MOLECULAR DETECTION OF ANAPLASMA MARGINALE IN THE EGYPTIAN WATER BUFFALOES (BUBALUS BUBALIS) BASED ON MAJOR SURFACE PROTEIN 1α

By

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Abstract

Anaplasmosis is an infectious, non-contagious disease caused by the rickettsial pathogen Anaplasma marginale. The organism is globally distributed and infects erythrocytes, resulting in anemia, jaundice, fever, abortions and death. Once infected, animals remain carriers for life. In developing countries anaplasmosis is of great economic losses as it is highly endemic.

The present study was designed to determine the prevalence of A. marginale in blood samples of buffaloes since they are important reservoir hosts for A. marginale and can serve as a source of infection for tick. A total of 150 buffalo blood samples was randomly collected from four governorates and was analyzed using PCR assay based on msp1α. Anaplasma marginale DNA was detected in 69.3% (104/150) of the sampled buffaloes, and 86.6% (130/150) of collected ticks. As anaplasmal infection is endemic in Egypt, it was recommended screening herds to detect A. marginale even when the signs and symptoms of infection were not visible.

Keywords: Egypt, Anaplasma marginale, Buffaloes, Ticks, msp1α gene, PCR.

Introduction

The domestic water buffalo (Bubalus bubalis) contributes a significant share of global milk production and is the major milk producing animal in several countries including Egypt. Buffaloes are kept mostly by small-scale producers in developing countries, who raise one or two animals in mixed crop-livestock systems.

Anaplasma marginale is an obligate intracellular Gram-negative bacterium classified in the family Anaplasmataceae of Order Rickettsiales. The socioeconomic impact of the disease and the restrictions on trading infected animals internationally led the Office International des Epizooties (OIE) Animal Health Code to categorize anaplasmosis as a disease that required the notification of its presence (OIE, 2008).

The epizootiology of anaplasmosis is complicated by the life-long carrier state which occurs in animals that have recovered from the clinical disease (Atif, 2015). It is transmitted biologically by tick species of genera (Ixodes, Dermacentor, Rhipicephalus, and Amblyomma) and mechanically by other blood-sucking insect-vectors and fomites (Liz et al, 2000; Walker et al, 2001; Skotarczak et al, 2003). It is the most prevalent pathogen transmitted by ticks in the third world. It was found on six continents (Kocan et al, 2010) and causes anap-lasmal infection globally in tropical and subtropical (Lew and Jorgensen, 2005; Ryma-szweska and Grenda, 2008) as well as temp-erate regions worldwide (Ohashi et al, 2005; Woldheiwet, 2006; Bakken and Dumler, 2008).

Although the clinical signs are rare in buffaloes, their treatment is essential as they may act as carriers for cattle (Vatsya et al, 2013). Clinical anaplasmosis is most notable in cattle, but other ruminants including water buffalo (Bubalus bubalis), American bison (Bison bison) were also affected (Kuttler, 1984; Zaugg et al, 1996). Various forms of the anaplasmal infection reduced the body weight of infected animals, causing abortions, reduction in milk production, and frequently led to death causing the economic losses to livestock owners (Sainz et al, 1999; Melendez, 2000; Stuen et al, 2002). The bovine anaplasmosis often resulted in devel-
opment of mild to severe anemia and icterus without hemoglobinemia and hemoglobinuria. The clinical symptoms included fever, weight loss, abortion, lethargy, icterus, and often death in the animals over two years old (Kocan et al., 2003; 2004).

The major surface protein 1 alpha (MSP1α) has been used as a marker to characterize the genetic diversity and identification of *A. marginale* strains (Silva et al., 2014). Also, because of its involvement in the interaction of the bacterium with vertebrate and 63 invertebrate host cells (de la Fuente et al., 2010), detection of persistently infected cattle is important to control the movement of infected cattle into and from the disease-free regions, however, not in buffaloes. Several reports are available on anaplasmosis in the Egyptian cattle (Kumar and Sangwan, 2010; Nair et al., 2013), however, there was little information regarding the buffaloes (Ashuma et al., 2013).

The present study screened the incidence of *Anaplasma marginale* in Egyptian buffaloes by using the reliable molecular tool in four representative Egyptian Governorates.

**Materials and Methods**

Ticks collection and Identification: Ticks were taken off each buffalo from which blood samples had been taken. Only the non-en-gorged adults were identified. Collected ticks were transferred to the laboratory where they were killed with hot water and identified to the species level according to Hoogstraal (1981) keys, using the stereo microscope. To overcome contamination problem, the surface of ticks can be sterilized by washing them in 70% ethanol and rinsed with sterile water (Wittenbrink et al., 1994; Higgins and Azad, 1995; Zhang et al., 1995). The identified sterilized ticks were stored individually in the 1.5-ml micro-centrifuge tubes containing 70% ethanol and stored at -20°C prior to the DNA extraction.

DNA extraction of collected ticks: DNA extraction was performed using Qiamp DNA extraction kit for tissue protocol (Qiagen, Cat No./ID: 56404). Firstly, the ticks were roughly torn to pieces in 180µl ATL buffer and treated with proteinase K (100µg/mL) for 16 h at 56°C. The subsequent steps were carried out according to the manufacturer’s instructions.

Blood samples collection: A total of 150 blood samples were collected from buffaloes highly infested by ticks from Giza, Qalyoubia, El-Wadi El-Gadeed and Menofia Governorates. Blood was collected from jugular vein in evacuated tubes containing EDTA.

DNA extraction of collected blood samples: DNA was extracted using QIAamp DNA blood mini kit (Qiagen, Cat No. ID: 51104) following the Manufacturer’s instructions. The DNA concentration from each sample was quantified by using a NanoDrop spectrophotometer and stored at -20°C.

Amplification of *A. marginale* DNA: The PCR amplification of *A. marginale* DNA based on *msp1α* (Lew et al., 2002). PCR was performed in 25µL final volume containing 5µ l of DNA template either from the ticks or from collected blood, 12.5µL of PCR Master Mix (Qiagen), 1.6µM of forward & reverse primers: 1733F (5’- TGTTGTATGCA CCATTITCC-3’), 3134R (5’-TCACGGTCA ACCTTGTTCACC-3’), and the nuclease-free water to the final volume of 25µl.

PCR was performed under the following conditions, an initial de-naturation at 94°C for 5min, 40 cycles of 94°C for 30s, 55°C for 1min, and 72°C for 2 min, followed by the final extension at 72°C for 7min. The PCR products were subject for agarose gels electrophoresis. After the staining with ethidium bromide the PCR products were visualized using UV transilluminator (IN Geniuse 3) connected with the Quantity-one 4.6.2 software image capturing and analysis.

**Results**

The results are shown in fig. (1).
Fig. 1: Amplification of Msp1α at 548bp. Lane 1: negative PCR control, Lane 2, 3, and 4 amplified DNA extracted from blood samples, lanes 5, & 6: amplified DNA extracted from collect ticks. Lane 9: positive control

Discussion

In the present study, the prevalence of Anaplasma sp. varied from one region to another and a number of factors were associated with incidence of this tick-borne diseases including age, sex, breed, tick density, season, geographical area, and management (Stokka et al, 2000; Khan et al, 2004; Kivaria, 2006; Magona et al, 2011; Atif et al, 2012).

The expected PCR product from amplified A. marginale msp1α gene was 548 bp (Fig. 1). A total of 500 adult hard ticks were collected and by using the pictorial key for the tick’s identification, those of buffaloes belonged to Rhipicephalus (280), Boophilus (150), Hyalomma anatolicum anatolicum (30), and H. a. excavatum (40). The high infection rates were detected in the ticks (86.6%) by amplified msp1α, using a microscopic examination, in Pakistan, the tick collected from the water buffaloes showed infection rate of 36.59%.

In spite of the fact that the water buffaloes were exposed to A. marginale, a low rate of A. marginale PCR-positive animals were reported, which might be related to the habitat where the sampled animals lived because they exhibited a low rate of attached ticks to skin. The amplification of A. marginale msp1α gene showed that 69.3% of collected blood from the Egyptian buffaloes was positive A. marginale although these animals were apparently healthy without any clinical manifestation. Sajid et al. (2009) and Ocaido et al. (2005) reported 11.8% prevalence of A. marginale in cattle and buffaloes of Sorti District, Uganda. This difference might be attributed to the climatic conditions and/or habitat. The results agreed with Haider and Bilqees (1988) who reported 61% Anaplasma sp. in Karachi and adjoining areas. This difference in the results might probably be due to the environmental and/or the climate conditions with relatively high humidity in Karachi that favor the tick-vector development and spreading (Shahnowaz et al, 2011). Also, serological and molecular de-
tection of *A. marginale* in seventy-three water buffalo in Brazil gave prevalence of 49.0% and 5.4%, respectively (Silva et al., 2014).

Adjou Moumouni et al. (2015) in Kenya by using Nested PCR and sequencing reported the prevalence and genetic diversity of *Babesia bovis*, *B. bigemina*, *Theileria* species and *A. marginale* parasites in 192 cattle blood samples. The *B. bovis* spherical body protein 4, *B. bigemina* rhoptry-associated protein 1a, *A. marginale* major surface protein 5, *Theileria* spp. 18S rRNA, *T. parva* p104 and *T. orientalis* major piroplasma surface protein were used as the marker genes. They concluded that results reaffirmed the endemicity and co-infection of cattle with tick-borne hemoparasites, and the role of wildlife in pathogens’ transmission and population genetics, the buffaloes could serve as a source of zoonosis.

Michael et al. (1987) in El-Minia Governorate reported the first case of human babesiosis in a farmer. El-Bahana and Morsy (2008) in Alexandria reported zoonotic ba-besiosis in a youth and El-Bahana and Morsy et al. (2011) in Cairo reported babesiosis in a boy who acquired the infection from his pet dog infested with ticks. Sazmand et al. (2016) in Iran reported that camels were identified as hosts for bovine Mediterranean theileriosis. Qiu et al. (2016) in China stated that domestic ruminants were infected with a variety of *Anaplasma* spp. and *Ehrlichia* spp. Matei et al. (2017) in Romania evaluated the annual prevalence of *Rickettsia* spp. and *A. phagocytophilum* in *I. ricinus* collected from humans over three consecutive years. They concluded that prevalence of *R. helvetica*, suggested an increasing risk for the humans zoonotic pathogen. Battilani et al. (2017) in Italy stated that *Anaplasma* are the etiological agents of tick-borne diseases of veterinary and medical importance in both tropical and temperate regions.

In Egypt, Loftis et al. (2006) detected *A. marginale*, *Coxiella burnetii*, *Rickettsia aetschlimannii*, and four novel genotypes similar to: "Anaplasma platys," *Ehrlichia canis*, *Hillichia* spp. reported from the Asian ticks, and a *Rickettsiales endosymbiont of Ixodes ricinus*. El-Ashker et al. (2015) in Dakahlia Governorate used a novel DNA microarray system for diagnosing bovine piroplasmosis and anaplasmosis in comparison with microscopy and PCR assay. They found that in the acutely ill cattle, the *A. marginale* was 10/164 and mixed infections with *Babesia/Theileria* spp. and *A. marginale* were in two more cases. Moreover, *A. marginale* infections were also detected in 23 of the apparently healthy cattle. Fareig et al. (2017) in southern Egypt reported antibodies against *A. marginale* in 25/90 (28%) of cattle.

**Conclusion**

Infection with *A. marginale* is more or less endemic not only in Egypt but also in many cattle rearing countries where tick-vectors are common. There is a lack of the adequate information on its genotypes.

The present outcome results declared that *A. marginale* is highly prevalent in the Egyptian water buffaloes especially those apparently healthy. Undoubtedly, the control of tick-vectors and the early diagnosis and treatment of infected cattle would save meat and meat production.

Conflict of interest: The authors have neither conflict of interest nor received financial support

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