DE GRUYTER
OPEN

DOI: 10.2478/fv-2018-0003

FOLIA VETERINARIA, 62, 1: 18—24, 2018

EVALUATION OF ENZYME LINKED IMMUNO-SORBENT ASSAY AND RAPID IMMUNO-DIAGNOSTIC TEST FOR RABIES ANTIGEN DETECTION IN ARCHIVED DOG BRAIN TISSUES

Okoh, G. R.[1], Kazeem, H. M.[2], Kia, G. S. N.[3], Mailafia, S.[1]

[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, Abuja
[2]Department of Veterinary Microbiology
[3]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria
Nigeria

godspower.okoh@uniabuja.edu.ng

ABSTRACT

Rabies urgently requires strengthening of new and existing diagnostic methodology in order to overcome the threat it poses. We evaluated the Enzyme Linked Immuno-Sorbent Assay (ELISA) and the Rapid Immunodiagnostic Test (RIDT) in detecting rabies viral antigens, comparing both tests with the Direct Fluorescent Antibody Test (DFAT) which is the gold standard in rabies diagnosis. Fifty dog brain tissues collected from the archives of the Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Nigeria, were utilized for this study. ELISA performed better than RIDT and recorded equivalent result with DFAT as compared with RIDT. There was a 96% agreement between ELISA and DFAT for rabies antigen detection (concordance coefficient 78% : 95% C.I. 0.6366 to 0.8654) while there was a 54% agreement between RIDT and DFAT (concordance coefficient 17%:95% C.I. 0.05138—0.2752). Compared to DFAT, the sensitivities of ELISA and RIDT were 95.5% and 47.6%, respectively, and the specificities of ELISA and RIDT were 100% and 87.5% respectively. The simple Cohen's kappa coefficient for ELISA related to the DFAT was found to be 0.834 (95% C.I. 0.613—1.0). For RIDT, the Kappa value was 0.170 (95% C.I. 0.003—0.337). The ELISA is as reliable a diagnostic method as the DFAT which is the gold standard for rabies diagnosis. It has an advantage of being able to analyse large number of samples at the same time, making it more suitable for epidemiological studies and for laboratories that cannot perform the DFAT. The unsatisfactory result of RIDT in this study reiterates the need to perform an adequate test validation before it can be used in the laboratory for rabies diagnosis.

Key words: Direct Fluorescent Antibody Test; Enzyme Linked Immuno-Sorbent Assay; rabies; Rapid Immunodiagnostic Test; sensitivity; specificity

INTRODUCTION

Globally, estimates show that human death due to endemic dog-mediated rabies is most prevalent in Asia, with

the highest occurrence and mortalities recorded in India. Next to Asia is Africa; however, the absence of dependable data has led to uncertainty in the estimation of the disease burden [23]. India has the most prevalent rate of human rabies in the world, basically due to large number of stray dogs [18]. Rabies which is known to be endemic in Nigeria has the domestic dog as the primary reservoir of the causative virus [3]. It first occurred in the country in humans in 1912 and was first diagnosed in the laboratory in a dog in 1925 [5], since then, human and animal rabies cases have been reported in all the regions and ecological zones of Nigeria annually [2, 35].

Rabies remains a threat underappreciated by healthcare practitioners in many endemic areas, often owing to lack of rapid diagnostic tools, post-mortem evaluations, and public health reporting. Although most veterinary laboratories in Africa have sufficient personnel capacity to diagnose rabies in animals, routine diagnosis is often limited by a lack of laboratory equipment and reagents [16, 24].

Diagnosing rabies can be demanding sometimes, this is because it is easily confused, especially at the early stages, with other diseases [8]. Proper history taking and clinical signs are very important in the diagnosis of rabies; however, confirmatory diagnosis of rabies depends on the laboratory identification of the virus or its specific components. Microscopic examination of specimens is one of the laboratory routines that allows for the rapid identification of rabies virus-specific antigen, irrespective of geographical location and condition of the host. The Direct Fluorescent Antibody Test (DFAT) is the 'gold standard' method for diagnosing rabies and its use has been recommended by the World Health Organization (WHO) [35]. However, Fooks et al. [13] noted that decomposed samples can affect the sensitivity and specificity of DFAT. To ensure reliable results, the brain tissues to be tested must be preserved by chilling or freezing. The transportation of the tissues to the rabies laboratory often presents difficulties; especially since facilities for refrigeration are usually limited [1, 36]. Even when ice or dry ice and insulated packages are available, the delays involved in transportation often result in deterioration of the tissue in route, which increases the likelihood of false negative results with DFAT. Because of these difficulties, many specimens are not submitted for laboratory examination, even though it is important that decisions on post-exposure prophylaxis should, whenever possible, be based upon the results of laboratory tests [36].

Rabies urgently requires strengthening of new and existing diagnostic methodology in order to overcome the threat it poses [34]. It is pertinent to note that the accurate laboratory diagnosis of rabies in an animal has a direct effect on human treatments [20]. Therefore, rapid and accurate diagnosis of rabies is vital to human post-exposure prophylaxis, steering epidemiologic surveillance and providing adequate information for the design of rabies control programs [12]. The DFAT has been regarded as the 'gold standard' method for rabies diagnosis for many years despite the numerous limitations associated with this technique [10, 13]. The Enzyme Linked Immuno-sorbent Assay (ELISA) is suitable for analysing samples not preserved in good conditions [22]. It is rapid, easy to use, and relatively safe because they do not require the use of infectious virus, making them suitable for use in developing countries [8]. A recently described method for the detection of RABV antigen from post-mortem samples is the Rapid Immuno-Diagnostic Test (RIDT), a useful method for rabies diagnosis without the need for laboratory equipment [11]. This RIDT is a one-step test that facilitates low-cost and rapid identification of viral antigens.

There is a need for more economical and user friendly tests, particularly for use in developing countries. Therefore, this study sought to evaluate the performance of ELISA and RIDT in relation to DFAT for the diagnosis of rabies in frozen dog brain tissues.

MATERIALS AND METHODS

Specimen collection

This experiment was conducted at the Rabies laboratory, National Veterinary Research Institute, Vom. Fifty dog brain tissues preserved at –20 °C were collected from the archives of the Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, for this study. The tissues were thawed at room temperature. Approximately 1 g of brain tissue samples were cut and homogenized in 10ml phosphate buffered saline (PBS), pH 8.5. Supernatants were carefully collected after centrifuging at 3000 × g for 20 minutes. All homogenate samples were stored at –70 °C until used.

Direct Fluorescent Antibody Technique

The direct fluorescent antibody test (DFAT) was performed as described previously [19, 28]. Impression smears

were made on appropriately labelled pre-cleaned slides by turning the slides over the assembled portion of the brain stem, hippocampus, cerebellum, and cerebrum. The slides were air dried at room temperature and fixed by placing them in a coplin jar containing acetone at –20 °C for 30 minutes. The slides were removed from the acetone and then air-dried at room temperature. The fixed slides were transferred to a humidified chamber and a drop of 150 µl of fluorescein-labelled monoclonal anti-rabies immunoglobulin (Fujirubio Diagnostics, Inc., USA) was used for staining. These were then incubated at 37 °C for 30 minutes. After incubation, the slides were washed three times with PBS (pH 8.5). The slides were then air-dried at room temperature and arranged in a slide carrier. A drop of 50 % mounting buffered glycerol and a cover slip were applied on each smear. The slides were visualized under a fluorescent microscope (Zeiss International, Germany). The presence of bright/dull/dim yellow-green, oval or ellipsoid fluorescing intracellular accumulations was considered positive. Fluorescence was scored by two separate individuals using a three-plus scoring system (scores were as follows: 3 +++ bright yellow green fluorescence; 2 ++ dull yellow green fluorescence; + dim but detectable yellow green fluorescence).

Enzyme Linked Immuno-Sorbent Assay

The test was carried out according to the manufacturer's instruction (MyBioSource, USA). Briefly, in the micro-ELISA strip plate, two wells were left as negative controls and another two wells as positive controls. Negative and positive controls in a volume of 50 µl were added to the negative and positive control wells respectively. In the sample wells, a 10 µl sample and a 40 µl Sample dilution buffer were added. 100 µl of HRP-conjugate reagent was added to the positive control, negative control and sample wells and then mixed very well by gentle shaking. The plate was incubated for 60 minutes at 37 °C after covering with an adhesive strip. Following incubation, the test wells were washed manually with 1 : 20 pre-diluted washing buffer solution (400 µl) by carefully peeling off the adhesive strip and washing 5 times. At each washing step, the wash solution was decanted after resting for one minute. After the last wash and decanting, any remaining wash solution was removed by aspirating. The plate was inverted and blotted against clean paper towels. Fifty microliter of Chromogen Solution A and 50 µl Chromogen Solution B were added to each well (shielded from light) and mixed by gently shaking. The plate was then incubated at 37 °C for 15 minutes. Fifty microlitres of the stop solution was added to each well to terminate the reaction and the wells were observed for colour changes.

The optical density (OD) of the test wells were then read at 450 nm using a microtitre plate reader ensuring that the bottom of the wells were clean prior to reading. The assay was carried out within 15 minutes after adding the stop solution. The critical value (cut off) was calculated as the average OD value of the negative control +0.15. The sample was canine rabies virus positive if the OD value ≥ cut off. Scoring was done based on the values of the sample OD and cut off.

Rapid Immuno-Diagnostic Test

The test was done according to the manufacturer's instruction (Quickings, China) and as described previously [11]. Briefly, swab stick was inserted into 10 % brain tissue fluid homogenates (prepared as described earlier) until saturated and then placed into the assay buffer tube where it was thoroughly agitated to ensure good sample extraction. The cassette was taken out from the foil pouch and placed horizontally. Gradually, 3 drops of sample extraction were dripped into the sample hole using a disposable dropper. The result was interpreted in 5—10 minutes. The presence of both control band and test band on the strip (whether test band is clear or vague) was considered positive. The test and control lines on the test strips were scored by two separate individuals using a three-plus scoring.

Data analysis

The intensity of the fluorescence was counted and given one point per cross (+: 1 point; ++: 2 points; +++: 3 points). The concordance coefficient and simple Cohen's kappa coefficient value were used for statistical comparison of the diagnostic tests. The concordance coefficient values were expressed as a percentage. The kappa value of the agreement levels was interpreted as follows: poor agreement ≤ 0.20; fair agreement 0.20—0.40; moderate agreement 0.40—0.60; good agreement 0.60—0.80; and very good agreement ≥ 0.80. The confidence interval was calculated by assuming a binomial distribution. All statistical procedures were done using the MedCalc Software (MedCalc Software bvba, Version 17.8).

RESULTS

A total of 50 archived dogs brain tissues were tested with ELISA, RIDT and also with DFAT, which was used as a reference method. Forty four (88 %) of the 50 brain samples tested positive by DFAT, 42 (84 %) tested positive by ELISA and 21(42 %) tested positive by RIDT (Table 1). Two (4 %) samples that were negative for rabies antigen by DFAT were positive by ELISA. Twenty two (44 %) samples that were positive by DFAT were negative by RIDT while 1 (2 %) sample that was negative by DFAT was positive by RIDT (Table 2). However, we found 96 % agreement (42 positives and 6 negatives) of ELISA and DFAT and 54 % agreement of RIDT and DFAT (20 positives and 7 negatives). Compared to DFAT, the sensitivities of ELISA and RIDT were 95.5 % and 47.6 % respectively, while the specificities of ELISA and RIDT were 100 % and 87.5 % respectively (Table 2).

The simple Cohen's kappa coefficient for ELISA relative to the DFAT was found to be 0.834 (95 % C. I. 0.613—1.0). For RIDT, the Kappa value relative to DFAT was 0.170 (95 % C. I. 0.003—0.337). The concordant result of the various techniques was shown in Figure 1. The concordance coefficient for ELISA and RIDT relative to DFAT were 78 % (0.6366 to 0.8654) and 17 % (95 % C. I.; 0.05138—0.2752) respectively.

Table 1. Rabies antigen detection by DFAT, ELISA and RIDT

	DFAT	[%]	ELISA	[%]	RIDT	[%]
Positive	44	88	42	84	21	42
Negative	6	12	8	16	29	58
Total	**50**	**100**	**50**	**100**	**50**	**100**

DFAT — Direct Immuno-Fluorescent Test; ELISA — Enzyme Linked Immuno-Sorbent Assay; RIDT — Rapid Immuno-Diagnostic Test

DISCUSSION

The "gold standard" method for diagnosing rabies worldwide is the direct fluorescent antibody test (DFAT), which is recommended by the World Health Organization (WHO) and OIE [27, 38]. The main advantages of DFAT are its high sensitivity and specificity, even on fixed specimen [37] and that results can be obtained within 3~4 hours [9]. Despite the detectable advantages of the DFAT in di-

Table 2. Sensitivity and Specificity of ELISA and RIDT for rabies antigen detection in archived brain samples

		ELISA			RIDT		
DFAT		P	N	Total	P	N	Total
	P	42	2	44	20	22	42
	N	0	6	6	1	7	8
Total		42	8	50	21	29	50
Sensitivity		95.5 %			47.6 %		
Specificity		100 %			87.5 %		
		ELISA			**RIDT**		
DFAT		P	N	Total	P	N	Total
	P	42	2	44	20	22	42
	N	0	6	6	1	7	8
Total		**42**	**8**	**50**	**21**	**29**	**50**
Sensitivity		95.5 %			47.6 %		
Specificity		100 %			87.5 %		

P — positive; N — negative; DFAT — Direct Immuno-Fluorescent Test
ELISA — Enzyme Linked Immuno-Sorbent Assay; RIDT — Rapid Immuno-Diagnostic Test

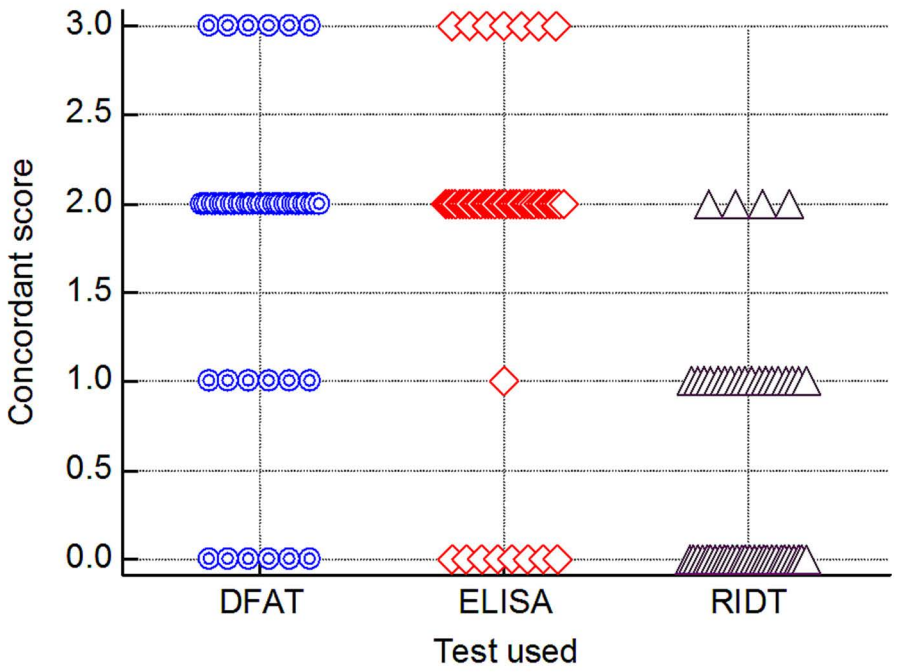

Fig. 1. Concordant results of dog brain tissues preserved at –20 °C

agnosing rabies, complementary diagnostic methods that can be more reliable as that of DFAT are needed. Any false negative results may lead to death and widespread under-reporting of the disease while false positive results can lead to unnecessary post exposure prophylaxis [15, 40]. Consequently, the true public health impact of rabies will be greatly underestimated and political commitment for its control would be lacking [7].

In this study, 88 % of the frozen brain specimens tested positive by DFAT, 84 % tested positive by ELISA and 42 % tested positive by RIDT. Similar findings have been recorded by earlier researchers; Whitefield et al. [37] recorded 66.9 % positive result by DFAT in frozen brain specimens. Of the 1253 specimens analysed in a trial by Perrin and Sureau [30], 651 were positive in both the DFAT and the ELISA. Two different studies conducted by Yang et al. [40] and Sharma et al. [33], gave 17 % and 64.7 % positive results respectively by RIDT on fresh samples. Generally, the accuracy of rabies diagnosis is dependent on the quality of the sample [13, 6], the type of anti-rabies conjugate used [31], virus antigen distributions in the brain and areas of the brain tested [4].

The present study evaluated the efficacy of RIDT to be used under laboratory and field condition for rabies diagnosis and obtained sensitivity and specificity of 47.6 % and 87.5 % respectively. This however, contradicts the findings of Nishizono et al. [25] who reported a sensitivity of 95.25 % and a specificity of 88.9 % using a type I RIDT kit which recognizes epitope II and III of the nucleoprotein of rabies virus. Similarly, Kang et al. [14] recorded a high sensitivity and specificity of 91.7 % and 100 % respectively. This variation in the sensitivity and specificity of RIDT was observed by Eggerbauer et al. [11] who compared six commercially available RIDTs for diagnostic and analytical sensitivity, as well as their specificity and concluded that the sensitivity and specificity varied considerably with different test kits. Also, none of the test kits investigated proved to be satisfactory, although the results somewhat contradicted previous studies, indicating batch to batch variations. Therefore, the low sensitivity and specificity of RIDT recorded in our study could be attributed to poor quality control and relatively low detection limit of the test kit used.

The ELISA is usable even on autolysed or partially degraded brain samples. It can be read qualitatively with the naked eyes and a large number of samples can be tested at the same time [9, 17]. However, false positive results due to cross reactivity with other antigens with very similar epitopes had been recorded [30]. In this study, the sensitivity and specificity of ELISA were shown to be 95.45 %

and 100 % respectively. This is in complete agreement with earlier studies [21, 26, 29, 32]. More recently, X u et al. [39] recorded a sensitivity and specificity of 97 % and 99.9 % respectively using a modified ELISA technique known as WELYSSA. In our study, 96 % agreement was observed between DFAT and ELISA. The very good strength of agreement between the ELISA and DFAT (Concordance coefficient 78 %; Kappa 0.834) implies that ELISA is as reliable as the DFAT and can be used in laboratories that cannot perform DFAT or whenever DFAT results are in doubt.

CONCLUSIONS

The ELISA is as reliable a diagnostic method as the DFAT which is the gold standard for rabies diagnosis. It has an advantage of being able to analyse large number of samples at the same time, making it more suitable for epidemiological studies and for laboratories that cannot perform DFAT. The unsatisfactory result of RIDT in this study reiterates the need to perform an adequate test validation before it can be used in the laboratory for rabies diagnosis.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Yakubu Dashe, the Head of Central Diagnostic Laboratory, National Veterinary Research Institute (NVRI), Vom, for his immense support. We would also like to appreciate Dr. Tekki, I.S and the entire staff of rabies laboratory, NVRI for their contribution and the technical support provided during the course of this work.

REFERENCES

1. **Aguiar, T. D. F., Teixeira, M. F. S., Costa, E. C., Vitaliano, A. B., Teles, C. H. A., Barroso, I. C. et al., 2013:** Medium-term cryopreservation of rabies virus samples. *Rev. Soc. Bras. Med. Trop.*, 46, 678—683.
2. **Atuman, Y. J., Ogunkoya, A. B., Adawa, D. A. Y., Nok, A. J., Biallah, M. B., 2014:** Dog ecology, dog bites and rabies vaccination rates in Bauchi State, Nigeria. *IJVSM*, 2, 41—45.
3. **Barecha, C. B, Girzaw, F., Kandi, V., Pal, M., 2017:** Epidemiology and public health significance of rabies. *Persp. Med. Res.*, 5, 55—67.
4. **Bingham, J., van der Merwe, M., 2002:** Distribution of rabies antigen in infected brain material: determining the reliability of different regions of the brain for the rabies fluorescent antibody test. *J. Virol. Methods.*, 101, 85—94.
5. **Boulger, L. R, Porterfield, J. S., 1958:** Isolation of a virus from Nigerian fruit bats. *Trans. R Soc. Trop. Med. Hyg.*, 52, 421—424.
6. **Cliquet, F., Freuling, C., Smreczak, M., van der Poel, W. H. M., Horton, D., Fooks, A. R. et al., 2010:** Development of harmonized schemes for monitoring and reporting of rabies in animals in the European Union. *EFSA Sci. Rep.*, 7, 1—60.
7. **Coleman, P. G., Fèvre, E. M., Cleaveland, S., 2004:** Estimating the public health impact of rabies. *Emerg. Infect. Dis.*, 10, 140—142.
8. **Dacheux, L., Wacharapluesadee, S., Hemachudha, T., Meslin, F.-X., Buchy, P., Reynes, J. M. et al., 2010:** More accurate insight into the incidence of human rabies in developing countries through validated laboratory techniques. *PLoS Negl. Trop.*, 4, 765.
9. **Duong, V., Tarantola, A., Ong, S., Meya, C., Choeung, R., Ly, S. et al., 2016:** Laboratory diagnostics in dog-mediated rabies: an overview of performance and a proposed strategy for various settings. *Int. J. Infect. Dis.*, 46, 107—114.
10. **Dürr, S., Naïssengar, S., Mindekem, R., Diguimbye, C., Niezgoda, M., Kuzmin, I. et al., 2008:** Rabies diagnosis for developing countries. *PLoS Negl. Trop.*, 2, 206.
11. **Eggerbauer, E., Benedictis, P., Hoffmann, B., Mettenleiter, T. C., Schlottau, K., Ngoepe, E. C. et al., 2016:** Evaluation of six commercially available rapid immunochromatographic tests for the diagnosis of rabies in brain material. *PLoS Negl. Trop. Dis.*, 10, 1—16.
12. **Ehizibolo, D. O., Nwosuh, C., Ehizibolo, E. E., Kia, G. S. N., 2009:** Comparison of the fluorescent antibody test and direct microscopic examination for rabies diagnosis at the National Veterinary Research Institute, Vom, Nigeria. *Afr. J. Biomed. Res.*, 12, 73—74.
13. **Fooks, A. R., Johnson, N., Freuling, C. M., Wakeley, P. R., Banyard, A. C., McElhinney, L. M. et al., 2009:** Emerging technologies for the detection of rabies virus: challenges and hopes in the 21st century. *PLoS Negl. Trop. Dis.*, 3, 530.
14. **Kang, B. K., Oh, J. S., Lee, C. S., Park, B. K., Park, Y. N., Hong, K. S. et al., 2007:** Evaluation of a rapid immunodiagnostic test kit for rabies virus. *J. Virol. Methods*, 145, 30—36.
15. **Lembo, T., Niezgoda, M., Velasco-Villa, A., Cleaveland, S., Ernest, E., Rupprecht, C. E., 2006:** Evaluation of a direct rapid immunohistochemical test for rabies diagnosis. *Emerg. Infect.*

Dis., 12, 310—313.

16. **Mallewa, M., Fooks, A. R., Banda, D., Chikungwa, P., Mankhambo, L., Molyneux, E. et al., 2007:** Rabies encephalitis in malaria-endemic area, Malawi, Africa. *Emerg. Infect. Dis.*, 13, 136.
17. **Mani, R. S., Madhusudana, S. N., 2013:** Laboratory diagnosis of human rabies: Recent advances. *Sci. World. J.*, 2013, 1—10.
18. **Menezes, R., 2008:** Rabies in India. *CMAJ*, 178, 564—566.
19. **Meslin, F. X., Kaplan, M. M., Koprowski, H., 1996:** *Laboratory diagnosis of rabies*. Geneva, World Health Organisation, 88—95.
20. **Messenger, S. L., Smith, J. S., Orciari, L. A., Yager P. A., Rupprecht, C. E., 2003:** Emerging patterns of rabies deaths and increased viral infectivity. *Emerg. Infect. Dis.*, 9, 151—154.
21. **Miranda, N. L., Robles, C. G., 1991:** A comparative evaluation of a new immunoenzymatic test (RREID) with currently used diagnostic tests (DME and FAT) for dog rabies. *Southeast Asian J. Trop. Med. Public Health*, 22, 46—50.
22. **Morvan, J., Mouden, J. C., Coulanges, P., 1990:** Rapid diagnosis of rabies by the ELISA method. Its application in Madagascar: advantages and disadvantages. *Arch. Inst. Pasteur Tunis*, 57, 193—203.
23. **Mshelbwala, P. P., Audu, S. W., Ogunkoya, A. B., Okaiyeto, S. O., James, A. A., 2013:** Detection of rabies antigen in the saliva and brains of apparently healthy dogs slaughtered for human consumption and its public health implications in Abia State, Nigeria. *ISRN Vet. Sci.*, 2013, 468043.
24. **Nel, L. H., 2013:** Discrepancies in data reporting for rabies, Africa. Emerg. Infect. Dis., 19, 529—533.
25. **Nishizono, A., Khawplod, P., Ahmed, K., Goto, K., Shiota, S., Mifune, K. et al., 2008:** A simple and rapid immunochromatographic test kit for rabies diagnosis. *Microbiol. Immunol.*, 52, 243—249.
26. **Oelofsen, M. J., Smith, M. S., 1993:** Rabies and bats in a rabies-endemic area of southern Africa: application of two commercial test kits for antigen and antibody detection. *Onderstepoort J. Vet. Res.*, 60, 257—260.
27. **Office International des Épizooties (OIE), 2008:** *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 6th edn., OIE, Paris, 304—322.
28. **Office International des Épizooties (OIE), 2013:** Rabies (Infection with rabies virus). *OIE Terrestrial Manual*, 1—28.
29. **Perrin, P., Gontier, C., Lecocq E., Bourhy, H., 1992:** A modified rapid enzyme immunoassay for the detection of rabies and rabies-related viruses: RREID-lyssa. *Biol.*, 20, 51—58.
30. **Perrin, P., Sureau, P., 1987:** A collaborative study of an experimental kit for rapid rabies enzyme immunodiagnosis (RREID). *Bull. World Health Organ.*, 65, 489—493.
31. **Robardet, E., Andrieu, S., Rasmussen, T. B., Dobrostana, M., Horton, D. L., Hostnik, P. et al., 2013:** Comparative assay of fluorescent antibody test results among twelve European National Reference Laboratories using various anti-rabies conjugates. *J. Virol. Methods*, 191, 88—94.
32. **Saxena, S. N., Madhusudana, S. N., Tripathi, K. K., Gupta, P., Ahuja, S., 1989:** Evaluation of the new rapid rabies immunodiagnosis technique. *Indian J. Med. Res.*, 89, 445—448.
33. **Sharma, P., Singh, C. K., Narang, D., 2015:** Comparison of immunochromatographic diagnostic test with heminested reverse transcriptase polymerase chain reaction for detection of rabies virus from brain samples of various species. *Vet. World*, 8, 135—138.
34. **Singathia, R., Dutta, P., Yadav, R., Gupta, S. R., Gangil R., Gattani, A., 2012:** Current update on rabies diagnosis. *IJAVMS*, 6, 229—240.
35. **Tekki, I. S., Ponfa, Z. N., Nwosuh, C. I., Kumbish, P. R., Jonah, C. L., Okewole, P. A. et al., 2016:** Comparative assessment of seller's staining test (SST) and direct fluorescent antibody test for rapid and accurate laboratory diagnosis of rabies. *Afr. Health Sci.*, 16, 123—127.
36. **Umoh, J. U., Blenden, D. C., 1981:** Immunofluorescent staining of rabies virus antigen in formalin-fixed tissue after treatment with trypsin. *Bull. World Health Organ.*, 59, 737—744.
37. **Whitfield, S. G., Fekadu, M., Shaddock, J. H., Niezgoda, M., Warner, C. K., Messenger, S. L., 2001:** A comparative study of the fluorescent antibody test for rabies diagnosis in fresh and formalin-fixed brain tissue specimens. *J. Virol. Methods*, 95, 145—51.
38. **World Health Organization (WHO), 1992:** WHO expert committee on rabies. *World Health Organ. Tech. Rep. Ser.*, 824, 1—84.
39. **Xu, G., Weber, P., Hu, Q., Xue, H., Audry, L., Li, C. et al., 2007:** A simple sandwich ELISA (WELYSSA) for the detection of lyssavirus nucleocapsid in rabies suspected specimens using mouse monoclonal antibodies. *Biol. J. In. Assoc. Biol. Stand.*, 35, 297—302.
40. **Yang, D. K., Shin, E. K., Oh, Y. I., Lee, K. W., Lee, C. S., Kim, S. Y. et al., 2012:** Comparison of four diagnostic methods for detecting rabies viruses circulating in Korea. *J. Vet. Sci.*, 13, 43—48.

Received September 18, 2017
Accepted December 6, 2017