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An epidemiological survey of bovine *Babesia* and *Theileria* parasites in cattle, buffaloes, and sheep in Egypt

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ABSTRACT

Cattle, buffaloes, and sheep are the main sources of meat and milk in Egypt, but their productivity is thought to be greatly reduced by hemoprotozoan parasitic diseases. In this study, we analyzed the infection rates of *Babesia bovis*, *Babesia bigemina*, *Theileria annulata*, and *Theileria orientalis*, using parasite-specific PCR assays in blood–DNA samples sourced from cattle (n = 439), buffaloes (n = 50), and sheep (n = 105) reared in Menoufia, Behera, Giza, and Sohag provinces of Egypt. In cattle, the positive rates of *B. bovis*, *B. bigemina*, *T. annulata*, and *T. orientalis* were 3.18%, 7.97%, 9.56%, and 0.68%, respectively. On the other hand, *B. bovis* and *T. orientalis* were the only parasites detected in buffaloes and each of these parasites was only found in two individual DNA samples (both 2%), while one (0.95%) and two (1.90%) of the sheep samples were positive for *B. bovis* and *B. bigemina*, respectively. Sequence analysis showed that the *B. bovis* Rhoptry Associated Protein-1 and the *B. bigemina* Apical Membrane Antigen-1 genes were highly conserved among the samples, with 99.3–100% and 95.3–100% sequence identity values, respectively. In contrast, the Egyptian *T. annulata* merozoite surface antigen-1 gene sequences were relatively diverse (87.8–100% identity values), dispersing themselves across several clades in the phylogenetic tree containing sequences from other countries. Additionally, the *T. orientalis* Major Piroplasm Surface Protein (*MPSP*) gene sequences were classified as types 1 and 2. This is the first report of *T. orientalis* in Egypt, and of type 2 *MPSP* in buffaloes. Detection of *MPSP* type 2, which is considered a relatively virulent genotype, suggests that *T. orientalis* infection may have veterinary and economic significance in Egypt. In conclusion, the present study, which analyzed multiple species of *Babesia* and *Theileria* parasites in different livestock animals, may shed an additional light on the epidemiology of hemoprotozoan parasites in Egypt.

1. Introduction

Piroplasmosis caused by different species of *Babesia* and *Theileria* in various wild and domestic animals affects the health status of the infected hosts [1]. Disease outbreaks in livestock animals related to infections with these parasites are of great economic significance. Among the *Babesia* species associated with *Bovine babesiosis*, *Babesia bovis*, *Babesia bigemina*, and *Babesia divergens* are considered the most virulent [2]. While *B. bovis* and *B. bigemina* are found in tropical and subtropical regions of the world, *B. divergens*, which is also defined as a zoonotic agent, is common in Europe [2,3]. *Babesia* sporozoites released from infected ticks during blood feeding infect the host's red blood cells (RBCs), where they transform into merozoites [4]. The asexual multiplication of merozoites within the RBCs results in hemolysis of the cells, leading to anemia and jaundice in a host animal [2]. The clinical picture associated with *B. bovis* infection includes nervous and respiratory symptoms caused by the sequestration of infected RBCs in the capillary beds of vital internal organs [5].

In cattle, *Theileria parva* and *Theileria annulata* are the main etiological agents of severe clinical theileriosis [6], but *Theileria orientalis*, a benign *Theileria* parasite, has also caused outbreaks of theileriosis in several countries [7,8]. In contrast to most *Babesia* species, *Theileria* sporozoites infect the host leukocytes, where they undergo schizogony and merogony [4,6]. Because *T. parva* and *T. annulata* schizonts induce rapid proliferation of leukocytes, these species are classified as transforming *Theileria* parasites [9]. In contrast, *T. orientalis* does not induce leukocyte

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proliferation and is therefore referred to as a non-transforming *Theileria* parasite [6]. Merozoites released upon schizont lysis are infective to RBCs [6]. While *T. annulata* and *T. orientalis* merozoites efficiently multiply in RBCs, merogony in RBCs is less pronounced in *T. parva* [6,10]. While *T. parva* is endemic in eastern, central, and southern Africa, *T. annulata* is common in north Africa, southern Europe, and Asia [11], and *T. orientalis* has a worldwide distribution [7,8,12–18].

Most of the animals that recover from the clinical diseases caused by *Babesia* and *Theileria* parasites remain carriers of these diseases [19,20]. Subclinical infections may also be common among animals that are resistant to clinical piroplasmosis [2]. Detection of carriers and subclinical infections is essential for estimating the level of risk posed by *Babesia* and *Theileria* parasites. Therefore, data from epidemiological surveys could be useful for gauging the efficacy of the parasite control programs implemented in the past. Based on the findings of such surveys, parasite control strategies could be modified where needed. Microscopic examination of Giemsa-stained blood smears is a simple and common method for identifying blood parasites. However, because of low parasitemias at the carrier stage, microscopy may not be an effective diagnostic tool as it lacks sensitivity and specificity [21,22]. Currently, DNA detection techniques, such as PCR assays are preferable for epidemiological investigations, because these methods are specific, sensitive, and capable of detecting active infections [23].

In Egypt, cattle, buffalo, and sheep are the main source of meat, milk, and their related products. Clinical diseases caused by *Theileria* and *Babesia* species are common among the cattle and buffaloes in this country [24–26]. Disease outbreaks often lead to economic losses from reduced productivity, require costly veterinary treatment, and can result in the death of affected animals. Previously, a number of epidemiological studies of *Babesia* and *Theileria* parasites have been conducted in Egypt. *B. bovis* and *B. bigemina* have been reported in cattle, buffaloes, and ticks [27,28]. However, the study areas in these investigations were usually limited to one or two provinces of the country. Furthermore, most of the past epidemiological surveys have focused on either *Babesia* or *Theileria*, but studies aimed at simultaneous detection of both parasites have not been conducted. Additionally, *T. orientalis*, which has been reported in several countries, has never been studied in Egypt. In the present study, therefore, we conducted an epidemiological survey of *B. bovis*, *B. bigemina*, *T. annulata*, and *T. orientalis*, using blood–DNA samples collected from cattle and buffaloes reared in four Egyptian provinces. Sheep populations were also surveyed to investigate the possible infections with these parasite species.

2. Materials and methods

2.1. Blood sampling and DNA extraction

A total of 594 blood samples were collected from cattle, buffaloes, and sheep during a period from August to October, 2013. In detail cattle (n = 439) were sampled in four different Egyptian provinces (Menoufia, Behera, Giza, and Sohag), while blood samples were collected from buffaloes (n = 50) reared in the same provinces, except for Giza (Fig. 1, Table 1). Similarly, sheep (n = 105) were sampled in the same provinces, except for Sohag. In Egypt, livestock animals are maintained under three major management practices, intensive, semi-intensive, and extensive systems. Under the intensive rearing system, large herds of exotic animals are kept within proper housing facilities, while cross-bred animals are managed by semi-intensive system. On the other hand, the extensive management is characterized by few numbers of local animals and low production inputs. Cattle in the sampled locations were maintained under intensive, semi-intensive, or extensive management systems, while the buffaloes were reared solely under the extensive system. In contrast, the sheep were managed by semi-intensive or extensive practices. All the animals were apparently healthy during the sampling period. The ages of the sampled animals ranged from 0.5 to 10, 0.5 to 7, and 1 to 5 years for cattle, buffaloes, and sheep, respectively. Blood samples were collected from the tail veins of the cattle and buffaloes, while the sheep blood samples were collected from their jugular veins. Approximately 2 ml of whole-blood was collected from each animal into a Vacutainer tube containing EDTA. The blood samples were labeled and stored at −20 °C, until the DNA extractions were conducted. DNA samples were extracted from 300 μl of the blood samples using a commercial kit (Promega, Madison,

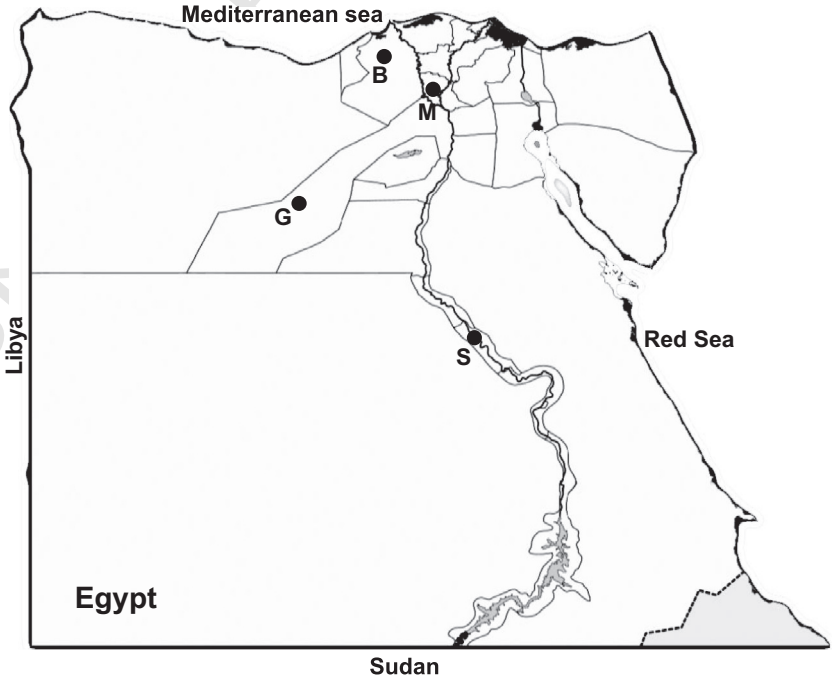

Fig. 1. Sampling locations in Egypt. Blood samples were collected from animals reared in Menoufia (M), Behera (B), Giza (G), and Sohag (S), as indicated by bullet points.

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