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Molecular characterization of *Cryptosporidium* species at the wildlife/livestock interface of the Kruger National Park, South Africa

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ABSTRACT

Molecular characterization of *Cryptosporidium* spp. was done on isolates from African elephant (*Loxodonta africana*), African buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*) and native domestic calves collected during May and June 2008 at the wildlife/livestock interface of the Kruger National Park (KNP), South Africa. A polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) analysis of the 18S rRNA gene was used in feces from 51 calves (3–12 months of age), 71 buffalo, 71 impala and 72 elephant, and sequencing of the 18S rRNA gene was done on PCR-RFLP-positive wildlife samples. *Cryptosporidium* spp. were detected in 8% (4/51) of the calves and identified as *C. andersoni* (2/4) and *C. bovis* (2/4). Four of the 214 wildlife samples were positive for *Cryptosporidium* with a prevalence of 2.8% each in impala and buffalo. *Cryptosporidium ubiquitum* was detected in two impala and one buffalo, and *C. bovis* in one buffalo. A concurrent questionnaire conducted among 120 farmers in the study area investigated contacts between wildlife species and livestock. Buffalo and impala had the highest probability of contact with cattle outside the KNP. Despite the fairly low prevalence found in wildlife and cattle, the circulation of zoonotic *Cryptosporidium* spp., such as *C. ubiquitum*, should be investigated further, particularly in areas of high HIV infection prevalence. Further studies should target younger animals in which the prevalence is likely to be higher.

1. Introduction

Cryptosporidium spp., protozoan parasites of the phylum Apicomplexa, have a wide spectrum of hosts including humans, domestic animals and wild mammals, birds, reptiles, amphibians and fish [1]. Cryptosporidiosis is a common cause of diarrhea in humans and animals, but is usually self-limitingin the immunocompetent host. However, in young or immunosuppressed hosts, such as HIV-infected patients, the parasite can cause severe and life-threatening diarrhea [26]. Humans can acquire *Cryptosporidium* infections via several transmission routes, such as direct contact with infected persons (person-to-person transmission), animals (zoonotic transmission), or contaminated fomites, or ingestion of contaminated food (foodborne transmission) or water (waterborne transmission) [2]. The role of animals in the transmission of human cryptosporidiosis is nevertheless not clear [1]. This is largely due to the fact that traditional diagnostic tools do not have the ability to differentiate between human-pathogenic and non-human-pathogenic species. Recently, molecular tools have been developed to detect and differentiate *Cryptosporidium* spp. at species/genotype and subtype level [3,4]. These tools have contributed to a

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better understanding of the transmission of cryptosporidiosis in humans and animals [2]. In a recent survey in four hospitals from South Africa, *Cryptosporidium* was detected in 12.2% of children with diarrhea. However, most of the identified strains were found to be non-zoonotic (*C. hominis* and *C. parvum*) [5]. At least 10 species (*C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. cuniculus*, *C. suis*, *C. muris*, *C. andersoni*, and *C. ubiquitum*) have been associated with human disease, although *C. hominis* and *C. parvum* remain the most common species [2,6].

Cattle are considered to be an important source of zoonotic cryptosporidiosis, since they are regarded as a major host of *C. parvum*. However, cattle are also commonly infected with *C. andersoni*, *C. bovis*, the deer-like genotype and *C. ryanae* and the occurrence of these different species is usually related to the age of the host: *C. parvum* is mostly found in pre-weaned calves, *C. ryanae* in post-weaned calves and *C. andersoni* in yearling and adult cattle [7]. However, *C. bovis* and the deer-like genotype have been detected in pre- and post-weaned calves and adult cattle of all ages, which suggests that the occurrence of these species may not be age-related [8]. A species similar to *C. bovis* was also detected in one adult yak (*Bos grunniens*) in China and genotyping revealed only three nucleotide mutations in the target gene [8]. Thus, cattle and yaks are the only animal species in which *C. bovis* has been reported.

Cryptosporidium spp. have commonly been reported in wild mammals worldwide. Using microscopy, *Cryptosporidium* oocysts have been detected in feces of African buffalo (*Syncerus caffer*), zebra (*Equus quagga*) and wildebeest (*Connochaetes taurinus*) in Mikumi National Park, Tanzania [9], and more recently in African buffalo, impala (*Aepyceros melampus*) and elephant (*Loxodonta africana*) in Kruger National Park, South Africa [17]. Recent molecular studies have described 11 *Cryptosporidium* species and nearly 30 genotypes of unknown species status in wildlife [10]. Some of the *Cryptosporidium* spp. detected in wild or captive mammals include *C. parvum*, *C. muris*, *C. canis*, *C. meleagridis*, *C. bovis*, *C. andersoni*, and *C. hominis*. Genotyping studies have mostly been done in captive artiodactyls and the following *Cryptosporidium* spp. have been found: *C. parvum*in red deer (*Cervus elaphus*), fallow deer (*Dama dama*), addax (*Addax nasomaculatus*), Arabian oryx (*Oryx leucoryx*), gemsbok (*Oryx gazella*) and sable antelope (*Hippotragus niger*); *C. ubiquitum* in sika deer (*Cervus nippon*), blesbok (*Damaliscus pygargus phillipsi*), nyala (*Nyala angasii*) and ibex (*Capra sibirica*); and *C. andersoni*in European wisent (*Bison bonasus*) [10]. The white-tailed deer (*Odocoileus virginianus*) is the only free-ranging artiodactyl in which genotyping has been done and *C. parvum*, *C. ubiquitum* and the deer genotype were the major species reported [11–13].

Most of the above genotypes are host specific, having been found only in closely related host species. Studies in various regions of the world suggest a strong host-adaptation by these parasites and limited potential for cross-species transmission among different species. Feng et al. [11] concluded that these host adapted species and genotypes of *Cryptosporidium* do not pose a major threat to public health. However, an exception is *C. ubiquitum* which infects a wide range of hosts, including wild and domesticated ruminants, rodents, carnivores and primates including humans [14]. *Cryptosporidium ubiquitum* has a wide distribution, is a common species in sheep and the most common *Cryptosporidium* species found in storm runoff water, and is therefore likely also to be present in wild mammals [15].

On the periphery of protected areas in Africa, abundant populations of wildlife cohabit with livestock and rural communities, with livestock and wildlife often sharing grazing and water sources. This wildlife/livestock/human interface is a suitable environment for the circulation of common pathogens between the three compartments. As is the case with other neglected zoonosis, studies of cryptosporidiosis in this context are scarce. In the case of the Kruger National Park (KNP) interface, studies on zoonotic diseases are particularly important, since the prevalence of HIV/AIDS in rural communities in the area is among the highest in the world [16]. Therefore, the primary goal of this study was to detect and characterize *Cryptosporidium* spp. in three common African wildlife species (elephant, African buffalo, impala) and indigenous cattle at the wildlife/livestock/human interface of the KNP. A second objective was to estimate, using a questionnaire, the extent of contact between livestock and cattle in communities close to the KNP boundary, and thereby to assess the potential for cross-species transmission of *Cryptosporidium* spp.

2. Materials and methods

2.1. Study area

The KNP covers nearly 20,000 km^2 of semi-arid savannah in the north-eastern Lowveld region of South Africa. It is bordered to the east by Mozambique and to the north by Zimbabwe. The western border adjoins communal grazing areas and private game reserves in Mpumalanga and Limpopo provinces (Fig. 1).

This study area for cattle comprised twelve diptanks in the communal grazing lands, within 5 km from the KNP boundary, in the Bushbuckridge area, the most populated region adjacent to the KNP. In those communal lands, livestock owners bring their cattle every week to diptanks, managed by the communities, to be dipped in order to protect them against tick-borne diseases. The most common cattle breed in that area is the local Nguni type, used for meat and milk consumption.

Three study areas were chosen: two within the KNP (Skukuza and Tshokwane), and the third within Sabi Sand, which is not fenced off from the KNP (Fig. 1). Two of the study areas (Skukuza and Sabi Sand) are within a distance of 500 m from the boundary fence which separates wildlife from communally grazed domestic animals. However, they differ substantially in terms of the interactions that can occur between wildlife and cattle. In the case of privately owned game reserves such as Sabi Sand, the 2.4 m high electric fence is generally well maintained, electricity is functional, and it has been reported that incidents of wildlife escaping the KNP are rare in those