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TICKS, DISEASES AND CONTROL WITH SPECIAL REFERENCE TO CEREBRAL DISORDERS IN DOMESTIC AND WILD RUMINANTS IN AFRICA

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Tick-borne protozoan and bacterial pathogens are causing a broad spectrum of diseases of veterinary and medical importance throughout the world. A limited number of tick-borne pathogens is responsible for cerebral disorders in domestic and wildlife species. For instance, *Babesia bovis* is a protozoan parasite causing a cerebral form of babesiosis in cattle, which is characterised by numerous intra-erythrocytic *Babesia* parasites inside brain capillaries. Another example of a protozoan disorder is bovine cerebral theileriosis, or turning sickness, a disease of cattle that is reported regularly but at a low incidence in East and southern Africa. The infectious agent responsible for cerebral theileriosis has been reported as either *Theileria parva* of *Theileria taurotragi*, two closely related tick-borne protozoan parasites sharing the same vector tick, *Rhipicephalus appendiculatus*. Typical cases contain numerous macroschizonts blocking brain capillaries causing thrombosis of meningeal blood vessels, infarcts and intraventricular haemorrhages. Furthermore, *Cowdria ruminantium*, which causes heartwater or cowdriosis, transmitted by ticks of the genus *Amblyomma* in vast areas of sub-Saharan Africa, is another cerebral disorder of cattle and small ruminants. Typical cases display nervous symptoms and post-mortem findings include transudates in the pericardium, thoracic and peritoneal cavities. Histopathological examinations of cerebral cortex reveal colonies of rickettsial organisms in the cytoplasm of endothelial cells lining the capillaries.

An accurate diagnosis of these hemoparasites is of prime importance in order to support adequate disease control intervention schemes. To this end, we have developed integrated molecular diagnostic tests to simultaneously

detect and differentiate known tick-borne pathogens. Reverse line blot (RLB) hybridisation assays can differentiate *Theileria* and *Babesia* species on basis of differences in the hyper variable V4 region of the 18S small subunit ribosomal RNA gene. Within this region, oligonucleotides are deduced for species-specific detection. PCR products are hybridized to a membrane onto which species-specific oligonucleotides were covalently linked. The sensitivity of the assay was determined at 0.000001 % parasitemia, enabling detection of the carrier state of most parasites. Moreover, bovine blood samples from cattle experimentally infected with different parasites, reacted only with corresponding species-specific oligonucleotides.

A similar approach was followed for simultaneous detection and differentiation of *Cowdria, Anaplasma* and *Ehrlichia* species, targeting the 16S ribosomal RNA gene. Although amplification takes place in separate PCR reactions, all probes specific for *Theileria*, *Babesia*, *Anaplasma*, and *Ehrlichia* species are applied onto the same membrane, which makes the RLB a versatile technique for integrated epidemiological monitoring of tick-borne pathogens.

We are using this technique for molecular disease investigations in cattle and wildlife in Tanzania and South Africa. In Tanzania, a fatal nervous disorder in adult African short-horn cattle of Maasai pastoralists, was initially diagnosed as heartwater caused by a *Cowdria* infection. However, brain smears collected from fatal cases contained numerous *Theileria* schizonts. When subjected to RLB, amplified products incriminated *Theileria taurotragi* as causal agent of this disorder. A longitudinal study monitoring a number of herds in northern Tanzania is currently ongoing, with the objective to determine the factors that may predispose or contribute to the high levels of pathogenicity in cattle.

A similar approach is used for disease investigations in wildlife in Tanzania and South Africa. Alarming rates of mortality occurred in 2000, particularly among buffalo, wildebeest and lion in the Ngorongoro Conservation Area in northern Tanzania. Among the animals that died, the deaths of 4 black rhinoceroses were of particular concern. Since two out of these 4 rhinoceros had high levels of intra-erythrocytic *Babesia*-like parasites in blood and brain smears, we used RLB on brain and spleen tissue of both animals.

When subjected to RLB, amplified products displayed a positive *Theileria/Babesia* catch-all signal, but without a species-specific signal, indicating that a novel protozoan parasite may be present. Phylogenetic analysis, based on the entire 18S gene sequence, confirmed the presence of a new species, designated *Babesia bicornis* sp.nov. With a *B.bicornis*-specific probe, we were able to detect the parasite in tissue of a fatal case of babesiosis, which occurred in a black rhinoceros a South African in 1995. Preliminary screening of black rhinoceroses currently living in South Africa revealed a second parasite, designated *Theileria bicornis* sp. nov. in addition to the presence of *B.bicornis*. These findings have stimulated us to embark on a epidemiological survey for tick-borne pathogens in the black and white rhinoceros populations in east and southern Africa.