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Usefulness of a rapid immuno-migration test for the detection of canine monocytic ehrlichiosis in Africa☆

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ABSTRACT

A rapid immuno-migration test for the serological detection of canine monocytic ehrlichiosis, Witness® Ehrlichia (WE) (Zoetis, France), was evaluated in 528 serum samples from dogs living in endemic areas of West and East Africa: Senegal ($N=208$), Ivory Coast ($N=7$), Sudan ($N=27$), and Djibouti ($N=286$). Of these dogs, 200 were French military working dogs (MWD) temporarily residing in Africa. The WE test results were compared with those obtained by indirect immunofluorescence (IFA). The sensitivity of WE was 97% [94.2, 98.7] with a specificity of 100% [98.6, 100]. In MWD, the seroprevalence (IFA) was 7%; in native dogs, it reached 77.1%. This significant difference can be explained by prophylactic measures from which MWD benefit. The WE test represents a simple, fast and reliable test for the detection of anti-*Ehrlichia canis* antibodies. Its implementation for the diagnosis of clinical cases has been validated in the field, and its use allows easy detection of asymptomatic dogs that may be carriers of *E. canis*.

1. Introduction

Canine monocytic ehrlichiosis (CME) is a disease caused by bacteria from the *Anaplasmataceae* family (*Ehrlichia canis*), which are transmitted by ticks. In 1935, Donatien and Lestoquard isolated the causative agent of a hemorrhagic fever from dog monocytes in Algiers, and they classified it in the *Rickettsia* genus (*Rickettsia canis*) [1]. In 1937, the mode of transmission (vector-borne disease) was recognized. In 1947, the bacteria were reclassified into the *Ehrlichia* genus in homage to Paul Ehrlich. During the following decades, the same agents were identified throughout the globe (in all the countries where the vector is present): on the African continent, in the Middle-East, in India and in the USA. A notable outbreak occurred in 1970 in Vietnam, when US Army dogs were devastated by canine tropical pancytopenia [2]. *Ehrlichia* genus bacteria are strictly intracellular Gram negative bacilli.

Three clinical forms appear successively in infected dogs [3]. An acute form appears after incubation, which ranges from 8 to 20 days (mean: 14 days). The

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symptoms, fever and lethargy, are not specific. Hemorrhagic signs are often observed, including epistaxis, petechia, hematomas and hematuria. Confirming diagnosis requires the exploration of additional biological parameters. First, thrombopenia is generally present (30×10^6/mL), and the thrombocytes often suffer from functional deficiency. The leukocyte count may also vary; initially the dogs present with leukopenia, and then, after few days, leukocytosis appears in response to the infection. A sub-clinical stage follows the acute form. Clinical symptoms disappear, and the general appearance of the dogs improves, but they do not recover from the disease. Indeed, the bacteria are able to persist in macrophages throughout an animal's life, maintaining chronic ehrlichiosis, although this depends on the immune response of the dog. A low proportion of dogs can eliminate the bacteria. The others remain infected, and thrombopenia, hyperglobulinemia and hypoalbuminemia are the main biological signs. A new clinical manifestation of relapse may appear when the immune system is suppressed, especially due to another infection. Severe weight loss, which may lead to cachexia, is often the trigger. Relapses may manifest with multiple organ dysfunction syndrome (often the liver and kidneys) and hemorrhagic symptoms. Chronic infections are responsible for autoimmune diseases such as glomerulonephritis, articular pain, uveitis, and hemorrhagic retinitis. Anemia is severe and non-regenerative. Thrombopenia is presented. Dogs can die from general hemorrhaging or from a secondary infection.

Diagnosis can be established due to several elements. Because the clinical presentations are not specific, veterinarians must perform a differential diagnosis, including ruling out babesiosis, pesticidal intoxication, auto-immune anemia and leishmaniosis. This list should be adapted based on local epidemiological situations. Epidemiological diagnosis is useful for the detection of acute forms because the agent is transmitted by tick bites and the incubation period is short. The probability of ehrlichiosis varies by area, season and infestation of the patient by ticks. Biological diagnosis relies on thrombopenia, hyperglobulinemia (a limited α2-globulin peak and a larger β-globulin peak, a hyper-gammaglobulinemia that appears 10 days after inoculation) and hypoalbuminemia (these two parameters may vary over several years). Elevated levels of alanine aminotransaminase (ALT: 300 UI/L) and alkaline phosphatase (ALP: 2000 UI/L) are transient but characteristic of the acute phase.

Laboratory tests are necessary to confirm suspected ehrlichiosis. Direct tests for bacteria can be performed on a blood sample or from a stained organ biopsy. Morulae may be observed in the cytoplasm of monocytes as basophil inclusions on a May–Grünwald–Giemsa stained smear. Unfortunately, this diagnosis is unreliable and not very sensitive [4], although leuko-concentration techniques can enhance the outcome by 10%. Laboratory investigations using culture or polymerase chain reaction (PCR) are more reliable diagnostic methods, but they are not widely used. Therefore, indirect tests using serological methods are the reference diagnosis technique, primarily indirect immunofluorescence (IFA). Dogs are considered sero-negative if the titer is less than 1/40, lightly positive between 1/40 and 1/80, moderately positive between 1/160 and 1/320 and strongly positive if the titer is equal to or more than 1/640 [5]. The sensitivity and the specificity of this assay are very high, although cross-reactions exist with *Ehrlichia chaffensis*, *Ehrlichia ewingii*, *Ehrlichia ruminantium* and *Anaplasma phagocytophilum*. Some rapid tests have been developed and are available for clinicians: SNAP 4Dx® (Idexx, USA), Witness® Ehrlichia (Synbiotics, France/Zoetis, USA), ImmunoComb® Canine Ehrlichia (Biogal, Galed Labs, Israel), and Speed® Ehrli (BVT – Virbac, France).

The aim of our study was to diagnosis CME using the Witness® Ehrlichia quick test in the field in Africa. We compared its results with those of the IFA test performed in a laboratory from the same sera. Our data allow analysis of the prevalence of CME in parts of Africa and the evaluation of the rapid test.

2. Materials and methods

2.1. Dogs and sera

From 2000 through 2012, blood samples were collected from 528 dogs: 328 native dogs and 200 French military working dogs (MWD). The native dogs were collected in Senegal (Dakar; $n = 181$), Djibouti ($n = 120$) and Soudan (Barbar el Fugara (N13°39′; E36°8′), $n = 27$). The dogs were investigated with the assistance of their owners. All dogs appeared to be in good health, except 9 dogs that were sick (2 in Senegal and 7 in Djibouti). Ticks were frequently present on these dogs. Two hundred MWD, mainly male Belgian Shepherds from 2–9 years old, were included in this study. These MWD had experienced short stays (four months) in Africa: in Senegal (Dakar; $n = 27$), Ivory Coast (Abidjan; $n = 7$) and Djibouti ($n = 166$). All of the dogs appeared to be in good health. These dogs were orally administered 100 mg (approximately 3 mg/kg bodyweight) of doxycycline daily for chemoprevention of CME [6]. An adapted acaricide product was used regularly, and the handlers did not report any important tick infestations.

A blood sample collected from the radial vein was centrifuged within 24 h of collection, and the serum was subsequently frozen at −20 °C.

2.2. Indirect fluorescence antibodies test (IFA)

Indirect immunofluorescence was performed as the reference method [7]. The *E. canis* antigen, supplied by the Synbiotics Laboratory (Lyon, France), was purified from bacteria grown on MDH cells. A minimum of 25% of the cells were infected. The cells were placed on immunofluorescent slides and fixed in cold acetone for 20 min. Standard techniques were used with a 20-fold pre-dilution of the serum. The examination was performed using an ultraviolet microscope (Olympus microscope – 100 W Hg lamp) at 500×. The presence of anti-*Ehrlichia* antibodies was detected by a clear fluorescence of intracytoplasmic morulae. A negative serum is either not fluorescent or the entire cytoplasm is only slightly fluorescent. Titers greater than or equal to 1:40 were considered to be positive.