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Spot diagnosis of canine monocytic ehrlichiosis: Need of the hour

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Abstract

Canine monocytic ehrlichiosis is an important rickettsial disease with worldwide distribution, mainly caused by *Ehrlichia canis*. Diagnosis of the disease is challenging due to its multiple clinical manifestations and occurrence of disease in different phases of the infection. Diagnosis of CME is routinely carried out by blood smear and buffy coat smear examination. Advanced techniques include serology and PCR. Hence an attempt was made to know the sensitivity and specificity of blood smear and buffy coat smear against SNAP 4Dx in detecting CME. In the present investigation, 16 dogs suspected for ehrlichiosis were included. Blood smear and buffy coat smear examination showed a sensitivity of 16.67 and 38.89 per cent respectively as against 100 per cent in ELISA based SNAP 4Dx kit. Thus, SNAP 4Dx kit with cent per cent reliability and minimum reaction time (8 minutes) makes it an effective in-clinic spot diagnostic test for confirming ehrlichiosis in dogs.

Keywords: Ehrlichiosis, blood, buffy coat smear, sensitivity, SNAP 4Dx kit

Introduction

Vector-borne agents comprise medically important infections affecting dogs throughout much of the world. Canine Monocytotropic Ehrlichiosis (CME), caused by *Ehrlichia canis* is an important canine rickettsial disease with a worldwide distribution. The disease manifests as acute, chronic and subclinical form with varied clinical signs. The diagnosis of the disease is challenging due to its different clinical phases along with multiple clinical manifestations (Harrus *et al.*, 2002) [5]. CME should be suspected with a compatible history (living in or traveling to an endemic region, previous tick exposure) and typical clinical signs. Traditional diagnostic techniques like hemato-biochemical parameters, blood smear and buffy coat smear examination are valuable tools for confirming CME. However a definitive diagnosis of *E. canis* infection requires serological techniques and molecular techniques though molecular technique is a time consuming method. Sensitive detection of antibodies directed at tick-borne disease-causing organisms in dogs is diagnostically important for veterinarians, pets and their owners, and epidemiologically important for public health surveillance. The SNAP 4Dx Plus Test (IDEXX Laboratories, Inc., Westbrook, ME) identifies antibodies to or infection with multiple tick-borne pathogens in a single assay. Hence the aim of the present work was to know the efficacy of different diagnostic techniques (blood smear, buffy coat smear examination and SNAP 4Dx) for diagnosis of infection caused by *E. Canis*.

Materials and Methods

Dogs presented to Out Patient Ward (Medicine), Veterinary College, Bidar and Veterinary Hospital, Disease Diagnostics and Information Centre, APMC (Agriculture Product Marketing Committee) yard, Bidar showing clinical signs of ehrlichiosis i.e. pyrexia, inappetance, lymphadenopathy, bleeding tendencies and history of tick infestation from July 2014 to June 2016 were analysed.

Collection of blood: Blood samples (2ml) were collected for examination with blood smear examination, buffy coat smear examination and SNAP 4Dx KIT.

Blood smear and buffy coat smear examination: Thin blood smear and buffy coat smears were prepared and examined after staining with Giemsa stain as per standard protocol (Coles, 1986) [3]. The stained smears were examined under oil immersion (100X) objective of the microscope to detect the morula of *E. canis*.

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Snap 4Dx Plus: Snap 4Dx Plus is an *in-vitro* serological diagnostic kit for detection of *Dirofilaria immitis* antigen, antibody to *Anaplasma phagocytophilum*, antibody to *Anaplasma platys*, antibody to *Borrelia burgdorferi*, antibody to *Ehrlichia canis* and antibody to *Ehrlichia ewingii* using serum, plasma or whole blood sample. In the present study, fresh blood samples and serum samples were used for the test and samples were run as per the protocol given by IDEXX Laboratories, Westbrook, USA. Results were read after 8 minutes indicated by the development of blue colour in respective slots.

Results and Discussion

In the present study, blood smear examination could detect only 15.00 per cent (3/16) suggestive of lower efficacy whereas, it was 35.00 per cent (7/16) in buffy coat examination (Fig. 1) indicating moderate efficacy. Snap 4 Dx Plus Kit (Fig. 2), an ELISA based kit, was found to be superior with cent per cent (16/16) efficacy against blood smear and buffy coat smear examination. Reports by Mudaliar (1994); Lakshmanan *et al.* (2006); Kumar *et al.* (2010) and Dhankar *et al.* (2011) [8, 7, 6, 4] also opined that blood smear examination was not an effective diagnostic technique for detection of *E. canis* infection in dogs. This is primarily due to the fact that in CME there is significant decrease in total blood cell count and further as the morula is mostly found in monocytes which normally were very less in number (only 1-2%) leading to lowered chances of detection of the parasite in blood smears. Mylonakis *et al.* (2003) and Dhankar *et al.* (2011) [9 & 4] reported buffy coat smear examination to be more effective diagnostic test compared to blood smear for Ehrlichiosis in dogs. The higher detection recorded by buffy coat examination helps in concentration of the leucocytes which in turn increases the chances of finding the morulae of ehrlichia organisms. In the present study, Snap 4 Dx Plus Kit an ELISA based antibody detection kit found to be superior with cent per cent (16/16) efficacy as against blood smear and buffy coat smear examination whereas, Belanger *et al.* (2002) and Borthakur *et al.* (2014) [1, 2] cited 79.2 per cent and 22.51 per cent as efficacy of Snap 4 Dx kit in diagnosing Canine Ehrlichiosis. As the efficacy was 100.00 per cent with Snap 4Dx kit, it was taken as standard and comparative evaluation was made for Blood smear and Buffy coat examination. Liu *et al.* (2018) reported the sensitivity the

SNAP4Dx assay for detection of *E. canis* antibodies was 97.1%. The sensitivity blood smear examination was 16.67 per cent whereas, it was 38.89 per cent in buffy coat examination. The specificity of both the diagnostic test was 100 per cent (Table 1). Harrus *et al.* (2002) [5] reported that the testing and comparison of ELISA based SNAP kit assay with known IFA- positive and negative samples indicated 100 per cent specificity and 79.20 per cent sensitivity for detecting *E. canis* antibodies in dogs.

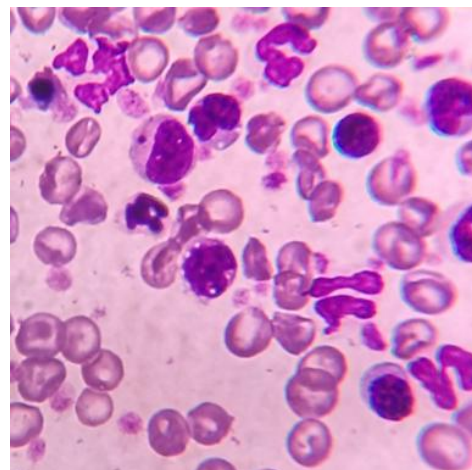


Fig 1: Presence of *E. canis* morula in a monocyte



Fig 2: Positive Snap 4 Dx kit showing blue colour *Ehrlichia* spp sample slot

Table 1: Sensitivity and specificity of blood smear and buffy coat smear examination

Screening test result (Blood smear examination)	Diseased	Non- Diseased	Total	Sensitivity (%)	Specificity (%)
Positive	03	0	03	16.67	100.00
Negative	15	02	17		
Total	18	02	20		
Screening test result (Buffy coat smear examination)	Diseased	Non- Diseased	Total	Sensitivity (%)	Specificity (%)
Positive	07	0	07	38.89	100.00
Negative	11	02	13		
Total	18	02	20		

Conclusion

Snap 4 Dx Plus detection kit was found to be superior with cent per cent efficacy in diagnosing Canine Monocytic Ehrlichiosis against blood smear and buffy coat examination. Rapid tests for the detection of ehrlichiosis are needed to monitor the health of dogs traveling through or living in India.

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