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Epidemiological survey following oriental theileriosis outbreaks in Victoria, Australia, on selected cattle farms

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ABSTRACT

This study investigated *Theileria orientalis* following outbreaks of oriental theileriosis in cattle in the state of Victoria, Australia, from September 2010 to January 2012, using traditional and molecular methods of diagnosis. A questionnaire was used to collect epidemiological information from cattle farms. Blood samples ($n = 301$), collected from individual symptomatic and asymptomatic cattle from 19 cattle farms, were examined for the presence of *Theileria* on stained blood smears and tested using a PCR-based approach, employing a region within the major piroplasm surface protein (*MPSP*) gene as a marker. The microscopic examination of stained blood smears detected stages consistent with *Theileria* piroplasms in 28.1% (79/281) of the samples. PCR products were amplified from 70.8% (213/301) of the samples. Mutation scanning analysis of all amplicons displayed seven distinct profiles. Following the direct sequencing of representative amplicons, the genotypes *ikeda*, *chitose*, *buffeli* and *type* 5 were detected in 91.1%, 32.9%, 2.4% and 1.4% of 213 blood samples, respectively. The distribution of these four genotypes varied among the 19 farms; genotype *ikeda* was detected on all farms, whereas genotypes *chitose*, *buffeli* and *type* 5 were detected on 14, 3 and 2 farms, respectively. Mix infections with genotypes *ikeda* and *chitose* were common (21.6%). Survey results revealed that oriental theileriosis affected mainly beef cows of more than two years of age, prior to calving, and disease was associated with abortion and cow deaths. Future investigations should focus on developing improved tools for investigating and managing oriental theileriosis.

1. Introduction

Theileriosis is a disease caused by protozoan parasites of the genus *Theileria* (Apicomplexa: Piroplasmida; Theileriidae), transmitted by ixodid ticks. Usually, the geographical distribution of *Theileria* species is restricted to tropical and subtropical regions where suitable tick vectors occur. *Theileria* species infect primarily wild and domestic ruminants, and cause economically significant diseases in cattle, sheep and goats.

For instance, *Theileria annulata* and *Theileria parva* (the causative agents of tropical or Mediterranean and East Coast Fevers, respectively) are known to be the most pathogenic species for bovines, whereas other species, such as *Theileria mutans*, *Theileria taurotragi* and members of the *Theileria orientalis* complex, often cause asymptomatic infections in bovids (Uilenberg et al., 1977; Uilenberg, 1981; Jongejan et al., 1986). However, in the recent years, *T. orientalis* has been linked to clinical cases of oriental theileriosis in the Asia–Pacific region (Sugimoto and Fujisaki, 2002; Izzo et al., 2010; Aparna et al., 2011;

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Islam et al., 2011; McFadden et al., 2011). Oriental theileriosis is associated with high fever, anaemia, jaundice, lethargy, weakness, abortion and/or mortality. The disease is believed to be transmitted by infected ixodid ticks of the genus *Haemaphysalis* (Izzo et al., 2010; McFadden et al., 2011). Since traditional diagnostic (i.e. microscopic and serological) methods do not allow the unequivocal identification of *T. orientalis* to species or subspecies (Sugimoto and Fujisaki, 2002), molecular tools need to be used for specific diagnosis and the genetic characterization of the causative agent/s (Kakuda et al., 1998; Gubbels et al., 2000). Based on DNA sequencing of PCR-amplified regions of the major piroplasm surface protein (*MPSP*) and/or small subunit of nuclear ribosomal RNA (*SSU*) genes, there are at least eight genotypes (designated *chitose* = *type* 1, *ikeda* = *type* 2, *buffeli* = *type* 3 and *types* 4–8) (Kakuda et al., 1998; Gubbels et al., 2000; Ota et al., 2009) within the *T. orientalis* complex. Only the genotypes *ikeda* and/or *chitose* are known to be associated with (pathogenic) oriental theileriosis, whereas other genotypes appear to relate to benign infections in cattle.

In Australia, the first clinical case of oriental theileriosis was reported in 1910 (Seddon, 1952; Stewart et al., 1992). It has been suggested that this form of theileriosis was introduced from Japan to Australia via cattle infested with the *Theileria*-infected ixodid tick, *Haemaphysalis longicornis* (see Stewart et al., 1992). A number of cases of oriental theileriosis, some of which were associated with fatalities, were also recorded in Queensland in the 1960s (Rogers and Callow, 1966). Although there were no further published reports of theileriosis from Australia after 1966, since 2006, there have been theileriosis outbreaks in both beef and dairy cattle, principally in the states of New South Wales (NSW) and Victoria (Izzo et al., 2010; Islam et al., 2011). Genotypes *ikeda* and *chitose* of the *T. orientalis* complex have been linked to theileriosis in these states (Islam et al., 2011; Kamau et al., 2011; Cufos et al., 2012; Eamens et al., 2013). The recent detection of these apparently virulent genotypes of *T. orientalis* in Victoria was suggested to be associated with the introduction of a few cattle from NSW (where the disease is endemic in some regions) to a beef cattle farm near Seymour, Victoria (Islam et al., 2011). Subsequently, Cufos et al. (2012) studied genetic variation in *T. orientalis* populations on the same cattle farm following a theileriosis outbreak, and found that most affected cattle (75% of 84) were infected with the genotype *ikeda*, 4.8% with *chitose*, and 20.2% with both *ikeda* and *chitose*. These studies suggest that these genotypes are propagating in Victorian cattle, previously believed to be free from members of the *T. orientalis* complex.

Oriental theileriosis outbreaks in Victoria 'exploded' from one case in September 2010 to more than 130 affected herds by February 2013. At present, the economic losses caused by theileriosis are difficult to estimate. However, that ~25% of cattle in herds have been clinically affected by this disease indicates a substantial, adverse impact on individual dairy and beef cattle farms in Victoria (Department of Primary Industries, unpublished data). This situation is exacerbated by the surprising lack of availability of any registered vaccine or treatment against this disease in Australia. Although there are major gaps in the knowledge of this disease and its impact, effective molecular diagnostic and analytical tools have been established (Islam et al., 2011; Yokoyama et al., 2011; Cufos et al., 2012; Eamens et al., 2013) to explore the epidemiology of this disease. In the present study, we logically extend previous work (Islam et al., 2011; Cufos et al., 2012) to genetically characterize *T. orientalis* populations from cattle on 19 farms affected by oriental theileriosis outbreaks between 2010 and 2012, employing a mutation scanning-based approach. In addition, we conducted a questionnaire survey to gain insights into some of the epidemiological factors that might be linked to this disease.

2. Materials and methods

2.1. Farms, and the collection of blood samples and epidemiological data

From September 2010 to January 2012, 56 beef and dairy cattle herds were reported to be affected by oriental theileriosis (Department of Primary Industries, unpublished data) (Fig. 1). According to geographic location, we grouped farms into different clusters (A–F), and selected 2–5 herds per cluster to study 19 cattle farms (Fig. 1). From each of these herds, 9–22 cattle were randomly selected for the collection of blood samples (Fig. 1). In addition, two farms in the surrounds of affected farms, with no history of any oriental theileriosis outbreak, were also selected as controls.

Blood samples ($n = 301$) were collected by registered, practicing veterinarians from 19 of 56 farms affected by theileriosis in Victoria at the time. In addition, 28 blood samples were also collected from cattle on two farms that were 2–5 km from affected farms and had no history of an outbreak. Demographic information (including the type of enterprise, herd size, area of grazing land, land adjoining the farms and cattle movement), presence of possible tick vectors and access of wild animals to the farms was collected for 18 of the 19 farms using a questionnaire; farmers or farm managers were interviewed by District Veterinary Officers of the Victorian Department of Primary Industries (DPI).

2.2. Haematological examinations

For each blood sample collected, a thin blood smear was prepared, stained with Giemsa (Weiser, 2012) and examined under oil immersion at 1000-times magnification for the presence of *Theileria* piroplasms (Allison and Meinkoth, 2010). In addition, haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts were determined for each sample using a haematology analyzer (Beckman Coulter AcT Diff, Beckman Coulter Inc., CA, USA). Anaemia was diagnosed if the RBC, PCV or Hb values were $<5.0 \times 10^{12}$/L, 0.24 L/L or 8 g/dL, respectively.

2.3. Molecular testing

2.3.1. Isolation of genomic DNA and PCR amplification

Genomic DNA was extracted from whole blood samples using the DNeasy blood or tissue kit (Qiagen, USA) following the manufacturer's instructions. A region of the *MPSP*