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Dr. Wanda Markotter has completed her PhD in Microbiology at the University of Pretoria, South Africa in 2007 where she is currently employed as a Senior lecturer. Her research focuses on the epidemiology and pathogenesis of African lyssaviruses as well as the development of new diagnostic techniques for the developing world. Other research interests include viruses associated with African bat species. She was awarded two competitive research fellowships that allowed her to complete part of her research towards a PhD degree in the USA, at the Rabies Unit of the Centers for Disease Control. She is the author of 14 scientific papers published in international journals (five as a direct result of her PhD studies) and four book chapters and has presented several papers at national and international conferences, most of them by invitation. In 2008, she also received a International Society for Infectious Diseases International Development Grant for Young Women to attend the 13th International Congress on Infectious Disease in Malaysia and present her PhD research. Wanda is also a research co-coordinator for the Northern Gauteng Bat Interest group and plays a key role in other national bodies such as the National Rabies Advisory Group.

ISID Small Grants Program ~ Final Report

Diagnostics and molecular epidemiology of wildlife rabies in remote areas of South Africa

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Rabies is caused by all members of the lyssavirus genus in the family *Rhabdoviridae*, a group of single stranded negative sense RNA viruses, currently consisting of seven genotypes of which genotype 1, 2, 3 and 4 occur in Africa (Nel and Markotter, 2007). The majority of animal and human cases in South Africa in recent decades had occurred in the KwaZulu Natal (KZN) province of South Africa where the disease is endemic in domestic dogs with occasional spillover reported in wildlife species. Recently more cases of rabies in wildlife species has been reported in species such as jackals, mongooses, foxes and hyenas in areas where rabies was not previously known to occur including nature reserves. These areas are very remote and far from a rabies diagnostic laboratory and it is therefore not always possible to send samples to a diagnostic laboratory and cases may therefore go unnoticed.

The gold standard for lyssavirus diagnostics, the fluorescent antibody test (FAT) is performed on brain tissue and can not be performed under field conditions since specialized equipment such as a fluorescent microscope is needed. In Africa and other developing regions of the world, lyssavirus diagnostics and surveillance are seriously hampered due to the lack of facilities and logistical support for reliable execution of the FAT. Furthermore, in some instances where a diagnostic facility does exist and is operational, the need to efficiently transport samples to a central facility can often not be met. These obstacles in obtaining a diagnostic result from field specimens have led to serious underreporting of the disease and have ultimately resulted in a lack of commitment to control the disease.

A rapid immunodiagnostic test kit (RIDT) that could offer advantages towards overcoming some of the difficulties mentioned above, has been developed by Kang et al., 2007 but has not been tested on the African lyssaviruses. It was therefore our aim to evaluate this test for its ability to detect the most diverse isolates of the African lyssavirus genotypes and variants known to us. Furthermore the epidemiology of wildlife rabies was determined by phylogenetic analysis of samples collected from wildlife in nature reserves in KwaZulu Natal, South Africa.

The rapid immunodiagnostic test kit (RIDT) was evaluated against a selection of isolates of lyssavirus genotypes occurring in Africa in parallel comparison with the fluorescent antibody test (FAT) (Dean et al., 1996). Isolates representing previously established phylogenetic groups from each genotype (gt 1-4) were included. The specificity of the rapid immunodiagnostic test compared favourably with the FAT and was found to detect all representatives of genotypes 1, 2, 3 and 4. Molecular epidemiology analysis was also performed on the G-L intergenic region (Coetzee and Nel, 2008) of the lyssavirus genome of wildlife samples that tested positive with the FAT. Phylogenetic analysis of hyena and jackal samples collected from a nature reserve indicated that the rabies virus present in these samples form part of the rabies cycle in dogs in this area. This indicates that wildlife cases in the reserve are due to spill over infection from domestic dogs in the area bordering the reserve. This information can now be used to intensify vaccination campaigns targeting domestic dogs in areas neighboring the nature reserves. Ultimately this will prevent spillover infections into wildlife species that may even include endangered species such as the African wild dog.

Rabies is under reported due to the lack of operational rabies diagnostic facilities or if it exists it is restricted to specific geographic locations and samples from remote areas seldom or never reach these laboratories. Under these circumstances, the RIDT may be a useful tool. This is a very simple test that can be performed in less than 10 min without any specialized equipment, infrastructure, or high level of training. There are no critical points to field use such as cold storage, since the test kit contains everything required for the diagnosis and is stable at ambient temperatures. The RIDT could specifically assist in better understanding of the epidemiology of lyssavirus infections in wildlife if application in an on-site manner is considered. Areas of wildlife reserve are often very remote, and it is rarely possible for conservationists, game rangers or farmers to duly collect and send samples to a diagnostic laboratory for testing. The technique has the potential of enhancing epidemiological surveillance of lyssaviruses under such conditions and in remote areas where lyssaviruses infections otherwise go unnoticed.

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