Multiple vector-borne pathogens of domestic animals in Egypt

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Abstract

Vector Borne Diseases (VBDs) are considered emerging and re-emerging diseases that represent a global burden. The aim of this study was to explore and characterize vector-borne pathogens in different domestic animal hosts in Egypt. A total of 557 blood samples were collected from different animals using a convenience sampling strategy (203 dogs, 149 camels, 88 cattle, 26 buffaloes, 58 sheep and 33 goats). All samples were tested for multiple pathogens using quantitative PCR and standard PCR coupled with sequencing. We identified Theileria annulata and Babesia bigemina in cattle (15.9 and 1.1%, respectively), T. ovis in sheep and buffaloes (8.6 and 7.7%, respectively) and Babesia canis in dogs (0.5%) as well as Anaplasma marginale in cattle, sheep and camels (20.4, 3.4 and 0.7%, respectively) and Coxiella burnetii in sheep and goats (1.7 and 3%; respectively). New genotypes of Anaplasma centrale, Anaplasma ovis, Anaplasma platys-like and Borrelia theileri were found in cattle (1.1, 3.4, 3.4 and 3.4%, respectively), Anaplasma platys-like in buffaloes (7.7%), Anaplasma marginale, Anaplasma ovis, Anaplasma platys-like and Bo. theileri in sheep (3.4, 1.7, 1.7 and 3.4%, respectively), Anaplasma platys, Anaplasma platys-like and Setaria digitata in camels (0.7, 5.4 and 0.7%, respectively) and Rickettsia africae-like, Anaplasma platys, Dirofilaria repens and Acanthochelonema reconditum in dogs (1.5, 3.4, 1 and 0.5%, respectively). Co-infections were found in cattle, sheep and dogs (5.7, 1.7, 0.5%, respectively). For the first time, we have demonstrated the presence of several vector-borne zoonoses in the blood of domestic animals in Egypt. Dogs and ruminants seem to play a significant role in the epidemiological cycle of VBDs.

Author summary

Vector Borne Diseases (VBDs) are considered emerging and re-emerging diseases that represent a global burden. Diagnosis of these diseases is challenging due to nonspecific febrile illness, difficulty of isolation, and cross-reactivity of serological methods. Therefore, the current study is the first large-scale epidemiological study in which molecular screening and characterization of multiple vector-borne pathogens in different animal hosts were performed to better understand the endemicity of VBDs in Egypt. We detected for the first time Anaplasma centrale, Anaplasma ovis, a novel Anaplasma platys-like and Borrelia theileri.
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Introduction

Vector Borne Diseases (VBDs) are emerging and re-emerging infectious diseases, that pose a health threat to humans, livestock, companion animals and wildlife [1]. VBDs are a global burden and cause severe economic losses through high mortality rates and production declines in the livestock industry, as well as impacts on human and animal health [2,3]. Moreover, about a quarter of vertebrate pathogens of veterinary importance are VBDs [4]. The World Organization for Animal Health (OIE) list includes many VBDs such as piroplasmoses, anaplasmoses and Q fever. The epidemiology and spread of VBDs are influenced by various factors such as globalization and increasing international trade, urbanization, climate change, travel and mobility of animals which pose unprecedented challenges to clinicians and veterinarians [5–6].

Piroplasmoses are tick-borne infectious diseases caused by apicomplexans of the order Piroplasmida, which includes three genera namely: Theileria, Babesia and Cytauxzoon [7]. Theileria annulata, T. ovis and Babesia bigemina are etiological agents of tropical theilerioses and babesiosis in ruminants especially cattle, buffalo and sheep [8]. Similarly, Ba. canis and Ba. vogeli are the main causative agents of canine babesiosis [9]. Piroplasmoses are common in Asia, Southern Europe and Africa [10]. The main clinical signs of piroplasmoses are fever and hemolytic anemia and deaths of up to 50% in the case of acute infection in susceptible herds [11,12]. Recovered animals may become asymptomatic carriers with long-term persistent infection [13,14]. Piroplasmoses have been detected in several provinces of Egypt and are widespread [15–18].

Anaplasmataceae include many tick-borne bacteria that infect mammals and consist of at least five genera: Anaplasma, Ehrlichia, Neoehrlichia Neorickettsia, and Aegyptianella [19–20]. Bovine anaplasmosis caused by Anaplasma marginale and An. centrale mainly in tropical and subtropical regions cause mild to severe anemia in ruminants [20,21]. Ovine anaplasmosis is a neglected mild disease in sheep, goats and wild ruminants caused by An. ovis and is common in different areas of the world [22,23]. In addition, there are many Anaplasmataceae bacteria pathogenic to dogs, such as An. platys and Ehrlichia canis [24,25]. Overall, these bacteria could cause persistent infection in mammals making them reservoir, which has lasting effect on the spread and new outbreaks of anaplasmosis [26,27]. In Egypt, anaplasmosis has been reported in cattle, water buffaloes and camels in different provinces [16,28–34].

Rickettsioses are bacterial infectious diseases that cause health problems in humans and animals worldwide [35,36]. Rickettsiae are divided into spotted fever group (SFG; mainly transmitted by ticks), typhus group (TG; transmitted by lice and fleas), Rickettsia bellii group and Rickettsia (R.) conorii and R. sibirica mongolitimonae, R. massiliae have been detected in ticks and animals in Africa [39–43]. In Egypt, SFG have been identified in vectors, animals and humans since 1989 [44–48]. SFG rickettsiae were found in ticks (Hyalomma sp. and Rhipicephalus
sanguineus) collected in Sinai province [49–51]. Moreover, R. siberica mongolitimonae was detected in a French traveler returning from Egypt [52]. Finally, R. africae was detected by molecular biology in Hyalomma sp. and camels [53–55].

Borrelioses are zoonotic infectious diseases and are divided into two groups: Lyme disease group (caused by Borrelia burgdorferi and related species) and relapsing fever group [56]. Relapsing fever borrelioses are arthropod-borne spirochetal diseases, usually transmitted by soft ticks; they are common in subtropical regions worldwide [57]. In Africa, relapsing fever is most common in the northern hemisphere and is caused by various Borrelia spp. such as Bo. hispanica, Bo. duttonii, and Bo. crocidurae [57–60]. Bo. theileri is the etiological agent of bovine borreliosis in ruminants, which causes anemia and fever and, unlike other members of the relapsing fever spirochetes, is transmitted by hard ticks [58]. In Egypt, data on borrelioses in animal hosts are sparse. Only the few studies have detected Bo. burgdorferi [61,62] and Bo. theileri in hard ticks [62].

Q fever is a zoonosis that infects humans and animals through direct contact or a tick bite [63]. Coxiella burnetii is the causative agent of Q fever that may be severe in humans [64]. Infection in animals it is usually subclinical except that reproductive diminution and abortions may occur [65]. Coxiella burnetii infects a wide range of animals, especially sheep, goats, cattle and camels, which serve as reservoirs [64,66]. In Egypt, the seroprevalence of C. burnetii was estimated in buffaloes, sheep, cattle and camels [67–70]. In addition, C. burnetii has been detected molecularly in goats, camels and ticks (H. dromedarii) [70–72].

Filarial nematodes are vector-borne helminths belonging to the order Spiruridae, suborder Spirurina and families Filaridae and Onchocercidae and pose a serious threat to humans and livestock [73,74]. Dirofilaria repens and D. immitis, followed by Acanthocheilonema sp. are the most important etiological agents of filarial infections in dogs [9,73,75]. Setaria digitata is a filarial nematode of cattle and buffaloes and is not pathogenic to these natural hosts, but when transmitted by mosquitoes to accidental hosts such as camels and horses, it can have serious pathological effects [76,77]. In Egypt, information on filarial infections in ruminants and dogs are scarce. In Africa, there are some reports of filarial infections in different places of the continent [78–80].

Diagnosis of all these diseases is challenging due to the non-specific febrile illness, difficulty in isolation and cross reactivity of serological methods [35,59]. Therefore, the advanced molecular techniques have been used to increase the sensitivity and specificity of diagnosis, to detect previously unknown pathogens and distinguish closely related species [5]. In Egypt, the epidemiology and prevalence of these diseases remain neglected and poorly understood. To date, few studies have been conducted on individual VBDs in vectors or animal hosts. Here, we provide the first data for molecular screening and characterization of multiple vector-borne pathogens in different animal hosts to better understand the epidemiological approach of VBDs in Egypt.

Materials and methods

Ethical approval

This study was approved by the Medical Research Ethics Committee at the National Research Centre, Egypt with the number 19058.

Study area and samples collection

We conducted a cross-sectional observational study with a total of 557 apparently healthy domestic animals (203 dogs, 149 camels, 88 cattle, 26 buffaloes, 58 sheep and 33 goats) using a convenience sampling strategy [81]. Animal blood samples were randomly collected from
different provinces in Egypt between 2016 and 2018. The details of the sample locations were presented in Fig 1 and Table 1. For each animal host, 5 ml of blood was collected in a sterile EDTA tube using a sterile syringe and stored at -20˚C for molecular purposes. The prevalence of infection of different pathogens by different animal hosts was calculated according to Thrusfield et al. [81].

**DNA extraction**

DNA was extracted from 200 μl of each blood sample using EZ1 DNA Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The extracted DNA was stored at -20˚C until use for molecular screening.

**Screening of multiple pathogen DNA by qPCR**

All samples were first screened for pathogen DNA by qPCR using genus-specific primers and probes targeting the 5.8S rRNA gene of piroplasms, the 23S rRNA gene of Anaplasmataceae, the gltA gene of Rickettsia sp., the 16S rRNA gene of Borrelia sp., the IS1111 of C. burnettii, BartoITS3 of Bartonella sp. and the pan-fil 28S rRNA gene of Filariidiae. For positive Filariidiae in dog samples, a triplex qPCR targeting Cox1 was used to detect *D. immitis*, *D. repenes* and *Ac. reconditum*. The sequence of primers and probes used in this study is showed in Table 2.

The qPCR was performed using a CFX 96 Real Time System (Bio-Rad Laboratories, Foster City, CA, USA). The total reaction volume of 20 μl included 10 μl of Eurogentec Master Mix Roche, 0.5 μl of each primer, 0.5 μl of FAM-labeled probe, 0.5 μl of UDG, 5 μl of DNA template, and 3 μl of DNAse- and RNAse-free water. Thermal cycling was performed according to the instructions provided by the manufacturer of the Master Mix PCR kit. To evaluate the PCR reaction, a positive control (pathogen DNA) and a negative control were added to each
reaction. The sample was considered positive if the cycle threshold (Ct) was less than 35 Ct [82].

**Standard PCR and sequencing**

All samples considered positive by qPCR were subjected to standard PCR and sequencing. Primers targeting 969 bp and 1200 bp region of the 16S rRNA gene, respectively, were used to identify *Piroplasma* and *Borrelia*. For the identification of Anaplasmataceae, standard PCR were performed with primers targeting a 520 bp fragment of the 23S rRNA gene. The positive samples with 23S rRNA gene were confirmed with *Anaplasma* genus-specific primers targeting the 525 bp fragment of the *rpoB* gene. *Rickettsia* genus-specific primers targeting the *gltA* gene were used and the positive samples were confirmed by the *ompB* gene. Moreover, multi-spacer typing (MST) for *C. burnetii* was performed by amplifying of three intergenic spacers (Cox2, Cox5 and Cox18). Identification of Filariidae was performed using 18S rRNA primers targeting 1155 bp. All primer sequences used in standard PCR and sequencing are listed in Table 2. All PCR reactions were performed in an Applied Biosystems 2720 Thermal Cycler model (Thermo Fisher Scientific Courttaboeuf, France) using AmpliTaq 360 Master Mix (Thermo Fisher Scientific Courttaboeuf, France) according to the manufacturer’s recommendations. Negative and positive controls were included in each reaction. PCR products were visualized by electrophoresis on a 1.5% agarose gel stained with Syper Safe stain (Invitrogen, USA) and analyzed using Lab Image software (BioRad, Marnes-La-Coquette, France).

PCR products were purified using NucleoFast 96 PCR plates (Macherery Nagel, EURL, Hoerdt, France), according to the manufacturer’s recommendation. The purified PCR products were sequenced using the Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems, Foster City, CA, USA) with an ABI automated sequencer (Applied Biosystems). The sequences obtained were assembled and edited using ChromasPro software.

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Table 1. The information data of collected samples.

| Provinces   | Geographic coordinates       | Animal Hosts | Locations                     | Numbers of Animals |
|-------------|------------------------------|--------------|-------------------------------|--------------------|
| Cairo       | 30° 03’ 45.47” N, 31° 14’ 58.81” E | Dog          | Police Academy (El-Abbassia) | 75                 |
|             |                              |              | Police Academy (El-Tagamoia)  | 67                 |
|             |                              |              | Police Academy (El-Dowaika)   | 61                 |
|             |                              | Camel        | Police Academy (Gasr-El Swiss) | 52                 |
| Giza        | 29° 58’ 27.00” N, 31° 08’ 2.21” E | Camel        | Police Academy (El-Haram)     | 96                 |
|             |                              | sheep        | households                     | 5                  |
|             |                              | Goat         | households                     | 6                  |
| Beni-Suef   | 29° 03’ 60.00” N, 31° 04’ 60.00” E | Cattle       | households                     | 63                 |
|             |                              | Sheep        | households                     | 48                 |
|             |                              | Goat         | households                     | 20                 |
|             |                              | Buffalo      | households                     | 20                 |
| Qalyubia    | 30.41’N, 31.21’E             | Cattle       | households                     | 2                  |
|             |                              | Buffalo      | households                     | 6                  |
|             |                              | Goat         | households                     | 2                  |
| Sinai       | 28° 32’ 13.79” N, 33° 58’ 14.39” E | Sheep        | households                     | 5                  |
|             |                              | Goat         | households                     | 5                  |
|             |                              | Camel        | Free rearing                   | 1                  |
| El-Wady El-Geded | 24°32’44”N, 27°10’24”E | Cattle      | households                     | 11                 |
| Qena        | 26° 09’ 60.00” N, 32° 42’ 59.99” E | Cattle      | households                     | 10                 |
| Beheira     | 30.61’N, 30.43’E             | Cattle      | households                     | 2                  |

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Phylogenetic analyses

Multiple sequence alignments were performed between the obtained sequences and other reference sequences in GenBank using CLASTAL W in MEGA software version X [83]. Phylogenetic trees were inferred using the Maximum-Likelihood method and Tamura-Nei model with 500 bootstrap replicates in MEGA X software [83,84].
Results

In this study, all samples (557) were screened by qPCR. None of the animals were positive for Bartonella sp., while different animal hosts were positive for piroplasms, Anaplasma sp., Rickettsia sp., Borrelia sp., C. burnetii and Filaria sp (Table 3).

Fifty of 557 (8.9%) animal hosts were positive for piroplasms based on 5.8S rRNA qPCR system. Standard PCR and sequencing based on 18S rRNA gene succeeded in amplifying and identifying two Theileria sp.; T. annulata in cattle (14/88), T. ovis in sheep and buffaloes (5/58 and 2/26, respectively) and two Babesia sp.; Ba. bigemina in cattle (1/88) and Ba. canis in dogs

| Animal Hosts | No. of examined Animals (Total = 557) | Pathogens amplified | No. of infected Animals (%) |
|--------------|--------------------------------------|---------------------|----------------------------|
| Cattle       | 88                                   | Piroplasmida        | 15/88 (17%)                |
|              |                                      | T. annulata         | 14/88 (15.9%)              |
|              |                                      | Ba. bigemina        | 1/88 (1.1%)                |
|              |                                      | Anaplasmataceae     | 25/88 (28.4%)              |
|              |                                      | An. marginale       | 18/88 (20.4%)              |
|              |                                      | An. centrale        | 1/88 (1.1%)                |
|              |                                      | An. ovis            | 3/88 (3.4%)                |
|              |                                      | An. platys-like     | 3/88 (3.4%)                |
|              |                                      | Borrelia sp.        | 3/88 (3.4%)                |
|              |                                      | Bo. theileri        | 5/88 (5.7%)                |
|              |                                      | Co-infection:       |                            |
|              |                                      | An. marginale + T. annulata | 2/88 (2.3%)           |
|              |                                      | An. marginale + Bo. theileri | 1/88 (1.1%)          |
|              |                                      | An. centrale + T. annulata | 1/88 (1.1%)           |
|              |                                      | An. platys-like + Ba. bigemina | 1/88 (1.1%)         |
| Buffalo      | 26                                   | Piroplasmida        | 2/26 (7.7%)                |
|              |                                      | T. ovis             | 2/26 (7.7%)                |
|              |                                      | Anaplasmataceae     |                            |
|              |                                      | An. platys-like     |                            |
| Sheep        | 58                                   | Piroplasmida        | 5/58 (8.6%)                |
|              |                                      | T. ovis             | 4/58 (6.9%)                |
|              |                                      | Anaplasmataceae     | 2/58 (3.4%)                |
|              |                                      | An. marginale       | 1/58 (1.7%)                |
|              |                                      | An. ovis            | 1/58 (1.7%)                |
|              |                                      | An. platys-like     | 2/58 (3.4%)                |
|              |                                      | Borrelia sp.        |                            |
|              |                                      | Bo. Theileri        |                            |
|              |                                      | Co-infection:       |                            |
|              |                                      | C. burnetii         | 1/58 (1.7%)                |
|              |                                      | An. platys-like + Bo. theileri | 1/58 (1.7%)       |
| Goat         | 33                                   | C. burnetii         | 1/33 (3%)                  |
| Camel        | 149                                  | Anaplasmataceae     | 10/149 (6.7%)              |
|              |                                      | An. marginale       | 1/149 (0.7%)               |
|              |                                      | An. platys          | 1/149 (0.7%)               |
|              |                                      | An. platys-like     | 8/149 (5.4%)               |
|              |                                      | Filaridae           | 1/149 (0.7%)               |
| Dog          | 203                                  | Piroplasmida        | 1/203 (0.5%)               |
|              |                                      | Ba. canis           | 7/203 (3.4%)               |
|              |                                      | Anaplasmataceae     | 3/203 (1.5%)               |
|              |                                      | An. platys          | 3/203 (1.5%)               |
|              |                                      | Rickettsia sp.      | 2/203 (1%)                 |
|              |                                      | Rickettsia africaine-like | 1/203 (0.5%)       |
|              |                                      | Filaridae           |                            |
|              |                                      | D. repens           | 1/203 (0.5%)               |
|              |                                      | Ac. reconditum      |                            |
|              |                                      | Co-infection:       |                            |
|              |                                      | R. africaine-like + Anaplasma | 1/203 (0.5%)       |

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(1/203). However, camels and goats were free of Piroplasmida DNA. The overall prevalence of piroplasmoses in different animal hosts was 23/557 (4.1%) as it was 17% in cattle, 8.6% in sheep, 7.7% in buffaloes and 0.5% in dogs. In our study, BLAST analysis revealed that cattle were positive for *T. annulata* and *Ba. bigemina*, including two genotypes of *T. annulata*, one genotype in 13 cattle with 100% (910/910) similarity to those of *T. annulata* detected in donkey blood in Turkey (GenBank: MG569892), a new genotype in one cattle with 99% (908/910) identity to the same reference dataset, and a new genotype of *Ba. bigemina* in one cattle with 99% (865/866) identity to those of *Ba. bigemina* detected in cattle blood from Switzerland (GenBank: KM046917). Similarly, we found that 5 sheep and 2 buffaloes were positive for a genotype of *T. ovis* with 100% (897/897) identity to *T. ovis* detected in wild sheep from Turkey (GenBank: KT851427). Finally, we identified *Ba. canis* in a dog with 100% (884/884) similarity to those of *Ba. canis vogeli* detected in a dog from Egypt (GenBank: AY371197). The phylogenetic tree of these genotypes was illustrated in Fig 2.

For Anaplasmataceae, 172 out of 557 (30.9%) animal hosts were positive for anaplasmoses by 23S rRNA qPCR system. Based on the 23S rRNA gene, only 87 out of 557 animal hosts were successfully amplified by standard PCR, consequently, sequencing identified only 48 out of 557. The overall prevalence of anaplasmoses in different animals was 8.6%, with 28.4% in cattle (25/88), 6.9% in buffaloes (4/58), 7.7% in sheep (10/149) and 3.4% in dogs (7/203), while goats were free of anaplasmoses (25/88), 6.9% in buffaloes (4/58), 7.7% in sheep (2/26), 6.7% in camels (10/149) and 3.4% in dogs (7/203), while goats were free of anaplasmoses (25/88). Points of reference 

In our study, BLAST analysis revealed that cattle, sheep and camels were positive *An. marginale*, including two different genotypes of *An. marginale*, the first originated from sixteen cattle, two sheep and one camel with 100% (455/455) similarity to those of *An. marginale* detected in *Rh. bursa* collected from cattle in France (GenBank KY498335), and another new genotype was detected in two cattle with 99% (454/455) identity to the same reference dataset (GenBank KY498335). Moreover, one case of cattle was positive for *An. centrale* with 100% identical to *An. centrale* strain Israel (GenBank NR076686). From cattle and sheep, a genotype of *An. ovis* was identified with 100% (454/454) similarity to *An. ovis* in sheep blood from Niger (GenBank KJ644694). We found that dogs and camels were positive for *An. platys*, including two different genotypes of *An. platys*, one genotype from six dogs and one camel with 100% (458/458) identity to *An. platys* in dog blood from France (GenBank KM021425) and another genotype from one dog with 100% (458/458) homology to *An. platys* in dog blood from France (GenBank KM021414). Finally, from cattle, buffaloes, sheep and camels, a new potential Anaplasma sp. was identified including, four different genotypes of this Anaplasma sp., the first genotype from six camels, the second from two camels, the third from one cattle and one sheep and the last from two cattle and two buffaloes with 98% (450/458), 98% (448/458), 98% (447/458) and 97% (446/458) similarity, respectively, to *An. platys* in dog blood from France (GenBank KM021414). Sequence analysis of this Anaplasma species revealed that this species has a homology score below 99% (more than 10 nucleotides different) and are closely related to *An. platys*, that means these sequences could be considered as potential new species of Anaplasma and can be called as *An. platys*-like. The phylogenetic tree showed that the new potential Anaplasma sp. in two separates and well-supported branches (bootstraps 99 and 96) belong to the cluster of *An. platys* (Fig 3).

To better characterize different Anaplasma genotypes, *rpoB* genus-specific PCR primers were applied and 23 good quality sequences were identified. The result revealed that, 12 cattle and one sheep were positive for a genotype of *An. marginale* with 100% (487/487) homology with *An. marginale* in *Rhipicephalus bursa* from France (KY498345), and another genotype of *An. marginale* from one cattle with 99% (486/487) similarity with the same reference dataset. We also identified that cattle and sheep were positive for *An. ovis*, one genotype was found in two cattle and another in a sheep with 100% (489/489) and 99% (487/489) identical to those of *An. ovis* in sheep blood from Niger (GenBank KY644695), respectively. From dogs, we...
identified a new genotype of An. platys obtained from two dogs with 99% (488/489) homology to An. platys in dog blood from France (GenBank KX155493). Finally, from cattle, buffaloes and sheep, a new potential species of Anaplasma was identified, its sequences had a homology score of less than 90%, confirming that these sequences are likely to be a new potential species of Anaplasma (like 23S rRNA gene). The only two different genotypes (one from two buffaloes and another from a cattle and a sheep) showed a low identity of 89% (432/486) and 88% (431/486), respectively, with An. platys in dog blood from France (GenBank KX155493), while identification of the genotype derived from camels failed. Phylogenetic analysis revealed a new potential Anaplasma sp. (An. platys-like) in a separate and well-supported branch (bootstraps 99) with the same clade belonging to An. platys (Fig 4).
Rickettsial infection was detected by qPCR targeting gltA gene in dogs (3/557; 0.54%); the other animal hosts were free of rickettsiosis. To identify *Rickettsia* sp., standard PCR and sequencing were performed using gltA gene, and it was possible to amplify a 728 bp fragment of this gene from these three positive samples. A BLAST search of the obtained sequences with those in GenBank revealed that two different genotypes, one genotype was 100% (728/728) identical with *R. africae* previously detected in *H. dromedarii* from Egypt (GenBank: HQ335126), and the other sequence had 99% (726/728) identity with the same reference. Moreover, *ompB* gene was used to confirm the detection of *R. africae*-like infection in dogs. Based on the BLAST search, the sequences obtained from dogs were identified as *R. africae* (GenBank: MN629894) and showed (757/758) 99% similarity with the reference strain of *R. africae* detected in a traveler returning from Tanzania (GenBank: KU721071). The phylogenetic tree of these *R. africae*-like in dogs based on gltA was shown in Fig 5.

Fig 3. 23S rRNA based phylogenetic analysis of genotypes identified in this study. Phylogenetic tree highlighting the position of *Anaplasma* sp. in the present study (Bold) related to other *Anaplasma* sp. and *Ehrlichia* sp. available in GenBank. The sequence of 23S rRNA were aligned using CLUSTAL W and phylogenetic inferences were constructed in MEGA X using Maximum Likelihood based on Tamura-Nei Model for nucleotide sequences with 500 bootstrap replicates. There was a total of 432 positions in the final dataset. The scale bar represents a 5% nucleotide sequence divergence.

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Screening of *Borrelia* sp. in all animal hosts we found that 3 cattle and 2 sheep were positive for *Borrelia* sp. (5/557; 0.9%). Standard PCR and sequencing using 16S rRNA gene identified it as *Bo. theileri*. Alignment of five obtained sequences of *Borrelia* sp. from our samples revealed that all sequences were identical to each other. Furthermore, comparison of the obtained sequences with sequences from the GenBank database showed that 1139/1143 (99%) identity with *Bo. theileri* detected in *Rh. geigyi* in Mali (GenBank: KF569941). The phylogenetic position of this new *Bo. theileri* genotype was shown in Fig 6.

Two out of 557 (0.36%) blood samples from one sheep and one goat tested positive for *C. burnetii* DNA by qPCR targeting IS1111. MST genotyping was performed using Cox2, Cox5 and Cox18, with only Cox2 successfully identified and the other spacers failing amplification.

![Fig 4. rpoB gene based phylogenetic analysis of genotypes identified in this study. Phylogenetic tree highlighting the position of *Anaplasma* sp. in the present study (Bold) related to other *Anaplasma* sp. and *Ehrlichia* sp. available in GenBank. The sequence of rpoB gene were aligned using CLUSTAL W and phylogenetic inferences were constructed in MEGA X using Maximum Likelihood based on Tamura-Nei Model for nucleotide sequences with 500 bootstrap replicates. There was a total of 534 positions in the final dataset. The scale bar represents a 10% nucleotide sequence divergence.](https://doi.org/10.1371/journal.pntd.0009767.g004)
A BLAST search for the two sequences obtained showed that (351/351) 100% identity with the reference sequences of *C. burnetii* recorded in GenBank.

Concerning Filariidae, four out of 557 (0.7%) animal hosts collected from three dogs and one camel tested positive for *Filaria* sp. DNA. By BLAST analyses, two dogs were found to have *D. repens* with 100% identity to those of *D. repens* previously detected in a Japanese woman returned from Europe (GenBank AB973229), and another sequence obtained from one dog showed 99% (1114/1119) similarity to *A. viteae* (GenBank: DQ094171). Moreover, *S. digitata* with (1107/1111) 99% identity to *S. digitata* from UK (GenBank: DQ094175) was found in a camel. The phylogenetic analysis of these *Filaria* sp. was constructed and presented in Fig 7.
Finally, seven of different animal hosts were positive for more than one vector-borne pathogen (co-infections; 7/557; 1.3%). In cattle, five co-infections were observed (5/88; 5.7%) as *An. marginale* plus *T. annulata* (2/88; 2.3%), *An. marginale* plus *Bo. theilerii* (1/88; 1.1%), *An. centrale* plus *T. annulata* (1/88; 1.1%) and *An. platis*-like with *Ba. bigemina* (1/88; 1.1%). Moreover, one co-infection in sheep was recorded as *An. platis*-like plus *Bo. theilerii* (1/58; 1.7%) and one case in dogs *R. africae*-like with *Anaplasma* (1/203; 0.5%) (Table 3).

**Discussion**

The sustainable and economic progress of developing countries depends mainly on domestic animal resources, as they provide vital food, draught power and manure for crop production, and generate income [85]. However, animal-associated diseases, especially, VBDs are a global burden [2]. Recently, the spectrum of VBDs affecting animals has expanded and the attention of clinicians and veterinarians is growing. Therefore, the diagnosis of VBDs is crucial to...
develop the epidemiological mapping of these diseases and this can be achieved through the advances in molecular biology [86].

Concerning piroplasmoses, the overall prevalence among animal hosts was 4.1%, including the highest prevalence among cattle 17%, then sheep 8.6%, buffaloes 7.7% and dogs 0.5%. Based on the 18S rRNA gene, two genotypes of *T. annulata* were detected in cattle from different provinces (El-Wady El-Geded, Beni-Suef, Qena and Beheira) and one case of *B. bigemina* was detected in cattle from Beni-Suef. In accordance to our results, many studies reported the high prevalence of *T. annulata* compared to other piroplasms in cattle from different provinces in Egypt [87–89]. In the current study, we observed that the majority of cases (10 out of 15) were detected in cattle from El-Wady El-Geded province that in accordance with Al-Hosary et al. [89], who stated that the prevalence of *T. annulata* in cattle from El-Wady El-Geded province was 63.6%. This finding might be due to the climate in this province, which is dry and sunny throughout the year, which is conducive to tick activity [89]. Likewise, we identified

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**Fig 7. 18S rRNA based phylogenetic analysis of genotypes identified in this study.** Phylogenetic tree highlighting the position of *Filaria* sp. in the present study (Bold) related to other *Filaria* sp. available in GenBank. The sequence of 18S rRNA were aligned using CLUSTAL W and phylogenetic inferences were constructed in MEGA X using Maximum Likelihood based on Tamura-Nei Model for nucleotide sequences with 500 bootstrap replicates. There was a total of 1110 positions in the final dataset. The scale bar represents a 10% nucleotide sequence divergence.

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Anaplasma ovis in sheep from Giza and Beni-Suef and buffaloes from Beni-Suef. In Egypt, there are few studies reporting T. ovis in sheep [90] and buffaloes [91]. In parallel, a recent study reported that T. ovis was detected in sheep from Menoufia and El-Wady El-Geded province [92], implying that this pathogen is widespread in sheep throughout Egypt. Finally, we detected one case of Ba. canis in a dog from Cairo province with 100% identity with Ba. canis vogeli detected in a dog from Egypt (GenBank: AY371197). Canine babesiosis is distributed worldwide and was later detected in Egypt by Passos et al. [93] and Salem and Farag [94]. In Africa, Ba. canis vogeli has been detected in different regions such as South Africa [95], Tunisia [96] and Côte d’Ivoire [80].

Family Anaplasmataceae was known to cause human and animal diseases, is transmitted by ticks and has a worldwide distribution [26,97]. In the current study, the overall prevalence of anaplasmosis was 30.9% (172/557) by qPCR, while we obtained only 48 samples with good quality sequences, possible due to the higher sensitivity of qPCR compared to standard PCR or due to the co-infection with family Anaplasmataceae. The overall infection rate of An. marginale was 3.8% (21/557) in cattle, sheep and camels from different localities (Beni-Suef, Qena, El-Wady El-Geded and Cairo). In Egypt, An. marginale was first mentioned in the national report in 1966, after which the disease was reported in numerous provinces [32–34,98]. Several studies reported endemicity of An. marginale in cattle [16,28,31–34], buffaloes [30] and camels [29]. However, An. marginale was detected for the first time in sheep. To our knowledge, An. marginale has not yet been described in sheep. For the first time, An. centrale was detected in a bovine from El-Wady El-Geded province, Egypt. Anaplasma centrale is closely related to An. marginale but less pathogenic, so it has been used as a live vaccine to protect against bovine anaplasmosis [99,100]. We also found that sheep and cattle from Beni-Suef province (upper Egypt) were positive for An. ovis with a prevalence rate of 0.7% (4/557). To the best of our knowledge, An. ovis has never been detected in cattle and sheep in Egypt. In parallel, a recent study reported that An. ovis was detected in sheep in Menoufia province (one of Delta provinces) [34], implying that this pathogen is widespread in cattle and sheep throughout Egypt. Anaplasma ovis is the etiological agent of ovine anaplasmosis in small ruminants and causes mild and subclinical infections [23]. In Africa, some studies reported An. ovis in sheep from Tunisia [101], Senegal [25] and Algeria [102,103], and in cattle from Algeria [103]. In addition, we found that dogs from Cairo and a camel from Giza province were positive for two genotypes of An. platys, with an infection rate of 1.4% (8/557). In Egypt, An. platys was never molecularly identified in dogs and camels. Later, Loftis et al. [51] detected An. platys in ticks collected from dogs. Anaplasma platys is the causative agent of canine anaplasmosis, which causes severe thrombocytopenia in dogs [104]. Interestingly, we detected that cattle, buffaloes and sheep from Beni-Suef province and camels from Giza and Cairo provinces were positive for a new potential Anaplasma sp. with a prevalence rate of 2.5% (14/557). This probably new species was genetically related to canine Anaplasma platis, which is why it was commonly referred to as An. platys-like. This An. platys-like genotype has never been detected in Egypt, except in a recent study where An. platys-like bacterium was detected only in cattle in Menoufia province [34], implying that this new potential pathogen circulates between different animal hosts (excluding dogs that seem to be susceptible for a type An. platys only) and different provinces in Egypt. Later, An. platys-like was detected in various animal hosts such as cattle in Italy [105], Algeria [106] and Tunisia [107], camels in Tunisia [108,109] and sheep and goats in South Africa [110] and Senegal [25]. Various Anaplasma sp. were identified by the 23S RNA gene and which further confirmed by the rpoB gene.

Rickettsioses are VBDs of humans and animals and are mainly transmitted by ticks [35]. In Africa, the human pathogens R. africae, R. aeschlimannii, R. conorii and R. massiliae have been identified in ticks and animals [39–41]. In our study, rickettsial DNA was detected in dogs
from Capital Cairo with a prevalence of 1.5% (3/203) in dogs. Phylogenetic analysis showed that our genotypes (R. africae-like) clustered in a separate and well-supported branch (bootstrap 94) with R. africae previously detected in Egypt (Fig 5) [53]. To the best of our knowledge, R. africae has not been previously detected in dogs anywhere in the world. Thus, this is the first detection of R. africae-like pathogens in dog anywhere in the world. African tick-bite fever, a benign disease with severe complications in elderly populations, and transmitted mainly in the south and West Africa by Amblyomma variegatum [35,111]. Likewise, R. africae was identified in other tick genera as Hyalomma sp. [42,53,54,112] and in Rh. sanguineus (the most common tick parasitizing dogs) [113].

Relapsing fever borrelioses caused by group of the spirochete group Borrelia sp. and is transmitted by soft and hard ticks [57]. In the present study, we identified Bo. theileri in bovine and ovine blood for the first time in Beni Suef province, Egypt, with an overall prevalence of 0.9% (5/557). Alignment of five sequences obtained revealed that there is a new potential genotype of Bo. theileri circulating between cattle and sheep in Beni-Suef province, which is 99% identical to Bo. theileri found in Rh. geigyi in Mali [58]. Borrelia theileri is considered one of the relapsing fever borreliae and the etiological agent of bovine borreliosis in cattle, transmitted by hard ticks, mainly Rhipicephalus sp. [114]. In Egypt, Bo. theileri was reported in Rh. annulata collected from donkeys in the same province [115]. Later, Bo. theileri was also detected in Rh. annulata in Egypt [62]. Recently, some studies have detected Bo. theileri in cattle such as Argentina [116] and Cameroon [117]. Similarly, Bo. theileri has been detected in the blood of sheep in Algeria [102]. It appears that, Bo. theilerii is not exclusively pathogenic to cattle.

Q fever is a tick-borne disease that is a major public health concern [65]. The infection in human manifests as acute or chronic febrile disease often associated with endocarditis and abortion [65]. In Egypt, Q fever was first detected in a high-risk group of cattle farmers [118]. Later, many reports demonstrated the prevalence of the disease in goats, sheep, cattle and camels [67–72,119,120]. In this study, the overall prevalence of Q fever in sheep and goats from Sinai province is 0.3% (3% in goats and 1.7% in sheep). This result was in accordance with Abdel-Moein and Hamza [71] who reported an overall prevalence of Q fever of 0.9% and 3.4% in goats. PCR and sequencing amplified only Cox2 with a 100% match with the reference recorded in GenBank. Therefore, we suspect that the identified species is, however, Rickettsia rickettii which is the natural filarial nematode of the Bovidae and the adult worm is resident...
in the peritoneal cavity [128,129]. Accidental transmission of _S. digitata_ to unnatural hosts such as horses, donkeys, sheep and goats causes worrisome pathological problems such as corneal opacity and blindness [74,130,131–133].

Finally, we reported 1.3% (7/557) co-infections in animals, with the highest percentage in cattle 5.7% (5/557). Co-infection in cattle is common and has been reported in many studies [33,34,117,134]. We observed that all cases of co-infections including _Anaplasma_ sp. with another pathogen such as piroplasms, _Borrelia_ or even _Rickettsia_. Regarding the endemicity of VBDs, we observed the most infected region in Beni-Suef province, where the same genotypes or even new potential pathogens circulated between different animal hosts with a risk of transmission to other adjacent provinces and to humans. Furthermore, we observed that the highest prevalence among animal hosts was anaplasmoses (48/557; 8.6%), followed by piroplasmoses (23/557; 4.1%). Molecular analysis revealed an interesting diversity of these VB pathogens in ruminants and dogs. Therefore, further studies are needed for a better understanding of the epidemiological mapping of pathogen-host-vector in this region or even in the whole Egypt.

In conclusion, the current study is the first large-scale epidemiological observational study that performed molecular screening and characterization of multiple vector-borne pathogens in different animal hosts for better understanding of the endemicity of VBDs in Egypt. We identified for the first time _An. centrale_, _An. ovis_, a new _An. platys_-like and _Bo. theileri_ in cattle, a new _An. platys_-like in buffaloes, _An. marginale_, _An. ovis_, a new _An. platys_-like and _Bo. theileri_ in sheep, _An. platys_, a new _An. platys_-like and _S. digitata_ in camels and _R. africae_-like, _An. platys_, _D. repens_ and _Ac. reconditum_ in dogs in Egypt. Therefore, ruminants and dogs in Egypt are reservoirs for multiple neglected, emerging and re-emerging vector-borne pathogens, especially new potential pathogens. Our observational study aimed to describe the repertory of possible vector-borne zoonotic pathogens in Egypt. However, convenient sampling approach did not permit us to evaluate the association of identified pathogens with host characteristics and to describe the geographic distribution of pathogens that limited our study. Further studies are needed to determine the pathogen-host-vector connections and other epidemiological factors of VBDs throughout Egypt, as well as to decipher the zoonotic potential of newly identified genotypes and their animals and public health significance.

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