An assessment of Zoonotic and Production Limiting Pathogens in Rusa Deer (Cervus timorensis rusa) from Mauritius

F. Jori¹,²,³, J. Godfroid⁴,⁵, A. L. Michel⁵,⁶, A. D. Potts⁶, M. R. Jaumally⁷, J. Sauzier⁸ and M. Roger¹,⁹

¹ Integrated Animal Risk Management Unit (AGIRs), International Agricultural Research Centre for Development (CIRAD), Montpellier, France
² Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa
³ Department of Animal Science and Production, Botswana College of Agriculture, Gaborone, Botswana
⁴ Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, Tromsø, Norway
⁵ Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa
⁶ Bacteriology Section, ARC-Onderstepoort Veterinary Institute, Pretoria, South Africa
⁷ Division of Veterinary Services, Reduit, Mauritius
⁸ Mauritius Deer Farming Cooperative Society Ltd, Curepipe, Mauritius
⁹ Centre de Recherche et de veille sur les maladies émergentes dans l’Océan Indien (CRVOI), Sainte Clotilde, La Réunion

Keywords:
ruca deer; Mauritius; leptospirosis; tuberculosis; Johne's disease; heartwater; Ehrlichia ruminantium; Brucella spp

Summary

A population of approximately 70 000 rusa deer (Cervus timorensis rusa) represents the most important mammal species reared for food on the island of Mauritius, being the main source of red meat for the local population. However, very limited information is available on the circulation of pathogens affecting the productivity and health of this species. To produce baseline data on the circulation of infectious pathogens in rusa deer under production, a serological survey and/or direct pathogen detection for six selected infectious diseases was undertaken in 2007 in a sample of 53% of the herds reared in semi-free-ranging conditions in hunting estates. Seropositive results were recorded for Johne's disease with an indirect ELISA test (1.7%, n = 351), heartwater with an immunofluorescence antibody test (IFAT) (95.5%, n = 178) and leptospirosis with a Microscopic Agglutination Test (MAT) (25.9%, n = 363). Significant associations were found between seroprevalence to some of the leptospiral serogroups detected (Tarassovi, Pomona, Sejroe and Mini) and age of the animals, animal density or location of the estates (being more prevalent in hotter and more humid areas). In addition, Mycobacterium bovis and M. avium subspecies paratuberculosis were confirmed in two deer carcasses by culture and PCR, respectively. No antibodies against Brucella spp. nor Rift Valley Fever virus were detected with the use of respective indirect ELISA’s. The results obtained suggest that the population of rusa deer from Mauritius is exposed to a wide range of pathogens which may affect their productivity. In addition, the results highlight the potential public health risks incurred by deer industry workers and consumers. This survey fills an important gap in knowledge regarding the health of tropical deer meat in Mauritius and justifies the need to implement more regular surveys of selected pathogens in the deer population.

Introduction

As human population increases, there is a greater need for food and diversified protein production. Some regions of the world, unsuitable for conventional livestock production, have developed breeding systems for the production of wildlife species as an alternative source of protein, particularly in developing countries (Chardonnet et al., 2002). In
this manner, a wide range of non-conventional species in the process of domestication are being bred for food in tropical regions of Asia (Shi and Hu, 2008; Brooks et al., 2010), Africa (Jori et al., 2005) and Latin America (Jori, 2001; Nogueira and Nogueira-Filho, 2011). However, scientists and farmers are confronted with limited knowledge on these production systems, productivity parameters and the pathogens to which they are exposed (Jori et al., 2001, 2005; Mayor et al., 2006). This is of concern as some pathogens can seriously impact their productivity. Moreover, 60% of emerging diseases are of zoonotic importance (Jones et al, 2008) and among the emerging pathogens identified in humans, 72% have wildlife species involved in their dissemination and/or maintenance (Taylor et al., 2001). Therefore, it appears logical and necessary to increase the surveillance of circulating pathogens among high-density wildlife populations reared for production of red meat, in order to identify potential zoonotic risks and production-limiting diseases.

The case of rusa deer (Cervus timorensis russa) production in Mauritius is a good example: Mauritius is a tropical island of 2 045 km² situated in the south-west Indian Ocean, 800 km east of Madagascar. In 2005, its human population was estimated at 1.24 million inhabitants (Puchhoa and Boodhoo, 2008). Conventional domestic animal production is very limited, particularly for mammals and the main source of red meat is provided by a comparatively large number of rusa deer raised under intensive or extensive production systems. The rusa deer is a tropical species originating from Indonesia, which has been introduced in many countries in the Indian Ocean, the Pacific (Australia, Mauritius, New Caledonia, New Zealand, Papua New Guinea and Reunion island) where it is utilized for food and hunting, although on different scales (Owen, 1977; Barré et al., 2001; Chardonnet et al., 2002). Introduced into Mauritius in the 16th century, rusa deer adapted very well to local ecological conditions and currently form part of the national cultural heritage. For approximately four decades, deer farming has been widespread in different ecosystems of the Mauritian territory, with more than 70 000 animals used as reproductive stock. The annual production in 2007 reached 550 tons of venison and it is expected to reach 600 tons by 2015. Ninety percent of this production comes from culling operations of semi-free-ranging deer herds in extensive farms during the hunting season (between 1st June and 30th September), while 10% is produced in intensive farms during the rest of the year. Around 20 000 people are directly or indirectly involved in the deer sector. In addition, venison consumption is widespread with a per capita consumption of 0.44 kg per annum (Puchhoa and Boodhoo, 2008), due to its affordable price and also due to the fact that venison consumption is not subjected to any religious or cultural barriers.

To date, despite the wide but scattered distribution of rusa deer on islands in the Pacific and Indian Oceans, limited information exists on the prevalence of infectious diseases within rusa deer populations in Mauritius and other countries. The limited information available including the detection of Mycobacterium bovis (Sibartie et al., 1983) and some clinical cases of heartwater (Poudelet et al., 1982) is now outdated or concerns only specific ectoparasites (Owen, 1977; Barré et al., 2001) and more recently orbiviruses (Jori et al., 2011).

Considering the high number of human consumers and the scarcity of data available on diseases affecting rusa deer, this research aimed to provide baseline data on zoonotic or production-limiting pathogens circulating in rusa deer in extensive farms on Mauritius. Disease surveillance performed routinely by the Mauritian Veterinary Services is based only on monitoring clinical cases and no budget is allocated for continuous monitoring based on laboratory tests.

Even though a large number of bacterial, viral or prion diseases can affect the health of deer species (Haigh et al., 2002; Mackintosh et al., 2002), the choice of the monitored diseases was based on previous knowledge of circulating pathogens that have an impact on deer productivity, livestock production or public health and are widespread among the Indian Ocean islands. Bovine tuberculosis has been previously described in deer from Mauritius (Jaumally and Sibartie, 1983) and is an important disease in deer reared in high densities worldwide and is a potential zoonosis (De Lisle et al., 2001; Mackintosh et al., 2002; Gortazar et al., 2006; O’Brien et al., 2006).

Leptospirosis, considered one of the most widespread and under-reported zoonoses worldwide (Bharti et al., 2003; Jobbins et al., 2013), is common in many tropical islands (Desvars et al., 2011; Desvars et al., 2011). Commonly reported and widespread in the deer populations farmed in New Zealand, it causes important production losses (Ayanegui-Alcérrec et al., 2010; Subharat et al., 2011) and cases have been reported in personnel in the deer farming industry (Ayanegui-Alcérrec et al., 2007). Brucellosis is an important zoonotic disease and common in free-ranging deer populations worldwide (Mackintosh et al., 2002; Munoz et al., 2010; Serrano et al., 2011; Nymo et al., 2013). Even though brucellosis has been eradicated in domestic animals from Mauritius, the status of this disease has never been assessed in the rusa deer population.

Paratuberculosis or Johne’s Disease (JD), caused by M. avium subsp. paratuberculosis (MAP), is not common in free-ranging deer and is only found occasionally in areas with significant numbers of domestic ruminants (Balseiro et al., 2008; Nebbia et al., 2000). Nevertheless, the disease is considered the most economically important infectious disease in deer species reared for venison worldwide.
Heartwater is a septicaemic disease caused by *Ehrlichia ruminantium* and is transmitted by several species of ticks from the genus *Amblyoma*, *Amblyoma variegatum* being the predominant species. White-tailed deer (*Odocoileus virginianus*), Fallow deer (*Dama dama*) (Dardiri et al., 1987) and Rusa deer are the only species of deer known to be susceptible to heartwater, and some fatal clinical cases have been reported in Mauritius (Poudelet et al., 1982; Peter et al., 2002). Finally, Rift Valley fever, a severe emerging zoonosis, has been recently detected in some Indian Ocean countries such as Madagascar and the Republic of Comoros (Andriamandimby et al., 2010; Roger et al., 2011).

Based on these choices, a serological survey screening for five infectious diseases having an impact on livestock production or public health (leptospirosis, JD, brucellosis, heartwater and RVF) was undertaken between April and December 2007. During the same period, veterinary inspections were performed on 500 deer carcasses to detect pathological lesions compatible with BTB or JD.

**Materials and Methods**

**Study area**

Mauritius benefits from a tropical climate, with an annual rainfall ranging between 200 and 2400 mm and an average temperature ranging between 23°C and 28°C. Altitude ranges from sea level up to 850 m in the south and influences the temperature and rainfall of the island (Nigel and Rughooputh, 2009).

About 93% of this land is dedicated to sugar cane production. Deer farms are mostly located in the private forested estates from the higher central areas of the island, ranging between 400 and 800 m above sea level, where rainfall is abundant. Most farms are registered at the Mauritian Meat Producers Association (MMPA). At the time of the study, the latest MMPA census estimated the total population of farmed deer at 70 000. Extensive deer farming accounts for 90% of the deer population of the Island which is distributed in 60 estates with a total surface area of approximately 24 000 hectares. Deer populations in these ranches are reared in free-ranging conditions. They are seldom handled, are not individually identified and the composition and structure of the herd is unknown. Deer in these extensive farms are mostly harvested during the hunting season, between the 1st of June and the 30th of September. Most of the hunted deer are more than 1-year old and generally males. The stocking rates for deer in extensive systems range between 1 deer per hectare and 3.3 deer per hectare in estate lands. In addition to the deer, a significant population of free-ranging feral pigs (*Sus scrofa*) has also developed in the vast majority (more than 90%) of rusa deer hunting grounds, and is also utilized for hunting purposes, although on a lower scale.

Domestic livestock census figures in Mauritius are limited but the following are available: 7 000 cattle, 24 000 goats, 1500 sheep and 16 000 pigs (CSO, 2010). Indeed, local production of domestic ruminants is limited in Mauritius and most livestock is imported from South Africa or East Africa and slaughtered after a fattening period of a few months in Mauritius. Therefore, rusa deer represent the most abundant ruminants under production in the island and have become the main source of red meat locally produced for the Mauritian population.

**Animal sampling**

The sampling approach was designed to detect the presence or absence of selected pathogens. An estimated population of 45 959 animals, distributed within 52 extensive ranches, provided by the MMPA was used to determine the number of animals to be sampled. To detect a seroprevalence ≥ 1% with 5% of error, a total of 299 animals were chosen randomly, using a random function from Excel. Considering an average sensitivity of 80% in the diagnostic tests, this sample size was increased to 363 animals from 28 extensive ranches (Fig. 1 and Table 2).

Animals were sampled out of a pool of animals culled for meat production. Sampling order was opportunistic and farms were chosen in order to adapt to the agenda of culling operations between June and July 2007. The average number of deer sampled per ranch was 13 animals [median 6, inter-quartile range (4; 16.25)] and the distribution of the 28 sampled ranches in Mauritius can be seen in Fig. 1. Median altitude in those ranches was 169 m, IQR (20; 284), and median deer estimated density was 2.56 individuals/km² IQR (0.6; 3.1). The details of sex and age distribution of the sampled animals are given in Table 2. Animals were sampled after being shot. When the carcass was hung up for evisceration, blood was collected from the thoracic cavity with a 20 ml sterile syringe and subsequently aliquoted in sterile 10 ml tubes. All blood samples were then centrifuged at 109 564 × g for 15 min. Serum samples were pipetted into cryotubes and stored at −20°C until analysis.

**Sample classification**

Animals older than 15 months were considered adults and below that age were considered as young. The proportion of age and sex in the samples is summarized in Table 1.

Estates were classified according to the density of the animals, temperature and rainfall. Temperature and rainfall in every location were determined based on rainfall and temperature distribution described in the literature (Nigel and
Density was calculated as the estimated number of animals divided by the surface area of the property. Densities of deer above 2 individuals/km² were considered as high (14 estates encompassing 191 individuals) and below that value were considered as low (172 deer from 14 estates). Climatic characteristics were estimated on the basis of average rainfall and location (coast or highlands) of the different areas of the island where the sampled estates are located. Twenty three estates encompassing 212 deer were classified as being in a humid environment (rainfall higher than 1200 mm) while five estates with 151 individuals were located in dry areas of the island where annual rainfall was below the 1200 mm rainfall threshold (Fig. 1).

Equally, eight estates were located on the hotter coastal areas below 150 m above sea level (n = 221 deer) while 20 estates (n = 142 animals) are found on the central highlands at altitudes ranging between 151 to 653 m above sea level (Fig. 1).

Serological analysis

All of the 363 sera were sent to South Africa (Onderstepoort Veterinary Institute (ARC-OVI) and University of Pretoria) and stored at −20°C until analysis. The sera were tested for five different diseases common in deer species under production or prevalent in the Indian Ocean region. The number of animals tested varied, depending on the resources and the volumes of sera available. Table 2 presents the number of sera tested for each disease and Table 1 the serological tests employed. For leptospirosis, a total of 363 sera was analysed using the Microscopic Agglutination Test (MAT) to detect antibodies against eight serovars (strains) belonging to eight serogroups (in brackets): *L. bratislava* (Australis), *L. canicola* (Canicola), *L. grippotyphosa* (Grippotyphosa), *L. icterohaemorrhagiae* (Icterohaemorrhagiae), *L. szwajizak* (Mini), *L. pomona* (Pomona), *L. hardjo* (Sejroe) and *L. tarassovi* (Tarassovi). For all serovars tested titers $\geq 1/100$ were considered positive (Faine, 1994).

A total of 351 sera was tested for antibodies against JD using a commercial indirect ELISA test (Pourquier® Laboratories, Montpellier, France) using a protoplasmic extract of *Mycobacterium avium paratuberculosis* (MAP) and a Protein G- horse radish peroxidase-labelled conjugate (Pierce Biotechnology, Rockford, IL 61105, USA), (Godfroid et al., 2000). This ELISA has been used in a previous study on deer species in Canada (Pruvot et al., 2013).

A total of 355 sera was tested for brucellosis at the Faculty of Veterinary Science, University of Pretoria using a commercial indirect ELISA (Pourquier® Laboratories) designed for the detection of *Brucella abortus* in cattle.
This test has a high specificity and sensitivity in livestock and is able to detect mainly IgG antibodies (OIE, 2008a; Godfroid et al., 2010). Indirect ELISA’s have been used to screen for brucellosis in populations of other deer species in Spain (Munoz et al., 2010) and Scandinavia (Nymo et al., 2013). In addition, in order to detect potential circulation of IgM indicative of recent infection, ninety-nine randomly chosen sera were tested in the Rose Bengal test (RBT).

Analysis for antibodies against heartwater (Ehrlichia ruminantium) was performed on 178 sera originating from farms in coastal areas, at the Parasitology laboratory of the ARC-OVI, using an in-house immunofluorescence antibody test -IFAT- (Yunker et al., 1988; OIE, 2008b). In this case, the number of sera was limited to the eight ranches (n = 178 animals) from the coastal area known to be a predilection site for Amblyomma variegatum. The sample of 178 sera from estates located in hotter areas included 59 animals with ticks attached to the carcass. Dilutions at 1/40 or higher were considered positive for the presence of Ehrlichia ruminantium antibodies.

Analysis for antibodies against RVF IgG was performed at the Virology laboratory of the ARC-OVI using an in-house indirect ELISA (Paweska et al., 2003) on 88 sera, Table 1.

<table>
<thead>
<tr>
<th>Table 1. The serological tests performed on rusa deer serum samples</th>
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<tbody>
<tr>
<td>Agent (group)</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Leptospira interrogans</td>
</tr>
<tr>
<td>Serogroup tested:</td>
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<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mycobacterium avium paratuberculosis</td>
</tr>
<tr>
<td>Brucella abortus</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Ehrlichia ruminantium</td>
</tr>
<tr>
<td>RVFV (Phlebovirus)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Seroprevalence, gender and age distribution of deer tested per disease</th>
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<tbody>
<tr>
<td>Leptospira interrogans</td>
</tr>
<tr>
<td>Sera collected</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Young</td>
</tr>
<tr>
<td>Adult</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

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randomly chosen from the original pool of collected sera and representing 10 different herds.

**Post-mortem inspection and tissue sampling**

During the same culling operations used for serological sampling, veterinary inspections were performed on a total of 500 deer carcasses to identify nodular or granulomatous lesions indicative of BTB or JD. When lesions were found, samples of affected tissue and lymph nodes located in proximity to the lesions were removed, and stored on ice for bacterial culture. Additional tissue samples from the same lesions were also preserved in a 10% buffered formalin solution for histopathological examination.

**Bacterial culture and PCR assays**

Suspect tissue samples were cultured for bovine tuberculous. Species identification of mycobacterial isolates was performed by PCR as reported previously (Bengis et al., 1996; Alexander et al., 2002). IS900 PCR amplification of JD was performed on DNA extracted from sections of formalin-fixed, paraffin embedded tissues as described previously (Sethusa, 2006).

To attempt isolation of leptospires, twenty samples were collected from carcasses originating from two different farms with higher densities (10 individuals per farm). A small sample (±1 g) of renal tissue was removed from deer at post-mortem and immediately placed in the tubes containing EMJH semisolid media with five fluorouracil (0.5 mg per ml) as a selective media. The tubes were then sent to the Bacteriology Laboratory at ARC-OVI for further culturing (at 29°C ± 1°C). Approximately 1 ml from the original tube was transferred to fresh media after 2 days. Growth of leptospires in the tubes was monitored weekly by viewing a small sample under the microscope (darkfield).

**Statistical analysis**

All statistical analysis was performed with Epi-Info v.3.5.3, 2011 (CDC, Atlanta, USA). Seroprevalence for *Leptospira* spp. serogroups, heartwater and JD were reported as percentages and 95% confidence intervals. Associations between seropositivity to leptospiral antibodies and age, sex, density, local rainfall and geographic location were tested with the chi square test calculations for homogeneity of two populations (Fischer exact test). Values of *P* < 0.05 were considered significant.

**Results**

Serological results for the different pathogens assessed are summarized in Table 2.

**Leptospirosis**

Ninety-four of 363 free-ranging deer (25.9%) showed titers ≥1/100, against anti-leptospiral agglutinins which were present in 71.4% (20/28) of herds (with at least one positive response to one of the serovars tested). Individual prevalences and distribution by age and sex are given in Table 3. Among all the positive responses, the most representative serogroups were Tarassovi [36.1%, *n* = 39, 95% CI (7.6; 13.9)], Pomona [27.8%, *n* = 30, 95% CI (5.4; 11.1)], Sejroe [16.7%, *n* = 18, 95% CI (2.7; 7.2)] and Mini [14.8%, *n* = 16, 95% CI (2.3; 6.5)]. Two animals were positive to serogroups Grippotyphosa and Canicola [1.9%, 95% CI (0.0; 1.3)] and one to serogroup Icterohaemorrhagiae [0.9%, 95% CI (0.0; 0.8)]. The serogroup Australis was not detected. The highest titers detected were for the serogroups Tarassovi (3 200), Pomona and Sejroe (1 600). Two serogroups were detected for ten animals and three serogroups for two animals (same herd).

The median prevalence observed in these 20 estates was 32.8% IQR (11.8; 50.0).

When merging all serogroups together, seroprevalence was higher in older animals and estates located in the hotter coastal region. Significant associations were also found between serogroup Tarassovi, coastal location and age. Density, location and rainfall of the different estates were significantly associated with some of the serogroup tested (Table 4).

Some growth was observed in the samples of renal tissue collected. However, contamination of the samples prevented the growth of purified cultures of leptospires and subsequent identification of leptospiral strains.

### Table 3. Prevalence of anti-leptospiral agglutinins per sex and age of deer tested

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Young</th>
<th>Adult</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Tarassovi</td>
<td>7</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>(6.2)</td>
<td>(6.2)</td>
<td>(17.2)</td>
</tr>
<tr>
<td>Pomona</td>
<td>5</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>(4.4)</td>
<td>(11.1)</td>
<td>(10.4)</td>
</tr>
<tr>
<td>Sejroe</td>
<td>6</td>
<td>5.3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>(6.4)</td>
<td>(8.3)</td>
<td>(6.4)</td>
</tr>
<tr>
<td>Mini</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(0.7)</td>
<td>(0.7)</td>
<td>(6.7)</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(0.7)</td>
<td>(0.7)</td>
<td>(0.7)</td>
</tr>
<tr>
<td>Canicola</td>
<td>1</td>
<td>0.9</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(0.7)</td>
<td>(0.7)</td>
<td>–</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(0.7)</td>
<td>(0.7)</td>
<td>(1.6)</td>
</tr>
<tr>
<td>Australis</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>17</td>
<td>44</td>
</tr>
<tr>
<td>%</td>
<td>18.6</td>
<td>27.0</td>
<td>32.8</td>
</tr>
<tr>
<td>IC 95%</td>
<td>(14.6)</td>
<td>(22.4)</td>
<td>(28.0)</td>
</tr>
<tr>
<td></td>
<td>(22.6)</td>
<td>(31.5)</td>
<td>(37.7)</td>
</tr>
</tbody>
</table>
Table 4. The P values of the associations between anti-leptospiral agglutinins to different serogroups and various characteristics of deer and hunting estates. Grey shading indicates P values < 0.05

<table>
<thead>
<tr>
<th>Total serogroups,</th>
<th>Tarassovi, n = 94</th>
<th>Pomona, n = 36</th>
<th>Sejroe, n = 18</th>
<th>Mini, n = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.044</td>
<td>0.0003</td>
<td>0.7</td>
<td>0.35</td>
</tr>
<tr>
<td>Sex</td>
<td>0.47</td>
<td>0.13</td>
<td>0.34</td>
<td>0.54</td>
</tr>
<tr>
<td>Density</td>
<td>0.08</td>
<td>0.43</td>
<td>0.008</td>
<td>0.04</td>
</tr>
<tr>
<td>Location</td>
<td>0.0001</td>
<td>0.4</td>
<td>0.002</td>
<td>–</td>
</tr>
<tr>
<td>Climate</td>
<td>0.52</td>
<td>0.01</td>
<td>0.53</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Johne’s disease

Antibodies against *M. avium* subsp. *paratuberculosis*, the etiological agent of JD, were detected in 1.7% of the sera tested [6/351; 95% CI (0.3; 3.1)] representing 14.8% of the herds assessed (4/27). Of the six positive animals, four were adult males and two young males. In addition, post mortem examination revealed one intestinal lesion in a female deer with symptoms of emaciation and diarrhoea, originating from an extensive farm. Infection with JD was confirmed by IS900 PCR performed on DNA extracted from tissue sections from the pathological sample. The ELISA result for this animal was negative.

Heartwater

Antibodies against *E. ruminantium* were detected in 95.5% [n = 170, 95% CI (92.5, 98.5)] of the 174 sera tested in coastal estates located at a maximum of 150 m above sea level. All the herds tested (n = 8) were found positive. The median altitude in those farms was 31.5 m above sea level, IQR (3; 100). No associations were found between seropositivity to *E. ruminantium* and any of the factors tested.

Brucellosis

No antibodies against *Brucella* spp were detected in the samples of animals tested.

Rift Valley fever

No antibodies against Rift Valley fever virus were detected in the samples of animals tested.

Bovine tuberculosis

During veterinary meat inspection, nodular lesions suggestive of tuberculosis were detected in the lymph nodes and lungs of one adult male deer. *Mycobacteria* spp. was isolated from the lung of this animal and confirmed as *Mycobacterium bovis* by PCR.

Discussion

Animal species, including wildlife species, when reared in captivity and at high densities are predisposed to a limited genetic diversity that can facilitate the circulation or emergence of unexpected pathogens. Several cases illustrate this phenomenon such as the circulation of avian influenza viruses in ostriches in South Africa (Thompson et al., 2008) or the occurrence of rabies outbreaks in kudu populations reared for hunting in Namibia (Mansfield et al., 2006). In the case of deer herds, management activities leading to a high density of individuals facilitates the circulation and spread of BTB (Miller et al., 2003; Vicente et al., 2007). For many such species, knowledge about the pathogens they harbour and to which they are exposed is often limited. A well-documented example of the potential risk of captive wildlife in facilitating the emergence and spread of zoonotic diseases is the role that palm civet (*Paguma larvata*) farming played in the replication of the SARS coronavirus before its transmission from bats to humans (Li et al., 2006; Shi and Hu, 2008).

In Mauritius, rusa deer represent the largest population of large mammals present in the island, reared at high densities and with regular contact with humans, sometimes under intensive conditions. This descriptive study is the first and most comprehensive health survey reported to date on rusa deer, and the results provide information on the circulation of pathogens that may have an impact on public health and animal production.

The selection of farms was not exhaustive but provided a good spatial and numeric representation of the total number of extensively farmed estates (Fig. 1). Sampling of animals on every farm was opportunistic and did not take into account the clustering of animals. This design did not provide quantitative prevalence data allowing conclusions to be drawn on the dynamics of the diseases studied at a national level. This study should be considered as a preliminary study which provides data on some of the potential pathogens affecting the productivity and health of farmed deer populations in Mauritius. One of its major weaknesses is that this survey was limited to 1 year and due to budget constraints, a follow up on those results has not been undertaken to date. However, the results provided in this study should serve to raise awareness among animal and public health stakeholders on the need to carry out regular monitoring studies and surveillance among deer populations under production and the personnel working for the deer industry.

In most cases, the diagnostic tests used are derived from veterinary tests used in livestock and have never been validated in the rusa deer. However, with the exception of the IFAT test for the detection of antibodies against *E. ruminantium* and the I-ELISA test used for RVF, most of the tests...
have been used in other deer surveys and were shown to be suitable for the detection of the pathogens chosen. This has been the case for leptospirosis (Ayanegui-Alcerreca et al., 2007; Ayanegui-Alcérreca et al., 2010), JD (Reyes-García et al., 2008; Boadella et al., 2010; Munoz et al., 2010; Nymo et al., 2013) and brucellosis (Colby et al., 2002; Medrán et al., 2012; O’Brien et al., 2013; Pruvot et al., 2013).

This survey documents the first serological report of animal leptospirosis in Mauritius, with seroprevalence values of 25.9% (n = 363) for individuals and more than 70% for deer herds tested. Prevalence was significantly higher in older animals. Equally, estates with higher density of animals or exposed to a higher rainfall or temperature (coastal areas) were significantly more affected (Table 4). These results suggest that the disease is fairly widespread in deer farms from Mauritius, particularly in estates located in more humid and hot locations. A positive correlation between seroconversion to serovars Hardjo-bovis and Pomona and humidity has been reported in cattle in Australia and New Zealand (Subharat et al., 2012). Intensive deer farms with higher densities are also likely to be more susceptible. The economic cost of human and animal leptospirosis in tropical islands is not negligible. In New Zealand, leptospirosis is known to cause mortality, reproductive failure and production losses in deer herds (Ayanegui-Alcerreca et al., 2007). Hardjo, Tarassovi and Pomona serovars found in this study have all been detected seroprevalence was exceptionally high (above 95.5% /170/178). Despite the fact that some clinical cases have occasionally been described (Poudelet et al., 1982), clinical disease is not commonly reported by deer farmers in Mauritius. This is due to the fact that most deer farms are in the central and higher areas of the Island where tick populations are less prevalent. Another hypothesis is that rusa deer could have acquired some form of natural resistance to E. ruminantium. A high tolerance to other blood parasites such as Trypanosoma evansi has been reported in the past in rusa deer (Reid et al., 1999). Indeed, the high seroprevalence observed suggests a possible enzootic stability which could be explained by repeated exposure of the deer population to ticks hosting the parasite. Infestation with this parasite induces severe disease in domestic ruminants and therefore, although numbers of domestic ruminants are scarce, the presence of heartwater in deer farms represents a potential a risk for the more sensitive ruminant population living in areas adjacent to deer ranches.

BTB is a major disease of deer species in the wild (Gortazar et al., 2007; Corn et al., 2010) and in captivity (De Lisle et al., 2001; Mackintosh et al., 2002; O’Brien et al., 2006). In Mauritius, the circulation of BTB was described 30 years ago in bovine herds (Jaumally and Sibartie, 1983). In the same year, a generalized case was described for the first time in the free-ranging deer population (Sibartie et al., 1983). It is not known whether BTB originally spread from cattle to deer or vice versa, as both species may maintain the disease (Renwick et al., 2007). However, considering the low numbers of cattle in Mauritius, the isolation of M. bovis from one suspected case in our study strongly suggests that the disease is still circulating in the deer population. During a health survey conducted in Mauritius in free-ranging macaques (Macaca fascicularis) in 2005, M. bovis was isolated from five individuals, suggesting that it could
be more widespread in free-ranging wild animals from Mauritius than currently known. Against this background, the significant feral pig population present in 90% of Mauritius’ hunting grounds should be considered at risk for spillover of *M. bovis* from infected deer herds. Indeed, in intensive hunting estates from the Iberian Peninsula with high densities of red deer (*Cervus elaphus*) and wild boar, both species have been shown to become infected with BTB result in high prevalences (Vicente et al., 2006). In these settings, wild boar can become infected by consuming deer carcasses and inter-species BTB transmission can also occur when both species aggregate at water and feeding sites (Vicente et al., 2007).

This is the first time that *M. avium* subsp. *paratuberculosis* has been serologically detected (1.7%, *n* = 351) and confirmed by PCR in rusa deer and provides evidence that the deer population from Mauritius is exposed to this pathogen. In addition, the infection seems to be fairly widespread in the deer herds in Mauritius (15% of the sampled herds affected). As serological methods do not seem to be very sensitive in deer populations (Marco et al., 2002; Woodbury et al., 2008), the apparent prevalence observed in our sample is likely to be underestimated, as suggested by the PCR positive but seronegative individual. Although evidence for its zoonotic potential is not strong, similarities between Johne’s disease (JD) in cattle and Crohn’s disease in humans cannot be ignored and deserve further research (Waddell et al., 2008). In addition, as is the case in New Zealand, JD infections could cause substantial production losses in Mauritian deer. To establish a surveillance programme in the future, and considering the low performance of serological tests in cervids, post mortem examination at the abattoirs and subsequent culture and histopathological examination should be the method of choice for monitoring this disease in the deer population farms (Reyes-Garcia et al., 2008).

Carta et al., 2013 reported serological cross-reactivity when detecting antibodies to *M. bovis* and *M. avium* subsp. *paratuberculosis* (MAP), respectively, which complicated the diagnosis of JD. In our study, deer sera were only tested for MAP and not for *M. bovis*, but the very low seroprevalence of 1.7% detected in the MAP ELISA suggested that cross-reactivity with regard to BTB was not a major problem in Mauritius. In addition, the inspection of 500 deer carcasses yielded lesions typical for BTB and JD in only one animal, supporting the hypothesis that the likelihood for cross-reactivity of *M. bovis* infected deer in the MAP ELISA was probably extremely small.

Mauritius has been reported to be free of brucellosis since 1981, following a successful vaccination programme (http://www.oie.int/wahis_2/public/wahid.php/Country-information/Animalsituation). It is known that in the absence of infection in domestic animals, other deer species are unable to maintain brucellosis and the disease tends to disappear from free-ranging deer populations (Serrano et al., 2011). The indirect ELISA shows the best sensitivity estimates of all the available brucellosis serological tests and therefore the ELISA is the test of choice for this type of study (Godfroid et al., 2010). The combination of the RBT and the indirect ELISA suggests that there was no circulation of IgG or IgMs in our sample. The absence of acute and chronic infections with *Brucella* spp. combined with the lack of historical evidence of brucellosis in Mauritius strongly suggests the absence of *Brucella* spp. in the deer population from Mauritius.

This work presents the first serological investigation of the circulation of Rift Valley fever in Mauritius. The results suggest that the virus has not been in contact with the rusa deer population sampled, despite potential vectors of the disease which are present in the Mauritian territory (M. Roger, personal communication). These results should be interpreted with caution because the sampled population was limited (88 individuals from 10 different herds) and the I-ELISA test has never been validated in deer species. Considering that periodic outbreaks are known to occur in East Africa and several outbreaks have been reported in neighbouring countries from the Indian Ocean region in recent years (Andriamandimby et al., 2010; Roger et al., 2011), surveillance of this disease should be encouraged in areas where potential mosquito vectors are known to occur.

**Conclusion**

These preliminary results from a representative but non-exhaustive survey suggest that the rusa deer population is exposed to three out of the six pathogens screened (leptospirosis, JD and heartwater). In addition, we found evidence of infection for two of the pathogens (BTB and JD). These results should be used as baseline data for future studies when financial opportunities become available. Considering the high numbers of deer reared in Mauritius and their national importance as a source of red meat, this species can act as a reservoir or an amplifying host for some circulating pathogens which can have an impact in other domestic animals and humans. Considering the reduced numbers of domestic ruminants in Mauritius, their possible economic impact in other livestock production systems is limited. However, from the public health perspective, awareness should be raised concerning the potential occupational hazard incurred by persons involved in animal husbandry, hunting and slaughter activities (Ayanegui-Alcerreca et al., 2007; Wilkins et al., 2008). In all cases, the importance of venison production for the local market and the large number of personnel involved in the deer meat industry justify the need to monitor the health of...
commercial semi-free and captive deer populations (and other wildlife species bred for human consumption such as feral pigs) more regularly and closely. It is critical that epidemiological data are regularly collected in a joint effort between the deer farming industry, the national veterinary services and public health institutions, to quantify more accurately the dissemination and potential impact of these pathogens at a national level.

Acknowledgements

We thank the staff of the Animal Health Laboratory, Division of Veterinary Services, for the indispensable assistance during field work. We also thank all the herders and hunters for having welcomed us during the hunting party. This project was a part of FSP EPIREGEN project supported by the French Ministry of Foreign Affairs.

Conflict of interest

The authors disclose any commercial associations that might create a conflict of interest in connection with the submitted manuscripts and declare that competing financial interests do not exist.

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