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Acaricide and ivermectin resistance in a field population of *Rhipicephalus microplus* (Acari: Ixodidae) collected from red deer (*Cervus elaphus*) in the Mexican tropics

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ABSTRACT

In the Neotropics the control of tick infestations in red deer (*Cervus elaphus*) is achieved primarily through the use of acaricides and macrocyclic lactones. In Mexico, resistance to one or multiple classes of acaricides has been reported in *Rhipicephalus microplus* infesting cattle, but information on acaricide susceptibility in *R. microplus* infesting red deer is lacking. In this study we report the level of resistance to different classes of acaricides and ivermectin in *R. microplus* collected from red deer in the Mexican tropics. Engorged *R. microplus* females were collected from a red deer farm in Yucatan, Mexico. The larval packet test was used to detect resistance to the organophosphates (OPs) chlorpyrifos and coumaphos, synthetic pyrethroids (SPs) cypermethrin and permethrin, and the phenylpyrazol, fipronil. Resistance to the formamidine amitraz (Am), and ivermectin was ascertained using the larval immersion test. Data were subjected to probit analysis to determine lethal concentrations and resistance ratios to kill 50% (RR_{50}) and 99% (RR_{99}) of the tick population under evaluation in relation to susceptible reference strains. Additionally, allele specific polymerase chain reaction was used to detect the sodium channel F1550I mutation associated with SP resistance in *R. microplus*. The *R. microplus* population from red deer in Yucatan showed very high resistance to the two SPs evaluated (RRs > 72.2 for cypermethrin; RR for permethrin resistance was so high a dose–response curve was not possible). All individual larvae tested to detect the sodium channel F1550I mutation associated with SP resistance in *R. microplus* were homozygous. The same tick population showed different levels of resistance to OPs (chlorpyrifos: RR_{50} = 1.55, RR_{99} = 0.63; coumaphos: RR_{50} = 6.8, RR_{99} = 5.9), fipronil (RR_{50} = 1.8, RR_{99} = 0.9), and amitraz (RR_{50} = 2.3, RR_{99} = 4.4). Resistance to ivermectin was regarded as moderate (RR_{50} = 7.1, RR_{99} = 5.0). This is the first report of *R. microplus* ticks collected from red deer in Mexico with different levels of resistance to four acaricide groups and ivermectin.

1. Introduction

New parasite–host relationships can be established in the range of introduced non-native species. Some of these new parasitic associations are of veterinary and economic importance when they involve ticks infesting livestock,

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especially when tick species serve as vectors of infectious agents causing significant morbidity and mortality (Tatchell, 1987). Cattle (*Bos taurus*, *Bos indicus*, and breeds resulting from their crosses), red deer (*Cervus elaphus*), and the southern cattle tick (*Rhipicephalus microplus*) are non-native species that were introduced to Mexico through the livestock trade (Primo, 1992). In 1994 the Mexican federal government imported a herd of about 900 red deer from New Zealand, to promote deer farming as an alternative form of diversifying livestock production (Vásquez et al., 2004). Breeding animals were distributed among different research institutions and red deer thus became exposed to a new suite of parasites including ticks. *R. microplus* is considered the most economically important tick species affecting red deer raised under semi-captivity conditions in the Mexican tropics (Rodríguez-Vivas et al., 2013). In some cases infested red deer in Mexico were shown to carry numbers of engorged female *R. microplus* that were eleven times higher than those recorded on cattle in the same ranch where tick infestation was diagnosed as the cause of red deer fatalities associated with severe anemia.

In addition to direct effects associated with its obligate blood feeding habit like anemia, toxicosis, and physical damage to hides, *R. microplus* also transmits the rickettsia *Anaplasma marginale* that causes anaplasmosis and the apicomplexan protozoa *Babesia bovis* and *Babesia bigemina*, which cause babesiosis in cattle. In tick endemic areas the practice of diversified livestock production in Mexico increases the risk for heavy *R. microplus* infestations in red deer where this species is raised together with cattle, especially in tropical regions where several tick generations can occur annually (Rodríguez-Vivas et al., 2005). Commercially available acaricidal formulations are used commonly by livestock producers in México to try to control *R. microplus* (Rodríguez-Vivas et al., 2006a). Acaricidal products are sold over the counter and are used generally according to label instructions, but the tendency is to treat infested animals at frequent intervals. The utility of acaricides to mitigate the economic impact of *R. microplus* on livestock production systems is undeniable. However, their indiscriminate use has resulted in populations of *R. microplus* that are resistant to all major classes of acaricides including the macrocyclic lactones (MLs) to which ivermectin (IVM) belongs (Jonsson et al., 2000; Rodríguez-Vivas et al., 2006a,b; Chevillon et al., 2007; Perez-Cogollo et al., 2010a).

Accumulating evidence indicates that the invasive characteristics of *R. microplus* include the ability to infest native and non-native cervids inhabiting areas where it is endemic now (De Meeus et al., 2010; Chevillon et al., 2013). In addition to being a natural competent host for *Rhipicephalus annulatus* (Ruiz-Fons et al., 2006), red deer are susceptible to heavy infestation with *R. microplus* in the Neotropics (Rodríguez-Vivas et al., 2013). Similar to the situation with rusa deer (*Cervus timorensis rusa*) in New Caledonia, red deer can perpetuate the infestation of ranches with ticks where cattle are sympatric by dispersing engorged female ticks that drop in its home range, which includes grazing pastures (Barré et al., 2001; Rodríguez-Vivas et al., 2013; Chevillon et al., 2013).

Like with cattle, acaricides are used in the Mexican tropics intensively to control *R. microplus* infestations on red deer. Products containing MLs like IVM are popular among producers in the region because of their potency and residual activity against susceptible tick populations when used according to label instructions (Rodríguez-Vivas et al., 2013). Resistance to IVM in *R. microplus* infesting cattle in Mexico was reported (Perez-Cogollo et al., 2010a,b); however, efforts to document acaricide resistance in *R. microplus* infesting red deer have not been reported. Anecdotal accounts of failure after treatment of red deer with acaricidal products like those containing IVM prompted this investigation. Thus, experiments were designed to determine the susceptibility of *R. microplus* collected from red deer to acaricides in veterinary products registered and sold to kill this ectoparasite. The implications of the various levels of resistance to OPs, SPs, fipronil, amitraz, and IVM reported here are discussed in the context of efforts for sustainable control of *R. microplus* in the Mexican tropics where diversified livestock production is practiced.

2. Materials and methods

2.1. Study area

The study was carried at a red deer farm located in the tropical region of Mexico (Yucatan State). The state is located between 19°30′ and 21°35′ north latitude and 90°24′ west longitude of the Greenwich meridian. The climate of the state is sub-humid tropical with a summer rainy season. The monthly maximum temperature varies from 35 °C to 40 °C (mean 26.6 °C). The relative humidity (RH) varies from 65% to 100% (mean 80%) and the annual rainfall varies from 415 mm to 1290 mm depending on the area. There are two different seasons: rainy (from June to October) and dry (from November to May) (INEGI, 2002). The state has 4629 cattle farms with a total of 624,488 head of cattle. The predominant livestock-production system is semi-intensive (beef farms), based mainly on year-round grazing on improved pastures, *i.e.* Guinea grass (*Panicum maximum*) and Star grass (*Cynodon nlemfuensis*), with supplementary feeding during the dry season. The use of acaricides to control ticks is a common practice in Yucatan, Mexico (Rodríguez-Vivas et al., 2006a), and acaricide resistance of *R. microplus* collected from cattle to organophosphates (OPs), synthetic pyrethroids (SPs), amitraz (Am) and IVM has been reported (Rodríguez-Vivas et al., 2006a,b, 2007; Perez-Cogollo et al., 2010a,b).

2.2. Background of parasite control management in the farm

The red deer farm has 10 ha divided in 54 individual paddocks, 23 of them with irrigation, each covering 0.8–1.5 ha. Every paddock is divided by a wire fence that is 3 m high. The farm has animal handling facilities used to perform different activities (identification, vaccination, parasite control and general management). Red deer are raised in a semi-intensive system based on year-round grazing of introduced pastures *i.e.* Guinea grass (*P. maximum*), Brizantha grass (*Brachiaria brizantha*),