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Field and Laboratory Detection of Rabies Antigens in Saliva and Brains of Dogs in Nigeria: An Approach Using Rapid Immunochromatographic Test

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Abstract

A rapid diagnosis associated with bite will quickly explain whether a bite constitute an exposure or not; brains and saliva of rabid animals has been documented to contain rabies antigens and serves as medium for the transmission of the infection to susceptible host and 98% of rabies in human is caused by bite from rabid dogs. In Kaduna State, Nigeria there are several active dog markets and slaughter areas where dogs are slaughtered for meat, there are also Veterinary Clinics where destroyed, euthanized and suspected rabid animals are send for examination or quarantined. Study was carried out in five slaughter areas and a clinic within 3 Local Government Areas in Kaduna State to evaluate the efficacy of field and laboratory detection of rabies antigens in saliva and brains of apparently healthy dogs slaughtered for consumption and suspected rabies cases that were brought to the Clinic dead or alive dogs using rapid immunochromagraphic diagnostic tool for detection of rabies antigen in the saliva and brains sample of rabies suspect, Saliva and brains of 50 apparently healthy dogs slaughtered for consumption were screened for rabies antigens and eight (16%) were found to be positive for rabies antigens using Rapid Immunochromatographic test for rabies antigens and Direct Flourescent Antibody Test (DFAT). In the clinic saliva of ten dogs involved in bite and showing signs consistent with rabies were tested for rabies antigen using Rapid Immunochromatographic Test following either natural death or euthenasia and were positive. The brain of same dogs was tested for rabies antigen using RICT. All extracted brains were tested for rabies antigens using the goal standard test, DFAT and all those that were positive for Rapid Immunochromatographic test for rabies antigen in the field and laboratory were also positive for DFAT. Considering the distance between diagnostic and reference centres from rural communities, we strongly recommend the use of this diagnostic test. It is handy, cost effective and can reduce cost of management of dog bite victims.

Keywords: *Brain; DFAT; Saliva; Rabies Antigen; RICT*

Introduction

Rabies is an acute, contagious and highly fatal disease of all warm blooded animals caused by a Rhabdo virus that is single stranded, negative sense RNA and belongs to lyssa genus [1,2]. The virus has tropism for peripheral nerve tissues and the central nervous system of both humans and animals [3-6]. The disease is endemic in Nigeria and the actual level of burden of the disease in the country has not been achieved due under reporting [7, 8]. The saliva of rabid dogs has been documented to contain high concentration of rabies viruses and serves as a medium for transmission of the infection [9-11]. Although, non-bite exposures can occur through licks or splash of infected saliva into wound, 98% of human deaths have been documented to have been caused by almost and always bite of a rabid dog [12]. The virus causes a non suppurative viral encephalomyelitis with characteristic clinical forms of rabies such as dumb, furious and asymptomatic (inapparent) forms [13-15]. Once clinical sign sets in, death is the end result. However, some recoveries have been reported both in Man and Animals world-wide [3,16].

The procedure for the direct Flourescent Antibody Test (DFAT) was first described by Goldwasser and Kissling

[17]. When reacted with fluorescein conjugated rabies immune serum and illuminated with ultra-violet light, rabies antigen in infected tissues appear as brightly-coloured apple green or greenish yellow, round to oval intracellular accumulations. The antibody primarily responsible for staining in the DFAT is that directed against the nucleo-capsid antigen of the virus. In addition to the larger stained bodies, infected tissues may contain smaller collections of antigen, which appear as granular or dust-like fluorescent particles or thread-like material [18,19]. Immuno fluorescent rabies antigen may be detected in all parts of the CNS of infected animals, but because of the frequently uneven distribution of antigen, the most reliable diagnosis is made from tests which include examination of the medulla (brain stem), cerebellum and the hippocampus (Koprowski, 1973). Antigen could be detected in the skin, the salivary glands and other tissues of an infected animal [20]. The DFAT is still the gold standard in rabies diagnosis has shown 100% sensitivity but it is limited by the cost of acquiring and maintaining a fluorescent microscope, conjugates and supply of electricity [17,19,21].

The immuno chromatographic lateral flow strip test is a one-step test that facilitates low-cost, rapid identification of various analytes including viruses [22]. Briefly, suspected material is subjected to a test device similar to a lateral flow device. Conjugated detector antibodies attached to two different zones on a membrane indicate the presence of viral antigen. Preliminary validation studies with a limited number of samples showed that the rapid immuno chromatographic test (RICT) might have great potential as a useful method for rabies diagnosis without the need for laboratory equipment and electricity [22].

A rapid diagnosis associated with bite will quickly explain whether a bite constitute an exposure or not [11,23]. The brain or saliva of suspected biting dog is used for the detection of the rabies antigen [24]. In African and Asian countries, at least 10% of brain samples received at the laboratories for rabies diagnosis are decomposed because of lack of storage facilities, proximity of the incident area to the laboratories, poor transportation, inadequate prompt diagnostic tests that are field based, all these factors lead to late and misdiagnosis. Diagnosis is fundamental in the surveillance and control of rabies. In Nigeria, dFAT is often performed on dogs at postmortem where dogs involved in bite cases are destroyed or euthenised to confirm if they are rabid or not. This avoidable practice often cause losses to dog owners and breeders [8,12,14,25]. Therefore, the absence of a rapid confirmatory test can result in the inappropriate management of animal bite, unnecessary administration of post exposure prophylaxis (PEP) which will cause an economic burden to the owner. It can also cause delay in rabies PEP which can cause human death. In Kaduna state, there are several active dog markets where dogs are slaughtered on a daily bases without ante-mortem and/or post-mortem examination, this pose as a potential danger to butchers and those involved in dog trading. Distance of diagnostic centres from most rural clinics serves as a major challenge to prompt diagnosis and management, in Kaduna state the only diagnostic centres are located in Mando and Zaria. A rapid diagnosis can initiate a rapid response. The study was carried to identify whether rapid immunochromatographic diagnostic test is suitable for use both in field and laboratory for diagnosis of rabies using the saliva and brain of dogs.

Materials and Methods

Study Method

Convenient random sampling as described by Mike (2011) was employed [26]. The most accessible units of the population were used. Live dogs bought or brought for slaughter were sampled in slaughter areas designated in a selected dog markets within 3 selected local Government areas of Kaduna State (Kaduna South, Chikun and Sabon Gari), Cases of dog attacking and biting school children, Biting the owner and family members, biting animals in the community, restless and attacking moving objects and humans and dog with hanging jaw with stringy saliva, unable to eat and use its hind limbs Dog bite cases with suspected exposure and history of mental disorientation were selected for the study.

Study Area

The study was conducted in Kaduna State which falls within the Guinea Savannah zone and covers an area of about 48,473.2 square kilometers and situated at longitude $06^{0}10'$ and $09^{o}00'$ East of Greenwich Meridian and Latitude 09^{o} 10' and $11^{0}30'$ North of the Equator. Dog markets/ slaughter slaps located in Basawa Army Barracks Mammy market, Ungwan Tabo Samaru and Jushi in Northern Senatorial district and Kamanzou, Trikania, Television Garage, in Central senatorial district based on availability.

Sampling Method

The study employed convenient random sampling technique as described by Mike (2001) [26]. The most accessible

unit of the population was used. Here, live dogs bought or brought for slaughter were sampled in the different slaughter areas. Saliva and brains of dogs were sampled for the study.

Saliva

Rapid Immunochromatographic Test kit Cat.No: RG18-0, Lot.No: 1801DD003 (Rabies Ag Test kit, Bionote) for the detection of rabies antigen in the brain and saliva of dogs was employed. Saliva from dogs presented for slaughter were sampled and tested as described by the manufacturer. Briefly, a sterile swab stick was inserted into the mouth of the dog before slaughtering. The wet swab was then inserted into an assay buffer tube and stirred to ensure a good sample extraction. The immunohromatographic test cassette was then removed from the foil pouch and placed horizontally. Using a sterile dropper, three drops of the extracted sample was then dripped into the sample hole in the cassette and result was interpreted within five to ten minutes.

Brain

Head of dogs that were brought to clinic with a request for rabies test following bite exposures and those whose saliva were tested in the field were all labeled and placed in polythene bags and transported to the postmortem room for brain extraction and brain was extracted using the method described by Kaplan and Koprowski (1980) at the post mortem room of the Department of Pathology Ahmadu Bello University, Zaria [27].

Laboratory diagnosis

All brain samples were tested using the rapid immunochromatographic test as described by the manufacturer. Briefly, a 10% suspension of brain sample was made in the buffer tube. The immunohromatographic test cassette was then removed from the foil pouch and placed horizontally on the table. Using a sterile dropper, three drops of the 10% sample was then dripped into the sample hole in the cassette and result was interpreted within five to ten minutes. All the brain samples tested using rapid immunochromatographic test were also subjected to Fluorescent antibody test (FAT) as described by Dean et al (1996) [21]. Briefly, DFAT was performed in the Viral Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, ABU, Zaria., Impression smear of the brain sample was prepared on a clean glass slide, air dried and fixed in cold acetone for 1hour at – 20oC. The acetone fixed immersion smear of the sample was then stained with Flourescien-labelled anti-rabies immunoglobulin (FITC anti-rabies monoclonal globulin, Fujirebio Diagnostic, Inc. (FDI), USA). The slides were incubated for 30 minutes at 37oC in a humid chamber and then washed with phosphate buffered saline (pH 8.5) three successive times over a period of 10 minutes. Slides were air-dried after rinsing with distilled water. The slides were viewed at X 40 using a Fluorescent microscope (ZEISS, Primo star Ileid, Carl Zeiss microimaging, Gottingen, Germany; series no. 3133000412). Results of the test were interpreted positive if a bright apple-green fluorescence of particles was observed under the microscope and negative when no specific apple-green fluorescence was observed on the slide under the fluorescent microscope.

Results

Using Rapid Immunochromatographic test, 8 out of 50 dogs sampled were positive for rabies antigen that means shedding the virus in their saliva (16% prevalence). The brains of these same dogs were subjected to RICT and the same result with that of the saliva was obtained. These brain samples were again subjected to DFA test and were positive for rabies antigens same as in RICT with varying degree of positivity from ++ to +++ (Figure 1).

Clinical samples were tested using RICT for rabies antigen and all those that were positive were also tested using Florescence Antibody Test were also positive with varying degree of positivity between +++ and ++++ (Figure 2).

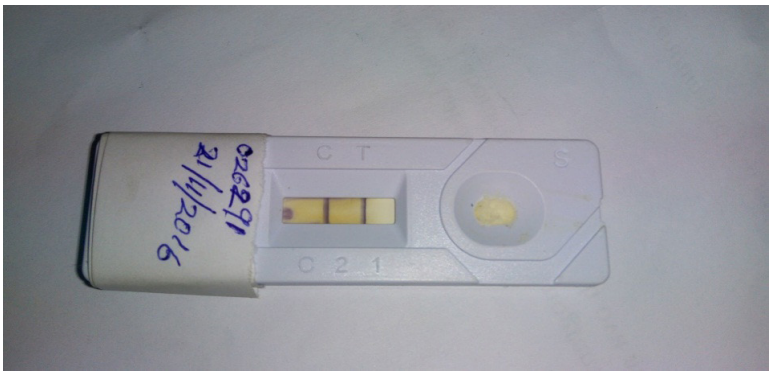


Figure 1: Rapid immunochromatographic test result of saliva of a dog showing positive

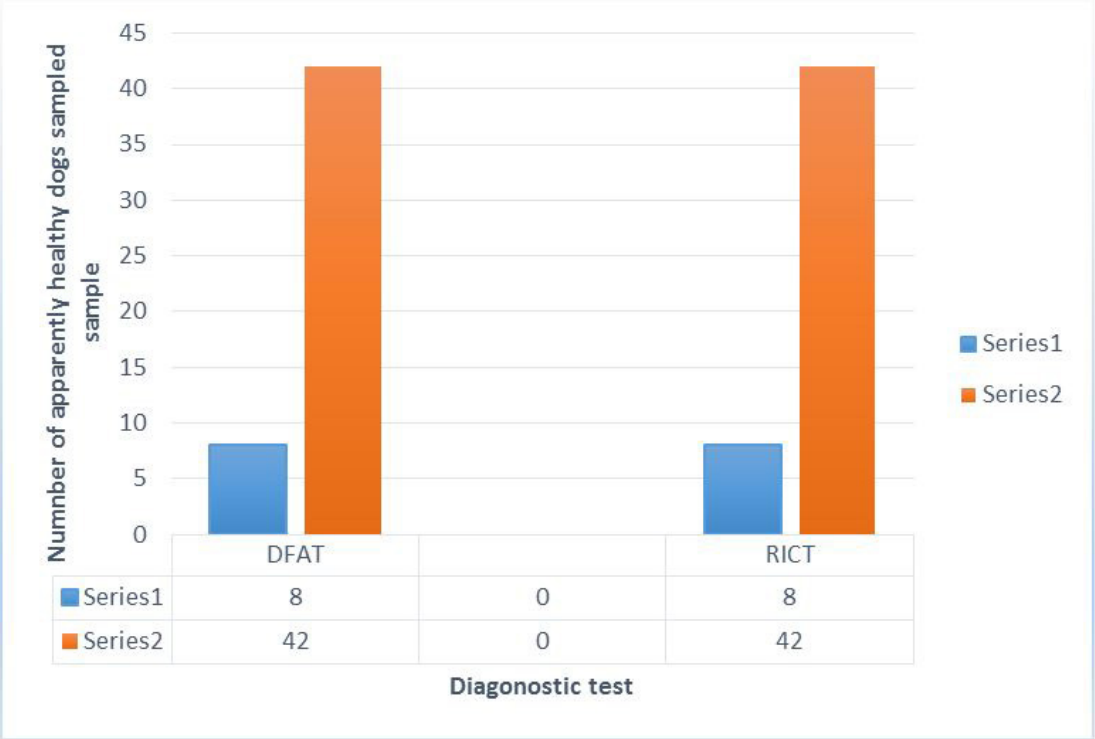
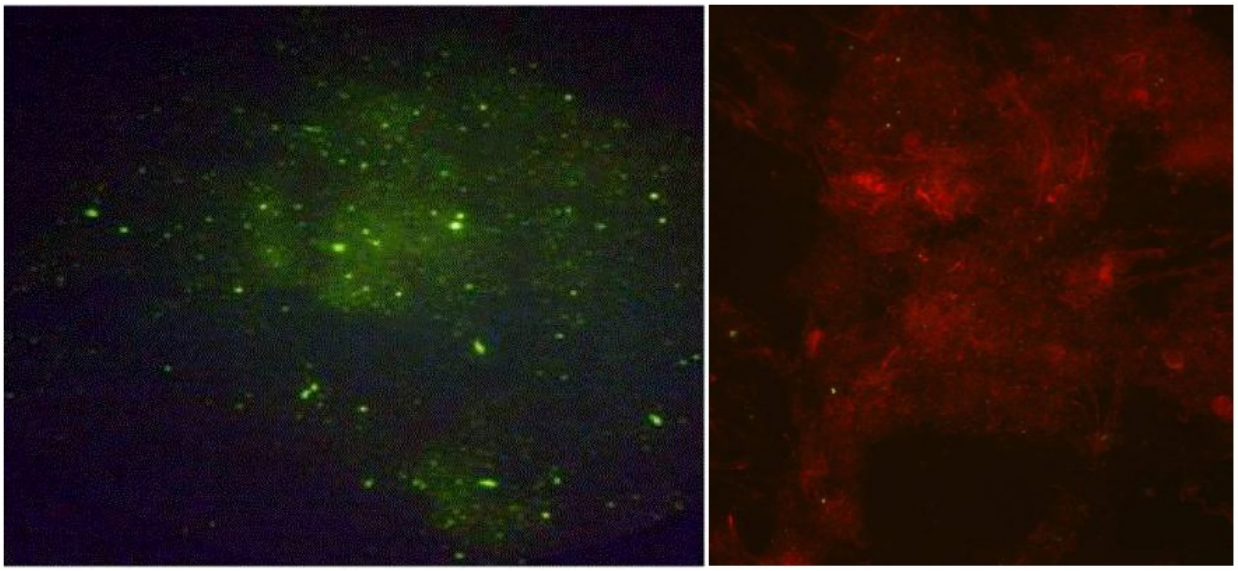


Figure 2: Comparative positive test result of brain of apparently healthy dog slaughtered for human consumption



Positive Negative

Figure 3: Shows a DFAT positive brain slide of a clinical rabies case with apple green appearance

Discussion

Poor diagnosis and reporting system of rabies cases either as an exposure or death despite being an endemic area may be as a result of poor surveillance and reporting system, inadequate, cheap, prompt, rapid and accurate laboratory and field-based diagnostic rabies tests. The findings of this study revealed that rabies is endemic in Kaduna state and is found in both dogs that shed the virus and yet look apparently healthy and those that were destroyed or brought for clinical examination as a result of mental derangement or sudden change in behaviour. These apparently healthy dogs are often slaughtered for human consumption in different active dog markets located in different parts of the state, this study agrees with findings reported by [8,25,28,29], that rabies is endemic in some dog markets in Nigeria. Rabies antigens was confirmed from saliva and fresh dog brain samples collected from apparently healthy dogs in slaughter areas and clinical cases presented to clinic. All dogs that were saliva positive for RICT were also brain positive for rabies antigen using RICT and DFAT. The result shows a strong agreement between the RICT and the goal standard test. The study also revealed that RICT used under field condition, was also found to yield the same result as when

used in the laboratory. Although the DFA test is the gold standard test frequently used for rabies diagnosis, the RICT therefore has proven to be equally efficacious and reliable as DFAT in Field and Laboratory with the added advantage of being cost effective and prompt, can be interpreted within 10 minutes which will save time and serve as an effective tool for rabies diagnosis in developing and underdeveloped countries, where there is limited or no surveillance on rabies [30,32].

In Conclusion, the study reports a 16% prevalence of rabies among apparently healthy dogs slaughtered for consumption and recommends the rapid immunochromatographic test as an important and useful diagnostic method for the rapid detection of rabies virus under field and laboratory conditions. It's cost effective, does not require special training and equipment and can be performed under room temperature and test result can be preserved for a long time under room temperature [33].

References

1. Bourhy H, Kissi B, Tordo N (1993) Molecular diversity of the Lyssavirus genus.Virology. 194(1): 70-81.

2. Sana Eybpoosh,1 Ali Akbar Haghdoost, Ehsan Mostafavi, Abbas Bahrampour, Kayhan Azadmanesh, and Farzaneh Zolala (2017) Molecular epidemiology of infectious diseases Electron Physician. 9(8): 5149–5158.

3. Ajayi BB, Rabo JS, Baba SS (2006) Rabies in apparently healthy dogs: histological and immunohistochemical studies. Niger Postgrad Med J. 13(2):128-34.

4. Jackson, A.C. (2002). Pathogenesis of rabies. In: Jackson A.C., Wunner A.H., eds Rabies. San Diego: Elsevier Science, Academic press, 245-282.

5. Dietzschold, B. Schnell, M. and Koprowski, H. (2005). Pathogenesis of rabies. Current topics in microbiology and immunology, 292: 45-56.

6. World Health Organisation (2005). Expert Consultation on rabies. World Health Organization Technical Report Series. 931: 1-88.

7. Umoh JU, Belino ED. (1979). Rabies in Nigeria: A historical review. International Journal of Zoonoses, 6: 41-48.

8. Mshelbwala PP, Ogunkoya, AB, Maikai BV, Nlebedum UC, and Abdullahi SU (2013). Detection of rabies antigen in the saliva and brains of apparently healthy dogs slaughtered for human consumption and its public health implication in Abia State, Nigeria. ISRN Veterinary Science Article ID 468043, 5 pages Doi. 10.1155/2013/468043.

9. Bishop GC, Durrhein DN, Kloeck PE, Godlonton JD, Bingham J, Speare R and the Rabies Advisory Group 2008 (2010). Rabies: Guide for Medical, Veterinary and Allied Professions, Government printers, Pretoria, South Africa. 32-89.

10. Centers for Disease Control (CDC) (2007). Human rabies: Indiana and California, 2006. Morbidity and mortality Weekly Report, 56: 361-365

11. World Health Organisation (2008). WHO expert consultation. Rabies- a neglected zoonotic disease. Geneva, Switzerland.

12. Dürr S, Naïssengar S, Mindekem R Diguimbye C, Niezgoda M, Kuzmin I, Rupprecht CE, Zinsstag J (2008). Rabies diagnosis for developing countries. PLoSNegleted Tropical Diseases. 26(206): e206.

13. Jackson AC, Warrell MJ, Rupprecht CE, Ertl HC, Dietzschold B, et al. (2003). Management of rabies in humans, Clinical Infectious Diseases, 36: 60-63.

14. Ehimiyein AM, Niezgoda M, Orciari L, Osinubi MOV,and Ehimiyein I., et al. (2014). Efficacy of a direct rapid immunohistochemical test (DRIT) for rabies detection in Nigeria. African Journal Biomedical Research. (17): 101- 110.

15. Baba SS, Ajayi BB, Rabo JS. (2006). Rabies in apparently healthy dogs: Histological and Immunohistochemical Studies. The Nigerian Postgraduate Medical Journal, 13(2): 128-134.

16. Fekadu, M. and Baer GM. (1980) Recovery from rabies of two dogs inoculated with rabies virus strain from Ethiopia. Am J Vet Res, 41(10): 1632-1634.

17. Goldwasser RA, Kissling RE. (1958). Fluorescent and staining of street and fixed rabies virus antigens. Proceeding of the Society for Experimental Medicine, 98: 219-223.

18. Fekadu M, Smith JS. (1985) Laboratory diagnosis of rabies. In: Winkler, W.G. (Ed.). Rabies Concepts for Medical Professionals. P 37.

19. Chhabra M, Mittal V, Jaiswal R, Malik S, Gupta M, Lal S. (2007). Development and Evaluation of an In vitro isolation of street rabies virus in mouse neuroblastoma cells as Compared to conventional tests used for diagnosis of rabies. Indian J Med Microbiol 25: 263-266.

20. Beran GW (1984) Rabies and infections by rabies related viruses. In: Beran, G.W. (Ed.). Hand book of Zoonoses, Viral, CRC Press. Boca Raton Florida, 307- 357.

21. Dean DJ, Albelseth MK and Atanasiu P. (1996). The Fluorescent Antibody Test. In: Laboratory Techniques in Rabies Ed. Meslin F.X., Kaplan M.M., Koprowski H. 4th ed Geneva World Health Organisation pp8889.

22. Zhang G, Guo J, Wang X. (2009) Immunochromatographic lateral flow strip tests. Methods of Molecular Biology, 504: 169-83.

23. Oboegbulem SI (1978). Rabies: Human exposure potential in Africa. International Journal of Zoonoses, 5: 80-90.

24. Kasempimolporn S, Saengseesom W, Lumlertdacha B, Sitprija V (2000) Detection of rabies virus antigen in dog saliva using a latex agglutination test. J Clin Micro 38: 3098-9

25. Audu SW (2011). Prevalence of Rabies virus in the saliva and brains of apparently healthy dogs slaughtered for human consumption in Kaduna State. M.Sc Thesis, Ahmadu Bello University Zaria, Nigeria Pp 75-80.

26. Mike B (2011) Convenience sampling: SAGE research methods online

27. Kaplan MM and Koprowski H (1980). Rabies. Scientific American, 2: 103-114

28. Garba A, Oboegbulem SI, Junaidu AU, Magaji AA, Umoh JU., et al. (2010). Rabies virus antigen in the brains of apparently healthy slaughtered dogs in Sokoto and Katsina States, Nigeria. Nigeria J Parasitol. 31: 123-125.

29. Aliyu TB, De N, Yenda EN and Lynn M (2010) Prevalence of rabies virus antigens in apparently healthy dogs in Yola, Nigeria, The Researcher 2(2): 1-14

30. Aghomo HO, Oduye OO, Tomori O and Ibe M (1989) Isolation of rabies virus from clinically healthy and previously unvaccinated dogs. Bulletin of Animal Health Production in Africa, 37: 131-136.

31. Okoh AEJ (2008). Current Knowledge of Rabies and Rabies Related Viruses in Nigeria: It's Importance with, Control and Eradication Strategy. Book of Proceedings of National Conference and Workshop on Rabies, IDR-ABU Zaria, Nigeria. pp 41-51.

32. Rupprecht CE (2008) Current and Future Trends In the Prevention, Treatment and Control of Rabies. Book of Proceedings of National Conference and Workshop on Rabies, IDR-Ahmadu Bello University, Zaria, Nigeria. 1-11, June 17-21st.

33. Warell MJ and Warell DA (2004) Rabies and other lyssavirus diseases. Lancet 363: 959-69.