

Application of 10% imidacloprid/50% permethrin to prevent *Ehrlichia canis* exposure in dogs under natural conditions

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Abstract

Canine monocytic ehrlichiosis (CME) caused by *Ehrlichia canis* is the most known canine tick-borne disease (TBD) spread throughout the world. Preventing tick bites is a priority to reduce the risk of TBDs and it was the aim of the present study to evaluate the efficacy of a combination of imidacloprid 10% and permethrin 50% (ImPer) (Advantix[®]; Bayer AG, Germany) in a spot-on formulation to control CME under field conditions. On January–March 2005, 845 dogs from two kennels in southern Italy (kennels of Bari (KB)- and Ginosa (KG)), with a history of tick infestation were initially tested by serology and PCR assay for *E. canis* infection. Data on *Leishmania infantum* infection were also available from a previous study carried out on the same dog population. One hundred twenty-six dogs (14.9%) presented anti-*E. canis* antibodies with a relative prevalence of 15.6% ($n = 65$ dogs in KB) and 14.2% ($n = 61$ dogs in KG). Five hundred thirty-five animals found negative both for *E. canis* and *L. infantum* infections were enrolled in three groups (Group A—treated with ImPer once a month; Group B—treated every 2 weeks; and Group C—untreated control animals) and monitored for *E. canis* infection by serology and PCR in November 2005 (first follow-up) and in March 2006 (second follow-up). The *E. canis* infection was serologically revealed, at the first and/or second follow-up, in 26 animals from Group C in KB and KG (mean incidence density rate (IDR), 13.24%) while in none of the animals from Group A (KB and KG) and only in one animal from Group B (IDR 1.13%) in KG. The final protection efficacy of ImPer ranged from 95.57% to 100% in Groups B and A. At PCR only 15 dogs from KG were positive for *Rickettsiales* only at the first follow-up and at the sequence analysis two (both in Group C) revealed 100% homology with *E. canis* sequences while 13 with *Anaplasma platys*. Four out of 13 *A. platys* PCR-positive dogs were also seropositive for *E. canis* at one or both follow-ups. ImPer, by virtue of its repellent and acaricidal activity against ticks, has been shown to be efficacious to prevent *E. canis* infection in treated dogs living under natural conditions in endemic areas.

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1. Introduction

Tick-borne diseases (TBDs), along with canine leishmaniosis (CanL), are among the most important canine vector-borne diseases (CVBDs) transmitted by arthropods (Sonenshine, 1991, 1993; Alvar et al., 1994; Lane and Crosskey, 1995). In particular, borreliosis, ehrlichiosis, rickettsiosis and viral encephalitis may be life threatening diseases in humans and dogs exposed to tick bites (reviewed by Shaw et al., 2001). TBDs are scattered in southern European countries although, over the last decade, the number of their reports (both autochthonous and imported) have been increasing through central and northern Europe (Trotz-Williams and Trees, 2003).

Canine monocytic ehrlichiosis (CME) caused by *Ehrlichia canis* Rickettsiae is a cosmopolitan TBD transmitted (trans-stadially) by *Rhipicephalus sanguineus*. This tick species, known as “Kennel tick” or “Brown Dog tick”, is the most commonly retrieved in subtropical and tropical regions around the globe. The Brown Dog tick serves as a vector for a wide range of organisms pathogenic to dogs. These are *Rickettsia conorii*, *Anaplasma platys* and *Hepatozoon canis* as reviewed by Shaw et al. (2001). CME is characterized by a wide range of clinical signs including lethargy, weight loss, anorexia, pyrexia, lymphaden- and splenomegaly while the most commonly retrieved haematological abnormalities are thrombocytopenia and anaemia (reviewed in Harrus et al., 1997). *E. canis* infection is worldwide distributed in tight relationship with the presence of its vector. Endemic areas for CME have been identified in several countries of the Mediterranean basin with the highest prevalence of 87.5% recorded in a population of kennelled dogs from Corsica (Trotz-Williams and Trees, 2003). Italy is an endemic area for CME as the seroprevalence percentage ranges from 15.5% (Buonavoglia et al., 1995) to 22.6% (Capuano et al., 2002) in southern regions. To the best of our knowledge, no information is available about the annual incidence rate for the CME in dog populations living in endemic areas.

To prevent the transmission of TBDs, prophylactic protection against ticks is needed. Among others, a combination of imidacloprid 10% and permethrin 50% (ImPer) has been developed (Advantix[®]; Bayer AG, Germany) in a spot-on formulation to provide treatment of and prophylaxis against ticks, fleas, mosquitoes and phlebotomine sand flies (Mencke et al., 2003). The efficacy of this combination against ticks has been experimentally demonstrated by using different approaches under laboratory conditions (Epe et al.,

2003; Mehlhorn et al., 2003; Young et al., 2003). Recently, the efficacy of ImPer to prevent the infestation by *R. sanguineus* in dogs living in a heavily tick infested area of southern Italy has been demonstrated under field conditions showing a protection against adults and immature stages up to 98.43% at day + 28 post-treatment (Otranto et al., 2005).

Studies on the prevention of TBDs in dogs are rare. In a laboratory study using ImPer it was demonstrated that transmission of *Borrelia burgdorferi* (s.s.) and *Anaplasma phagocytophilum* from naturally infected *Ixodes scapularis* ticks to dogs could be prevented (Spencer et al., 2003; Blagburn et al., 2004, 2005). Elfassy et al. (2001) studied the preventive effects of an amitraz impregnated collar against *I. scapularis*. In a single multi-center study the efficacy of fipronil to prevent *E. canis* infection has been investigated by seroconversion in 248 dogs over one season of tick exposure in Africa (Davoust et al., 2003). No information is available about the efficacy of ImPer to prevent CME under natural conditions while it has been recently demonstrated that it has a high efficacy to prevent CanL (Otranto et al., 2007).

Thus, it was the aim of the present work to evaluate under field conditions the efficacy of the 10% (w/w) imidacloprid/50% (w/v) permethrin topical spot-on solution (ImPer) as a control measure to prevent CME in dogs from an endemic area of southern Italy.

2. Materials and methods

2.1. Study design and procedures

The trial was conducted from February 2005 to April 2006 on dogs living in Apulia region, southern Italy (latitude: 42° and 39° North, longitude 15° and 18° East) following the same design and procedure of a previous study aimed to evaluate the efficacy of ImPer to prevent CanL in endemic areas (Otranto et al., 2007). In dogs from that area, heavy tick infestations by *R. sanguineus* have been previously reported (Otranto et al., 2005). In particular, in this area dog infestations by adult Brown Dog ticks peak from April to October (Manilla, 1998) and a seroprevalence for *E. canis* of 15.5% was previously reported (Buonavoglia et al., 1995).

Briefly, dogs included in the trial were housed in two kennels from the above area, namely Bari (KB) (latitude: 41°5' North, longitude: 16°5' East) and Ginosa (KG) (latitude: 40°3' North, longitude: 16°4' East). The field study was carried out as a negative-controlled trial to test ImPer spot-on for the prevention

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