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Short Communication

Detection of rabies in camel, goat and cattle in Sudan using Fluorescent antibody test (FAT) and hemi nested Polymerase Chain Reaction (hnRT-PCR)

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ABSTRACT

Objective: The objective of this study was to identify rabies virus in camels and other animals in Sudan.
Materials and methods: Four camel samples were collected from Garraht Elzawia, Kab-kabia and North Darfur areas in Sudan. The samples were collected based on clinical signs. In addition, two camel samples were obtained from Khartoum and Tambool, one goat sample was collected from El-Fashir, and one cattle sample was obtained from Atbara. The samples were transported to the Veterinary Research Institute (VRI) at Khartoum, Sudan for further studies. The samples were subjected for nested and hemi nested RT-PCR (hnRT-PCR) along with the gold standard Fluorescent antibody test (FAT) to diagnose rabies.
Results: Out of eight samples, seven were found to be positive by both FAT and RT-PCR methods. The remaining one sample was positive by FAT but negative by hnRT-PCR indicating the suitablity of hnRT-PCR along with FAT for accurate diagnosis of rabies in animals.
Conclusion: The study concluded that FAT and RT-PCR are useful tools for research and diagnosis of rabies.

KEYWORDS

Camel, FAT, Rabies, hnRT-PCR

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INTRODUCTION

Rabies is one of the most important viral diseases affecting all warm blooded animals and man; laboratory diagnosis of the disease is the corner stone for the post-exposure treatment regimen. Fluorescent antibody test (FAT) is the WHO and OIE recommended test for the diagnosis of rabies infection in both humans and animals, (Dean and Abelseth,1973; Kissling, 1975).

Early RT-PCR methods for lyssaviruses amplification were described by Sacramento et al (1991) who applied specific and sensitive amplification of infected brain material by using primer set mapping in the nucleo-protein cistron, and for molecular epidemiological studies, the authors selected another set of conserved primers flanking the highly evolutive pseudogene (Ψ gene) region. This set was found to be efficient for all tested fixed or wild rabies virus strains as well as the rabies-related Mokola virus, subsequently more detailed protocols have been reported.

In Sudan, laboratory diagnosis of rabies was early based on histopathological examination, since the last three decades FAT is routinely used. The use of molecular biology techniques for rabies diagnosis was not applied. In this study, hemi nested PCR (hnRT-PCR) was used beside the gold standard FAT to detect rabies viral antigen and genome, respectively, nested and hemi nested PCR (hnRT-PCR) can enhance the sensitivity of PCR. Nested PCR is the one in which the product of a PCR is subjected to a second round of amplification that uses primers internal to those employed for the first round (Kamolvarin et al., 1993). The aim of this study was to apply PCR technique in rabies diagnosis beside the gold standard technique for the first time in Sudan.

MATERIAL AND METHODS

Sources of specimens: Four samples were collected from camels at Garraht Elzawia, Kab-kabia, North Darfur, the signs of the disease were stated since 2006. Sporadic cases of paralysis in camels were reported at different areas of north Darfur state. The last cases were reported at Garraht elzawia in March 2010. Signs observed in four camels at this area were as follow: stiff gait, in-coordination, falling to the ground due to paresis of the hind quarters, then complete paralysis of hind limbs and the pelvis. Death usually occurs between 10-15 days but most cases were slaughtered. Four animals were sacrificed for postmortem examination. Other two camel samples were obtained from Khartoum and Tambool, one goat from El-Fashir and one cattle from Atbara. **Table 1** shows the details of specimens used.

Collection and preparation of samples: All samples were submitted to Veterinary Research Institute (VRI)/ Khartoum, Sudan for rabies diagnosis and prepared according to Le'pine and Atanasiu (1996).

Fluorescent antibody technique (FAT): FAT was done according to Kissling (1975). Anti-rabies conjugate was kindly provided by Claude Sabeta of Rabies unit Onderstepoort Veterinary Laboratory, South Africa.

Rabies viral RNA extraction from tissues using TRIZOL™: The procedure was followed as provided by Animal Health and Veterinary Laboratories Agency (AHVLA), UK. Along with the samples being extracted, a negative control, uninfected mouse brain was also extracted at the same time.

Reverse Transcription of rabies viral RNA: All reagents used in PCR were kindly provided by Dr Tony Fooks of Animal Health and Veterinary Laboratories Agency (AHVLA), UK. The procedure used includes the steps involved in the synthesis of rabies specific complementary DNA (cDNA) from rabies viral RNA using the enzyme reverse transcriptase, PCR to be run alongside the JW6/12 PCR (Heaton et al., 1997). One negative mouse brain was used; water was used as the RT "no template control" (NTC). Previously prepared CVS RNA (1 µg/µL) was used as the RT positive control.

PCR-Pan-Lyssavirus JW6/12 first round PCR: Primers used are JW12, JW6 (DPL), JW6 (E), and JW6 (M). The expected size of amplicon is 606-bp. Primers sequence are shown in **Table 2**.

Table 1: Samples used in the study

Specimen	Animal species	Location
Brain	Camel	Khartoum
Spinal cord	Camel	El-Fashir
Spinal cord	Camel	El-Fashir
Spinal cord	Camel	El-Fashir
Spinal cord	Camel	El-Fashir
Brain	Camel	Tambool
Brain	Goat	El-Fashir
Brain	Bovine	Atbara

PCR-Pan-Lyssavirus JW10/12 second round PCR: This procedure is used for second round amplification (hnPCR) to increase the sensitivity of detection of rabies and rabies related viruses and/or to confirm the specificity of the first round (JW6/JW12) PCR product (Heaton et al., 1997). A primer sequence (JW10) which lies within the sequence of the first round PCR product is

Table 2: Oligonucleotide primers for hnRT-PCR amplification of RV and RRVs.

Primer	Sequence (5′ - 3′)	Sense	Position in genome
JW12	ATGTAACACC(C/T)CTACAATTG	M	55-73
JW6 (DPL)	CAATTCGCACACATTTTGTG	G	660-641
JW6 (E)	CAGTTAGCGCACATCTTATG	G	660-641
JW6 (M)	CAGTTAGCGCACATCTTATG	G	660-641
JW10 (ME1)	GTCATCAATGTGTG(A/G)TGTTC	G	636-617
JW10 (DEL2)	GTCATCAAAGTGTG(A/G)TGCTC	G	636-617
JW10 (P)	GTCATTAGAGTATGGTGTTC	G	636-617

used in conjunction with the first round primer JW12 in the hnPCR. The resulting smaller PCR product confirms the specificity of the first round PCR product whilst potentially improving sensitivity of the assay.

Primers used are JW12, JW10 (ME1), JW10 (DEL2), and JW10 (P), Primers sequence are shown in **Table 2**. The expected size of amplicon is 586-bp.

RESULTS AND DISCUSSION

The postmortem examination of the four camels from (Garraht Elzawia) showed: subcutaneous petechial hemorrhage, the urinary bladders were fully distended in two animals, the myocardium was flabby in one animal, congestion of myocardium in one animal, moderate degree of pneumonia was seen, the brain vessels were congested, all cases showed liquifactive necrosis in the spinal cord especially at the sacral region.

All samples showed positive fluorescence and recorded as rabies positive materials (**Table 3** and **Figure 1**).

Table 3: FAT and hnRT-PCR results for detection of rabies virus.

Sample No.	FAT result	PCR result	
		1st round	2nd round
508	+ve	+ve	+ve
513	+ve	+ve	+ve
509	+ve	-ve	+ve
510	+ve	+ve	+ve
511	+ve	- ve	-ve
512	+ve	- ve	+ve
514	+ve	- ve	+ve
515	+ve	- ve	+ve

Four out of eight samples were negative in the first round but positive in the second one and one sample was negative in the two rounds (**Table 3** and **Figure 2**).

Rabies is endemic in Sudan since 1904 (Anon, 1997). Different animal species were involved in rabies epidemiology in Sudan with the predominance of dogs. Reports of camel rabies in Sudan are very rare due to nomadic nature of camel owners and distances of breeding sites from the centre. Camel rabies was reported in Kordofan at the west in 2001 (El Mardi and Ali, 2001) and in Kassala at the east in 2006 (Omer et al, 2006). All cases were diagnosed based on clinical signs and confirmed using FAT and histopathology. Recently PCR is used as initial step of all molecular biology studies; in the present study we used this test successfully for rabies diagnosis for the first time in Sudan to introduce the technique to be applied for further molecular work. FAT was correlated well with PCR. Seven out of eight tested samples were positive using FAT and RT-PCR, one sample was FAT positive but PCR negative which could be due to RNA degradation. Our results are inline with some recent reports (Emmanuelle et al., 2013).

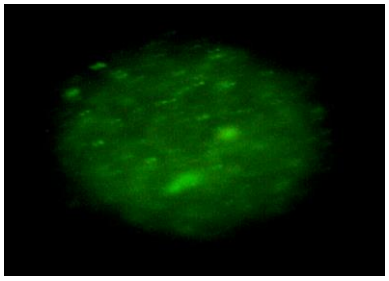

Figure 1: Detection of rabies antigen in animal brain and spinal cord specimens using FAT.

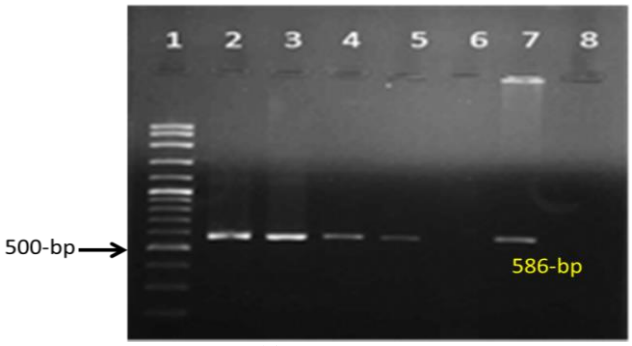

Figure 2: Second round hnRT-PCR. For the detection of rabies virus genome. Lane 1: DNA ladder, Lane 2-5 samples No. 508, 513, 510, 512, Lane 6 sample No. 511, Lane 7: +ve control, Lane 8: -ve control. Positive amplicon size is 586-bp.

Emmanuelle et al. (2013) compared different techniques for rabies diagnosis, the study demonstrated that the hnRT-PCR techniques produced the lowest rate of false negative results, and were consequently the most sensitive, conversely false positive rate in RT-PCR technique was the highest level among techniques used. The high sensitivity of PCR, which makes PCR a highly sensitive research tool means that extreme care must be taken to avoid generating false positive results. FAT was found to be a good compromise as only a few false positive and false negative results were obtained. Yang et al. (2012) evaluated four diagnostic methods. to detect rabies in animal brain homogenates. Compared to the FAT the sensitivity and specificity of RT-PCR were found to be 100%.

CONCLUSION

PCR is sensitive, accurate but expensive and time consuming technique to be used for routine diagnosis purposes but it is useful for research purpose.

CONFLICT OF INTEREST

None of authors have any conflict of interest.

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