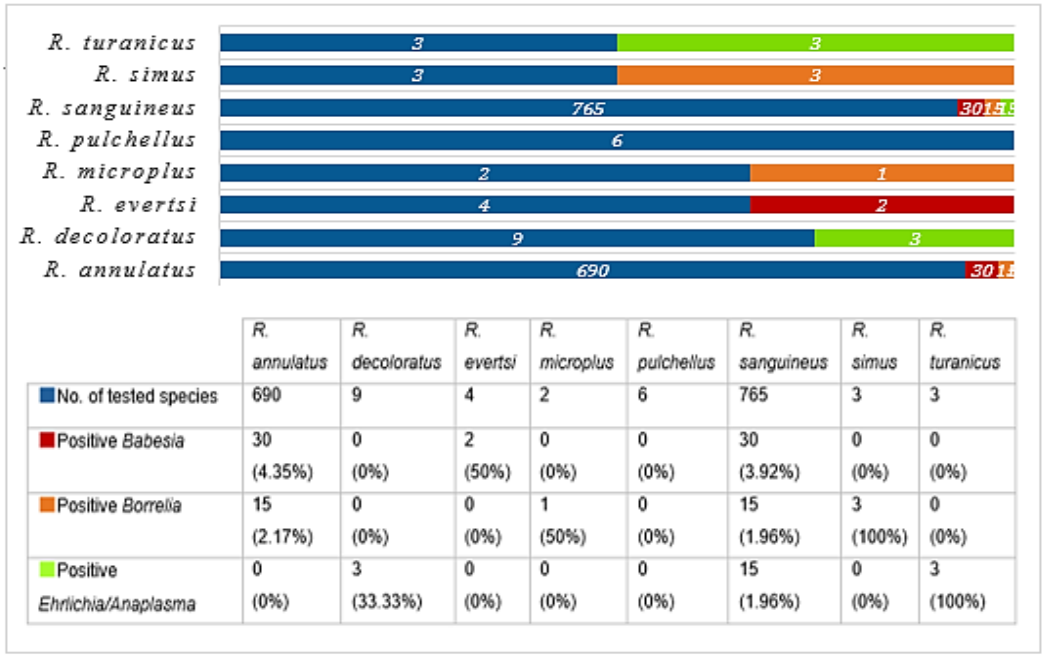


Fig. 8. *Rhipicephalus turanicus* Male: A) Dorsal view; B) Ventral view; C) Broad spiracle and spiracular area with setae; D) Festoons and three distinct grooves; E) Postero-dorsal region displaying the laterally wide end of the spiracle; F) Coxa 1 spur not visible, the posterior view of the coxa is divided into two spurs

Fig. 9. Dorsal view of *Rhipicephalus pulchellus* male.



	R. annulatus	*R. decoloratus*	*R. evertsi*	*R. microplus*	*R. pulchellus*	*R. sanguineus*	*R. simus*	*R. turanicus*
No. of tested species	690	9	4	2	6	765	3	3
Positive *Babesia*	30 (4.35%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	30 (3.92%)	0 (0%)	0 (0%)
Positive *Borrelia*	15 (2.17%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	15 (1.96%)	3 (100%)	0 (0%)
Positive *Ehrlichia/Anaplasma*	0 (0%)	3 (33.33%)	0 (0%)	0 (0%)	0 (0%)	15 (1.96%)	0 (0%)	3 (100%)

Fig. 10. Prevalence of Tick-Borne Pathogens in *Rhipicephalus* Tick Species Based on Molecular Detection
The bar chart displays the total number of tested ticks **(blue)** and the number of positive samples **(red for *Babesia*, orange for *Borrelia*, green for *Ehrlichia/Anaplasma*)** for each species

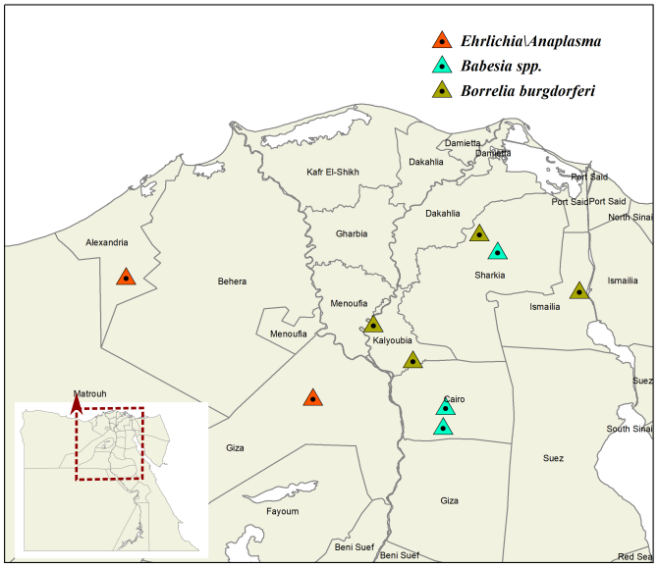


Fig. 11. Geographic distribution of tick-borne pathogens detected in Egypt.

The map shows the location where the ***Ehrlichia/Anplasma*** (orange triangle), ***Babesia spp.*** (blue triangle) and ***Borrelia burgdorferi*** (green triangle) were identified in tick samples collected during the study.

TABLE 3. Distribution of *Rhipicephalus* Tick Species and their infection with tick-borne pathogens detected by PCR testing in various regions of Egypt.

Tick genus	Tick species	Animal host	Detected pathogen	location
Rhipicephalus	***annulatus***	*Cattle*	*Babesia and Borrelia burgdorferi*	Sharkia
	decoloratus	*Cattle*	*Ehrlichia/Anaplasma*	Giza
	evertsi	*Cattle*	*Babesia*	Cairo
	microplus	*Cattle*	*Borrelia burgdorferi*	Kalyoubia
	pulchellus	*Camels*	-	Cairo
	sanguineus	*Dogs*	*Ehrlichia/Anaplasma*	Alexandria
			Babesia	Cairo
			Borrelia burgdorferi	Ismailia
	simus	Cattle	*Borrelia*	Kalyoubia
	turanicus	Cattle	*Ehrlichia/Anaplasma*	Giza

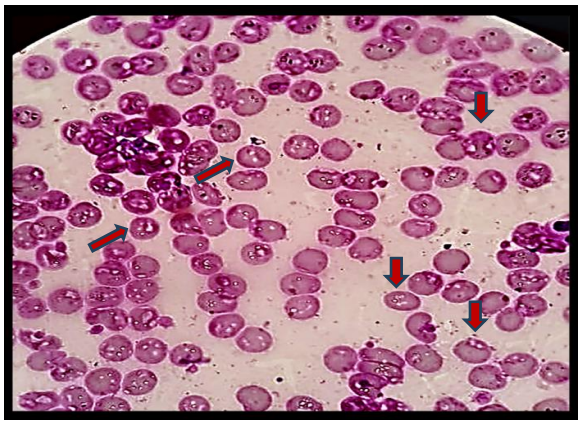


Fig. 12. Microscopic *Babesia spp.* in the hemocytes of an infected dog stained with Hemacolor® Rapid staining. Arrows show the infected red blood cells by *Babesia spp.*

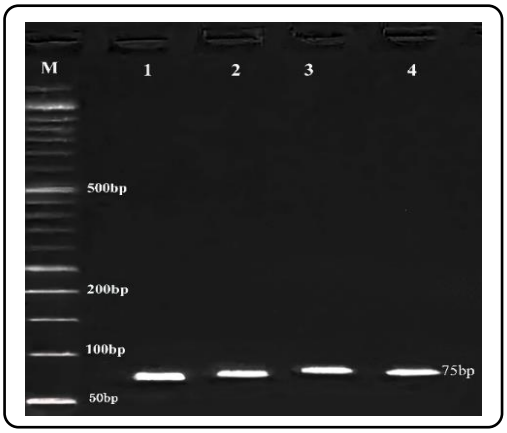


Fig. 13. Gel electrophoresis showing positive PCR amplification bands of *Babesia spp.* DNA detected in *Rhipicephalus spp.* and a dog blood sample. Positive bands are observed in the first four lanes:
Lane1. Infected dog blood sample, **Lane2.** *Rhipicephalus annulatus*, **Lane3.** *Rhipicephalus sanguineus* **Lane4.** *Rhipicephalus evertsi*

Fig. 14. Gel electrophoresis showing positive PCR amplification bands of *Borrelia burgdorferi* DNA detected in *Rhipicephalus spp.* Positive bands are observed in the first four lanes:Lane1. *Rhipicephalus annulatus,* **Lane2.** *Rhipicephalus microplus,* **Lane3.** *Rhipicephalus sanguineus* **Lane4.** *Rhipicephalus simus*

Fig. 15. Gel electrophoresis showing positive PCR amplification bands of *Ehrlichia/Anaplasma* DNA detected in *Rhipicephalus spp.* Positive bands are observed in lanes 2,4, and 5: Lane2. *Rhipicephalus sanguineus.* **Lane4.** *Rhipicephalus decoloratus* **Lane5.** *Rhipicephalus turanicus.*

References

1. Esteve-Gasent, M.D., Rodríguez-Vivas, R.I., Medina, R.F., Ellis, D., Schwartz, A., Garcia, B.C., Hunt, C., Tietjen, M., Bonilla, D., Thomas, D., Logan, L.L., Hasel, H., Martínez, J.a.A., Hernández-Escareño, J.J., Gualito, J.M., Díaz, M.a.A., Rosario-Cruz, R., Céspedes, N.S., Charrez O.M., Howard, T., Niño, V.M.C. and De León, A.A.P., Research on Integrated management for cattle fever ticks and bovine babesiosis in the United States and Mexico: Current status and opportunities for binational coordination. *Pathogens*, **9**, 871 (2020). https://doi.org/10.3390/pathogens9110871
2. Ahmed, S., Numan, M., Manzoor, A.W. and Ali, F.A., Investigations into Ixodidae ticks in cattle in Lahore, *Pakistan Veterinary Journal*, **48**(2), 185-191 (2012).
3. Brites-Neto, J., Duarte, K.M.R. and Martins, T.F., Tick-borne infections in human and animal population worldwide. *Veterinary World*, **8**,301–315(2015). https://doi.org/10.14202/vetworld.2015.301-315
4. Smith, Theobald, and Fred Lucius Kilborne. Investigations into the nature, causation, and prevention of Texas or southern cattle fever. No. 1. *US Government Printing Office* (1893).
5. Baneth, G. Tick-borne infections of animals and humans: a common ground. *International Journal for Parasitology*, **44**, 591–596 (2014). https://doi.org/10.1016/j.ijpara.2014.03.011
6. Ghafar, M.W. and Amer, S.A., Prevalence and first molecular characterization of *Anaplasma phagocytophilum*, the agent of human granulocytic anaplasmosis, in *Rhipicephalus sanguineus* ticks attached to dogs from Egypt. *Journal of Advanced Research*, **3**, 189–194 (2011) https://doi.org/10.1016/j.jare.2011.08.002
7. Yu, Z., Wang, H., Wang, T., Sun, W., Yang, X. and Liu, J. Tick-borne pathogens and the vector potential of ticks in China. *Parasites & Vectors*, **8**, 628x (2015). https://doi.org/10.1186/s13071-014-0628-x
8. Tan, L.P., Hamdan, R.H., Hassan, B.N.H., Reduan, M.F.H., Okene, I.A., Loong, S.K., Khoo, J.J., Samsuddin, A.S. and Lee, S.H. *Rhipicephalus* Tick: A contextual review for Southeast Asia. *Pathogens* **10**,821(2021). https://doi.org/10.3390/pathogens10070821
9. Dantas-Torres, F. and Otranto, D. Further thoughts on the taxonomy and vector role of *Rhipicephalus sanguineus* group ticks. *Veterinary Parasitology*, **208**, 9–13 (2014). https://doi.org/10.1016/j.vetpar.2014.12.014
10. Abdelbaset, A.E., Nonaka, N. and Nakao, R. Tick-borne diseases in Egypt: A one health perspective. *One Health*, **15**, 100443 (2022). https://doi.org/10.1016/j.onehlt.2022.100443
11. Marques, R., Krüger, R. F., Peterson, A. T., de Melo, L. F., Vicenzi, N. and Jiménez-García, D. Climate change implications for the distribution of the babesiosis and anaplasmosis tick vector, *Rhipicephalus (Boophilus) microplus. Veterinary Research*, **51**, 1-10 (2020). https://doi.org/10.1186/s13567-020-00802-z
12. Walker, A. R. Ticks of domestic animals in Africa: a guide to identification of species (Vol. 74). *Bioscience Reports*, (2003).
13. Estrada-Peña, A., Mihalca, A. D. and Petney, T. N. (Eds.). Ticks of Europe and North Africa: a guide to species identification (2018).
14. Horak, I. G., Heyne, H., Williams, R., Gallivan, G. J., Spickett, A. M., Bezuidenhout, J. D. and Estrada-Peña, A. The ixodid ticks (Acari: Ixodidae) of southern Africa. *Springer*. (2018). doi: 10.4102/ojvr.v86i1.1781. PMID: 31714141;
15. Senbill, H., Tanaka, T., Karawia, D., Rahman, S., Zeb, J., Sparagano, O. and Baruah, A. Morphological identification and molecular characterization of

economically important ticks (Acari: Ixodidae) from North and North–Western Egypt. *Acta Tropica*, and, 106438 (2022). https://doi.org/10.1016/j.actatropica.2022.106438

16. Kazim, A. R., Low, V. L., Houssaini, J., Tappe, D. and Heo, C.C., Morphological abnormalities and multiple mitochondrial clades of *Rhipicephalus haemaphysaloides* (Ixodida: Ixodidae). *Experimental and Applied Acarology*, **87**(1), 133-141 (2022). https://doi.org/10.1007/s10493-022-00731-w

17. Cooper, A., Stephens, J., Ketheesan, N. and Govan, B. Detection of Coxiella burnetii DNA in wildlife and ticks in northern Queensland, Australia. *Vector-Borne and Zoonotic Diseases*, **13**(1), 12-16 (2013) https://doi.org/10.1089/vbz.2011.0853.

18. Yabsley, M.J., McKibben, J., Macpherson., C.N., Cattan, P.F., Cherry, N.A., Hegarty, B.C. and Breitschwerdt, E.B., O'Connor, T., Chandrashekar, R., Paterson, T., and Perea, M.L. Prevalence of *Ehrlichia* canis, *Anaplasma platys, Babesia canis vogeli*, *Hepatozoon canis*, *Bartonella vinsonii berkhoffii*, and *Rickettsia spp* in dogs from Grenada. *Veterinary Parasitology*, **151**(2-4), 279-285 (2008). https://doi.org/10.1016/j.vetpar.2007.11.008

19. Courtney, J. W., Kostelnik, L. M., Zeidner, N. S. and Massung, R. F. Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. *Journal of Clinical Microbiology*, **42**(7), 3164-3168(2004). https://doi.org/10.1128/jcm.42.7.3164-3168.2004

20. Paules, C. I., Marston, H. D., Bloom, M. E. and Fauci, A. S. Tickborne diseases—confronting a growing threat. *The New England Journal of Medicine*, **379**(8), 701-703 (2018). https://doi.org/10.1056/nejmp1807870

21. Dantas-Torres, F., Chomel, B. B. and Otranto, D. Ticks and tick-borne diseases: a One Health perspective. *Trends in Parasitology*, **28**(10), 437-446 (2012). https://doi.org/10.1016/j.pt.2012.07.003

22. Aboelhadid, S. M., Arafa, W. M., Mahrous, L. N., Fahmy, M. M. and Kamel, A. A. Molecular detection of *Rhipicephalus (Boophilus) annulatus* resistance against deltamethrin in middle Egypt, *Veterinary Parasitology: Regional Studies and Reports*, **13**, 198-204 (2018).https://doi.org/10.1016/j.vprsr.2018.06.008

23. El-Ashram, S., Aboelhadid, S. M., Kamel, A. A., Mahrous, L. N. and Fahmy, M. M. First report of cattle tick *Rhipicephalus (Boophilus) annulatus* in Egypt resistant to ivermectin, *Insects*, **10**(11), 404 (2019).

24. Hassanen, E.A. Clinical, morphological and molecular characterization of canine babesiosis and its compatible tick vector in naturally infected dogs, in Egypt Zagazig, *The Veterinary Journal*, **48**(3), 242-253(2020). https://doi.org/10.21608/zvjz.2020.24982.1103

25. Selim, A., Alanazi, A.D., Sazmand, A. and Otranto, D. Seroprevalence and associated risk factors for vector-borne pathogens in dogs from Egypt, *Parasites & Vectors*,**14**,1-11(2021). https://doi.org/10.1186/s13071-021-04670-0

26. El-Ansary, R.E., El-Dabae, W.H., Bream, A. S. and El Wakil, A. Isolation and molecular characterization of lumpy skin disease virus from hard ticks, *Rhipicephalus (Boophilus) annulatus* in Egypt, *BMC Veterinary Research*, **18**(1), 302 (2022).

27. Elhelw, R., Elhariri, M., Hamza, D., Abuowarda, M., Ismael, E. and Farag, H. Evidence of the presence of *Borrelia burgdorferi* in dogs and associated ticks in Egypt. *BMC Veterinary Research*, **17**, 1-9 (2021). https://doi.org/10.1186/s12917-020-02733-5

28. Senbill, H., Karawia, D., Zeb, J., Alyami, N.M., Almeer, R., Rahman, S., Sparagano, O. and Baruah, A. Molecular screening and genetic diversity of tick-borne pathogens associated with dogs and livestock ticks in Egypt. *PLOS Neglected Tropical Diseases*, **18**(6),e0012185(2024). https://doi.org/10.1371/journal.pntd.0012185

29. Abdullah, H. H., El-Molla, A., Salib, F. A., Allam, N. A., Ghazy, A. A. and Abdel-Shafy, S. Morphological and molecular identification of the brown dog tick *Rhipicephalus sanguineus* and the camel tick *Hyalomma dromedarii* (Acari: Ixodidae) vectors of Rickettsioses in Egypt. *Veterinary World*, **9**(10), 1087 (2016). doi: 10.14202/vetworld.2016.1087-1101. Epub 2016 Oct 18. PMID: 27847418; PMCID: PMC5104717.

30. Hoogstraal, H. and Kaiser, M. N. Observations on Egyptian *Hyalomma* ticks (Ixodoidea, Ixodidae). I. Parasitism of lizards by nymphs. *Annals of the Entomological Society of America*, **51**(1), 7-12 (1958). https://doi.org/10.1093/aesa/51.1.7

31. Abdel-Shafy, S. Is the cattle tick *Rhipicephalus annulatus* Say, 1821 reared on the rabbit? *The Journal of Parasitic Diseases*, **42**(2), 297-302 (2018). https://doi.org/10.1007/s12639-018-1000-4

32. Arafa, W.M., Klafke, G. M., Tidwell, J.P., de León, A.A.P. and Esteve-Gassent, M. Detection of single nucleotide polymorphism in the para-sodium channel gene of *Rhipicephalus annulatus* populations from Egypt resistant to deltamethrin, *Ticks and Tick-borne Diseases*, **11**(5), 101488 (2020). https://doi.org/10.1016/j.ttbdis.2020.101488

33. Hoogstraal, H. African Ixodoidea: Ticks of the Sudan, US Naval Medical Research Unit No 3, *Cairo* (1956).

34. Jongejan, F., Zivkovic, D., Pegram, R.G., Tatchell, R.J., Fison, T., Latif, A.A. and Paine, G. Ticks (Acari: Ixodidae) of the Blue and White Nile ecosystems in the Sudan with particular reference to the *Rhipicephalus sanguineus* group. *Experimental and Applied Acarology*, **3**(4), 331-346 (1987).https://doi.org/10.1007/BF01193169

35. Seidi, S., Omidi, A. H. and Esmaeili, S. Distribution of different Rickettsia species in countries of the WHO Eastern Mediterranean (WHO-EMRO) region: An overview. *Travel Medicine and Infectious Disease*, 102695 (2024). https://doi.org/10.1016/j.tmaid.2024.102695

36. Madder, M., Adehan, S., De Deken, R., Adehan, R. and Lokossou, R. New foci of *Rhipicephalus microplus* in West Africa. *Experimental and Applied*

Acarology, **56**, 385-390 (2012). https://doi.org/10.1007/s10493-012-9522-4

37. Adakal, H., Biguezoton, A., Zoungrana, S., Courtin, F., De Clercq, E. M. and Madder, M. Alarming spread of the Asian cattle tick *Rhipicephalus microplus* in West Africa—another three countries are affected: Burkina Faso, Mali and Togo. *Experimental and Applied Acarology*, **61**, 383-386 (2013).

38. Yessinou, R.E., Akpo, Y., Adoligbe, C., Adinci, J., Assogba, M.N., Koutinhouin, B., Karim, I.Y. and Farougou, S. Resistance of tick *Rhipicephalus microplus* to acaricides and control strategies. *Journal of Entomology and Zoology Studies*, **4**(6), 408-414 (2016).

39. Hassan, M. I., Gabr, H. S., Abdel-Shafy, S., Hammad, K.M., & Mokhtar, M.M. Prevalence of tick-vectors of *Theileria annulata* infesting the one-humped camels in Giza, Egypt, *Journal of the Egyptian Society of Parasitology*, **47**(2), 425-432 (2017). https://doi.org/10.21608/jesp.2017.77797

40. Pegram, R.G., Walker, J.B., Clifford, C. M. and Keirans, J.E. Comparison of populations of the *Rhipicephalus simus* group: *R simus, R praetextatus*, and *R muhsamae* (Acari: Ixodidae), *Journal of Medical Entomology*, **24**(6), 666-682 (1987). https://doi.org/10.1093/jmedent/24.6.666

41. Hussein, H.S. and Mustafa, B.E. *Haemaphysalis spinulosa* and *Rhipicephalus simus* (Acari: Ixodidae): seasonal abundance of immature stages and host range in the Shambat area, Sudan, *Journal of Medical Entomology*, **22**(1), 72-77 (1985). https://doi.org/10.1093/jmedent/22.1.72

42. Swelum, A.A., Ismael, A.B., Khalaf, A.F. and Abouheif, M.A. Clinical and laboratory findings associated with naturally occurring babesiosis in dromedary camels, *The Journal of Veterinary Research*, **58**(2), 229-233 (2014). http://dx.doi.org/10.2478/bvip-2014-0034

43. Hasanien, E. A. A. Clinical, Morphological and Molecular Characterization of Canine Babesiosis and its Compatible Tick Vector in Naturally Infected Dogs, in Egypt. *The Zagazig Veterinary Journal*, **48**(3), 242-253 (2020): https://dx.doi.org/10.21608/zvjz.2020.24982.1103

44. Hegab, A.A., Fahmy, M.M., Omar, H.M., Abuowarda, M.M. and Gattas, S.G., Investigation of tickborne pathogens within naturally infected brown dog tick (Ixodidae: *Rhipicephalus sanguineus*) in Egypt by light and electron microscopy. *International Journal of Veterinary Science and Medicine*, **9**(4), 476-482 (2020): http://dx.doi.org/10.37422/IJVS/20.064

45. Al-Hosary, A., Răileanu, C., Tauchmann, O., Fischer, S., Nijhof, A. M. and Silaghi, C. Epidemiology and genotyping of *Anaplasma marginale* and co-infection with piroplasms and other Anaplasmataceae in cattle and buffaloes from Egypt. *Parasites & Vectors*, **13**(1), 495 (2020). https://doi.org/10.1186/s13071-020-04372-z

46. Bastos, R.G., Ueti, M.W., Knowles, D.P. and Scoles, G.A. The *Rhipicephalus (Boophilus) microplus* Bm86 gene plays a critical role in the fitness of ticks fed on cattle during acute *Babesia bovis* infection. *Parasites & Vectors*, **3**, 1-11 (2010). https://doi.org/10.1186/1756-3305-3-111

47. Miraballes, C. and Riet-Correa, F.A. review of the history of research and control of *Rhipicephalus (Boophilus) microplus*, babesiosis and anaplasmosis in Uruguay. *Experimental and Applied Acarology*, **75**(4), 383-398 (2018).

48. Obregón, D., Corona-González, B., Díaz-Sánchez, A.A., Armas, Y., Roque, E., de Sena Oliveira, M.C. and Cabezas-Cruz, A. Efficient transovarial transmission of *Babesia spp* in *Rhipicephalus microplus* ticks fed on water buffalo (Bubalus bubalis). *Pathogens*, **9**(4), 280 (2020). https://doi.org/10.3390/pathogens9040280

49. Dalgliesh, R. J. and Stewart, N.P. The use of tick transmission by Boophilus microplus to isolate pure strains of *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale* from cattle with mixed infections. *Veterinary Parasitology*, **13**(4), 317-323 (1983) https://doi.org/10.1016/0304-4017(83)90047-X

50. Laha, R., Das, M. and Sen, A. Morphology, epidemiology, and phylogeny of *Babesia*: An overview. *Tropical Parasitology*, **5**(2), 94-100 (2015). https://doi.org/10.4103/2229-5070.162490

51. Hove, T., Sithole, N., Munodzana, D. and Masaka, S. Isolation and characterization of a *Babesia* species from *Rhipicephalus evertsi evertsi* ticks picked off a sable antelope (Hippotragus niger) which died of acute babesiosis. *Onderstepoort. Journal of Veterinary Research*, **65**, 75-80 (1998).

52. Ica, A., Vatansever, Z., Yildirim, A., Duzlu, O. and Inci, A. Detection of *Theileria* and *Babesia* species in ticks collected from cattle. *Veterinary Parasitology*, **148**(2), 156-160 (2007). https://doi.org/10.1016/j.vetpar.2007.06.003

53. Rodriguez, A. E., Schnittger, L., Tomazic, M. L. and Florin-Christensen, M. Current and prospective tools for the control of cattle-infecting *Babesia* parasites. *Protozoa: Biology, Classification and Role in Disease; Castillo, V., Harris, R., Eds*, 1-44(2013).

54. M'ghirbi, Y. and Bouattour, A. Detection and molecular characterization of *Babesia canis vogeli* from naturally infected dogs and *Rhipicephalus sanguineus* ticks in Tunisia. *Veterinary Parasitology*, **152**(1-2), 1-7 (2008). https://doi.org/10.1016/j.vetpar.2007.12.018

55. Razmi, G. and Nouroozi, E. Transovarial Transmission of *Babesia* ovis by *Rhipicephalus sanguineus* and *Hyalomma marginatum* Iranian. *Journal of Parasitology*, **5**(3), 35 (2010).

56. Chao, L.L., Liao, H.T., Ho, T.Y. and Shih, C.M. First detection and molecular identification of *Babesia gibsoni* from *Rhipicephalus sanguineus* ticks. *Acta Tropica*, **166**, 356-362 (2017). https://doi.org/10.1016/j.actatropica.2016.09.022

57. Dong., Y., Zhou, G., Cao, W., Xu, X., Zhang, Y., J.i., Z., Yang, J., Chen, J., Liu, M., Fan, Y., and Kong, J. Global seroprevalence and sociodemographic characteristics of *Borrelia burgdorferi sensu lato* in human populations: a systematic review and meta-

analysis BMJ. *Global Health Journal*, **7**(6), 476-482 (2022). https://doi.org/10.1136/bmjgh-2021-007744

58. Nasr, A., & Ghafar, M.W. Detection of *Anaplasma platys* and *Ehrlichia canis* in *Rhipicephalus sanguineus* ticks attached to dogs from Egypt; a public health concern. *The Veterinary Medical Journal (Giza)***, 66**(1), 1-9 (2020). https://doi.org/10.21608/vmjg.2020.157540
59. Hegab, A.A., Omar, H.M., Abuowarda, M., Ghattas, S.G., Mahmoud, N.E. and Fahmy, M.M. Screening and phylogenetic characterization of tick-borne pathogens in a population of dogs and associated ticks in Egypt. *Parasites & Vectors*, **15**(1), 222 (2022) https://doi.org/10.1186/s13071-022-05348-x
60. Choubdar, N., Golshani, M., Jalili-Baleh, L., Nadri, H.., Küçükkilinç, T.T., Ayazgök, B., Moradi, A., Moghadam, F.H., Abdolahi, Z., Ameri, A. and Salehian, F. New classes of carbazoles as potential multi-functional anti-Alzheimer's agents. *Bioorganic Chemistry*, **91**, 103164 (2019). https://doi.org/10.1016/j.bioorg.2019.103164
61. Silva, E.M.C., Marques, I.C.L., de Mello, V. V.C., do Amaral, R.B., Gonçalves, L.R., Braga, M.D.S.C.O. and de Carvalho Neta, A.V., Molecular and serological detection of *Anaplasma spp* in small ruminants in an area of Cerrado Biome in northeastern Brazil. *Ticks and Tick-borne Diseases*, **15**(1), 102254 (2024). https://doi.org/10.1016/j.ttbdis.2023.102254
62. King'ori, E., Obanda, V., Chiyo, P.I., Soriguer, R.C., Morrondo, P. and Angelone, S. Molecular identification of *Ehrlichia*, *Anaplasma*, *Babesia* and *Theileria* in African elephants and their ticks. *PLoS One*, **14**(12), e0226083 (2019). https://doi.org/10.1371/journal.pone.0226083
63. Jongejan, F. and Uilenberg, G. The global importance of ticks. *Parasitology*, **129**(S1), S3-S14. (2004). https://doi.org/10.1017/s0031182004005967
64. McCoy, K.D., Léger, E. and Dietrich, M. Host specialization in ticks and transmission of tick-borne diseases: a review. *Frontiers in Cellular and Infection Microbiology*, **3**, 57(2013).

جنس *Rhipicephalus* في مصر: الوصف المورفولوجي للأنواع والكشف الجزيئي عن مسببات الأمراض المهددة لصحة الحيوان

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الملخص

يعتبر القراد ناقل للعديد من العوامل الممرضة، والتي تسبب امراضا تتراوح من أمراض خفيفة إلى شديدة لدى البشر والحيوانات الاليفة والمفترسة. ويعد وصف الشكل الظاهري الدقيق للقراد أمراً بالغ الأهمية لتقييم الحالة الوبائية للأمراض المنقولة بالقراد. وفي مصر، لا تزال مساهمة القراد في نقل الأمراض الحيوانية المنشأ غير واضحة بسبب ندرة البيانات المتعلقة بتنوع القراد. تهدف هذه الدراسة إلى تقديم وصف مورفولوجي للقراد والكشف الجزيئي للأمراض المنقولة بالقراد ضمن جنس *Rhipicephalus* الذي يصيب الحيوانات الأليفة. في الفترة من أكتوبر 2021 إلى مارس 2024، تم جمع القراد من الماشية والكلاب والإبل في عشر محافظات في مصر: القاهرة، والقليوبية، والإسكندرية، وكفر الشيخ، والبحيرة، والغربية، والمنوفية، والجيزة، والإسماعيلية، والشرقية. تم وصف القراد الذي تم جمعه مورفولوجيا واختباره بحثًا عن وجود مسببات الأمراض *Babesia* و *Anaplasma/ Ehrlichia* و *Borrelia burgdorferi* باستخدام تفاعل البلمرة المتسلسل (PCR). تم تجميع 4,488 قرادة من الحيوانات الاليفة، وتمثل ثمانية أنواع من جنس *Rhipicephalus*. تم اكتشاف *Babesia* و *Anaplasma/ Ehrlichia* و *Borrelia burgdorferi* في عدة عينات من القراد. في الدراسة الحالية، أظهرنا تسجيل دخول عدة أنواع من قراد الـ *Rhipicephalus* لم يُبلغ عنها سابقًا في مصر، بما في ذلك *Rhipicephalus microplus*، و*R. simus*، و*R. evertsi*، و*R. turanicus*، كما أكدنا وجود الممرضات باستخدام تقنيات تفاعل البوليميراز المتسلسل (PCR).

الكلمات الدالة: *Rhipicephalus*، الامراض المنقولة بالقراد، تفاعل البلمرة المتسلسل، مصر.