

Assessment of Compliance of Referral Veterinary Hospitals to Sample Collection, Preservation and Reporting of Suspected Cases of Rabies in South-West Nigeria

Ojo DT^{1*}, Olugasa BO² and Mshelbwala PP³

¹Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, Borno State, Nigeria

²Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Oyo State, Nigeria

³Department of Veterinary Medicine, University of Abuja, Nigeria

*Corresponding author: Ojo DT, Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, Borno State, Nigeria, Tel: +2348035745529; E-mail: sitomed4sure@yahoo.com

Received date: Dec 09, 2015; Accepted date: Feb 13, 2016; Published date: Feb 15, 2016

Copyright: © 2016 Ojo DT, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Retrospective records of suspected cases of rabies were retrieved from Veterinary Hospitals in Osun, Ekiti, Ogun and Oyo states in south-west Nigeria; to establish the number of clinically suspected cases reported between January 2009 to December 2013. Preserved brain specimens from dogs suspected of rabies submitted to the Veterinary Hospitals in Ogun and Oyo states were collected for rabies antigen test using the Direct Rapid Immunohistochemistry Test (DRIT). Key informant interview (KII) was conducted in these hospitals on the routine performance of case confirmation through laboratory methods which involve sample collection, preservation and transport to nearby laboratories.

Veterinary Hospitals in Osun, Ekiti, Ogun and Oyo states respectively had six, two, five and four cases of rabies reported within the period under study. Retrieved records revealed that only 5.9% (1/17) of diagnosis made were based on a confirmatory laboratory test. Of all the 47 brain specimens that were subjected to DRIT, 12.8% (6) of the specimens were indeterminable while 10.6% (5) of the specimen tested positive for rabies antigen. The KII revealed that 86.7% (13/15) of the interviewee claimed the Veterinary hospital where they work diagnosed rabies based on the history and presentations of clinical signs. Specimens for diagnostic purposes were reportedly not collected from suspected cases at respective Veterinary hospitals. Only 33.3% (2/15) of the interviewee claimed the Veterinary hospital where they work were involved in habitual specimen collection but were usually restrained by inadequate power, transport and facilities for preserving specimens in their respective Veterinary hospitals.

Findings in this study clearly show that rabies is still prevalent in the south-west Nigeria. The findings also show that methods of storage of specimens from suspected rabies cases are poor.

Keywords: Rabies; DRIT; Antigen; Veterinary hospital; South west

Introduction

Rabies is a fatal viral disease that affects all warm blooded vertebrates including man [1]. It is the most severe acute viral infection of humans with case fatality rate of almost 100% [2]. Rabies is caused by an infection with the rabies virus (RABV), a single stranded, negative sense, neurotropic RNA virus of the genus *Lyssavirus* type 1 and family *Rhabdoviridae*; and in rare cases by other non-rabies virus *Lyssaviruses* (e.g., *Duvenhage virus*) [2,3].

The domestic dog (*Canis familiaris*) plays a principal role as a reservoir and transmitter of the disease to humans accounting for over 99% of human transmissions [4].

Rabies which is an important public health problem especially in the developing countries and very much so in Nigeria has been linked to how common and regular dog bites occur and how adherence to routine vaccination of dogs are very low in Nigeria [5]. About 10,000 annual human cases were reported in Nigeria [6] making the disease a persistent endemic problem in Nigeria since it was first reported in

1912 [6-8]. The first laboratory confirmation of rabies in Nigeria was in 1925 by the demonstration of negri bodies in the brain smear of a rabid dog [9].

Confirmation of suspected rabies cases include demonstration of negri bodies which are intracytoplasmic eosinophilic inclusions, in histological sections or fresh bilateral smears of samples from hippocampus (Ammon's horn), brain stem and the cerebellum after staining with seller, haematoxylin & eosin or Mann stain which was first carried out in 1903. This method which has been proven to have 50-80% reliability in detecting antigens in infected animals [10] has been superseded by other methods. The Direct fluorescent antibody test (DFAT) is the current OIE and WHO prescribed method for rabies virus detection [4,11] because of its reliability and sensitivity. It is a quick test but the expensive nature of equipment's such as fluorescent microscope and the expensive antibody conjugate makes its usage a challenge in Africa.

Several immunohistochemical tests able to detect rabies antigen in formalin-fixed sections are available. These tests detect rabies antigen using peroxidase-labelled antibodies which has been demonstrated in many studies to be as sensitive as FAT [12], while

other studies demonstrated higher sensitivity using peroxidase [13]. Histochemical tests are easy to perform and can be carried out on archived materials [10]. The direct rapid immunohistochemical test (DRIT) is a histochemical test developed by the Center for Disease Control and Prevention (CDC, USA) for detection of rabies virus using an immunoperoxidase technique [14]. Lembo et al. [15] had 100% sensitivity and 100% specificity when he compared DRIT and DFAT in the diagnosis of rabies in Tanzania under field and laboratory conditions.

In Nigeria and other developing countries of Africa, confirmations of suspected cases of rabies meet with various challenges such as obtaining reagents and breakdown of major equipment's, i.e. florescent antibody test (FAT) microscope [16,17]. Hence, many suspected cases are never confirmed. Specimens are preserved in various forms with the expectation that an opportunity to validate may arise.

The advent of the Direct Rapid Immunohistochemistry Test (DRIT) and other rapid diagnostic tests provides an opportunity to validate the prevalence of the disease among presented cases at referral veterinary hospitals in South-western Nigeria. Against this background, this study was designed to assess such specimen and to test for rabies antigen at the University of Ibadan Center for Control and Prevention of Zoonoses (CCPZ) with the following objectives, to; examine compliance of referral veterinary hospitals to rabies reporting, ascertain appropriate sample collection and specimen preservation for confirmation of clinically suspected human and dog cases of rabies, determine the prevalence of rabies in animals in south-west Nigeria and examine vaccination status of animals suspected of rabies, that were presented to the referral hospitals detect rabies antigen in clinical preserved brain samples suspected for rabies using DRIT.

Materials and Methods

Study location

The study was carried out in four (4) states in south-west Nigeria namely:

1. Osun state
2. Ekiti state
3. Ogun state
4. Oyo state

Study design and scope

The study design was a retrospective, cross-sectional and laboratory based study.

Data collection process

Retrospective data from suspected rabies cases were retrieved from selected referral veterinary hospitals in the four South-western states. Hospital records dating back from January 2009 to December 2013 were purposively reviewed.

A Key Informant Interview (KII) containing a set of questions was outlined to key persons in referral veterinary hospitals. These persons included: Veterinarians, hospital record officers, and/or laboratory technicians. Questions were outline in themes focusing on rabies specimen collection, specimen preservation, specimen transport and storage and diagnosis.

Stored specimens were obtained from selected referral veterinary hospitals. Specimens not stored for more than 12 months and still in good condition were collected and transported to the Rabies Diagnostic Laboratory Unit at the University of Ibadan Centre for Control and Prevention of Zoonoses (CCPZ).

Direct Rapid Immunohistochemistry Test (DRIT)

Test was carried out according to Standard Operating procedure prescribed by Centers for Disease Control and Prevention (CDC) Rabies Section.

Results

Hospital records

Number of cases: A total of seventeen cases were reported between January 2009 to December 2013, in the respective referral hospitals visited in the four south-west states. In Osun state, Ministry of Agric and food security clinic, Osogbo had five (5) cases reported while Ministry of Agric and food security clinic, Ilesha had one (1) case. In Ekiti state, the State veterinary Hospital, Ado-ekiti and the State veterinary Hospital Ijero had one (1) case each reported. Ogun state had a total of five (5) cases; State Veterinary Hospital, Abeokuta had two (2) while the Veterinary Teaching Hospital FUNAAB had three (3). In Oyo state, all four (4) cases were from the Veterinary Teaching Hospital UI. The State Veterinary Hospital, Mokola kept no record of suspected rabies cases as all cases brought to them were referred to the VTH, UI.

Method of diagnosis: 100% (17) of cases were suspected of rabies based on the history and clinical signs of the disease. 11.7% (2/17) of cases were taken for histopathology to detect the presence of Negri-bodies in the brain tissues. Only 5.9% (1/17) of diagnoses made was based on a confirmatory laboratory test, Fluorescent Antibody Test (FAT).

Vaccination history: 5.9% (1/17) of suspected cases reported had an up to date anti-rabies vaccination history. 52.4% (9/17) of suspected cases reported did not have up to date anti-rabies vaccine history. 41.2% (7/17) of suspected cases reported had an unknown anti-rabies vaccination history.

DRIT

All six (6) formalin preserved specimen obtained from VTH FUNAAB showed indeterminable results with DRIT. Six (6) of the forty two (42) dog brain specimen obtained from VTH UI tested positive for rabies antigen and twenty(20) were negative.

Prevalence of rabies

$$\begin{aligned} \text{Prevalence} &= \frac{\text{Total no. of samples positive}}{\text{Total no. of samples collected}} \times 100 \\ &= \frac{6}{47} \times 100 \\ &= 12.8\% \end{aligned}$$

Key informant interview

Method of diagnosis and sample collection and preservation: All interviewees agreed that at least one suspected case of rabies has been brought to their facility between January 2009 to December 2013.

86.7% (13/15) of persons interviewed admitted to the major method of arriving at a diagnosis in a suspected case of rabies in the Veterinary hospital where they are employed as use of history and clinical signs. 33.3% (2/15) of persons interviewed claimed the Veterinary hospital where they work collect samples from suspected cases of rabies reported for laboratory confirmation. 33.3% (2/15) of interviewed persons claimed the facility where they work have at one time or the other confirmed suspected rabies case(s) reported to their facility.

None of the 33.3% of interviewed persons that claimed that the health facility where they work collect samples from suspected cases of rabies reported, claimed to possess the proper equipment's and conditions needed for preservation of samples collected.

Rabies reporting: None of the interviewees knew if their facility followed the proper reporting system for rabies (Figures 1 and 2).

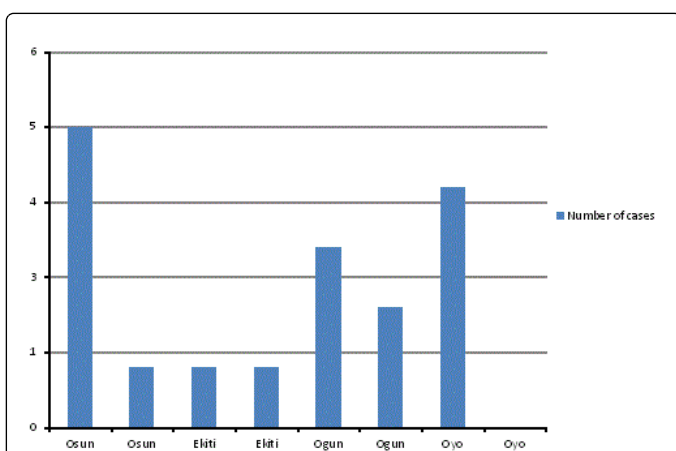


Figure 1: Number of cases reported between 2009 and 2013.

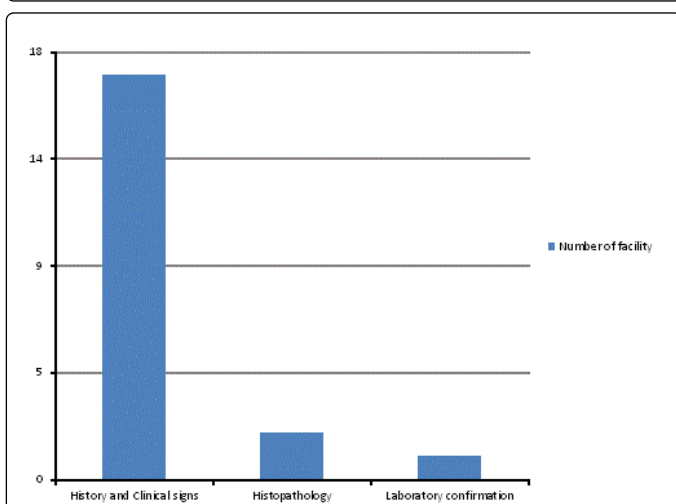


Figure 2: Frequency of diagnostic methods used at referral hospitals.

Discussion

Hospital records obtained from the referral hospitals visited, revealed that rabies is still endemic in Nigeria, even though the picture

of the true incidence cannot be obtained from the clinical records. Kitala et al. [18] compared the results of active surveillance put up in Kenya for a period of one year between 1992 and 1993, and the result of the passive surveillance that was on ground before and found that approximately 860 rabid dogs per 100 000 dogs were confirmed in this study, compared to approximately 12 per 100000 confirmed rabid dogs reported by the existing passive-surveillance system. This active surveillance also underestimated the true rabies incidence, because only 41% (130/317) of the potential specimens could be diagnosed but it was way higher and probably more efficient than the passive surveillance system.

Laboratory confirmation of suspected rabies cases was carried out in very few cases (5.9%) which agree with the findings by Wilde et al. [19]. Idachaba [20] reported an average of 100 confirmed of rabies cases annually in National Veterinary Research Institute (NVRI) Vom, Nigeria, which is highly indicative of poor attitude towards confirmatory diagnoses of rabies cases, when compared to the 10,000 persons who have been estimated to be exposed to rabies annually in Nigeria: that is apparently also underestimated. Ehizibolo et al. [21] records state that this is undoubtedly an underestimation of rabies prevalence in Nigeria which stems from lack of submission of specimens collected from suspected rabies cases to NVRI or other laboratories for confirmation. Lack of confirmation of rabies cases through any laboratory diagnostic method does not allow the true incidence of rabies in the nation to be estimated and this is a major hindrance in the planning and execution of any control measure.

Diagnoses of rabies in southwest Nigeria is largely carried out by history and clinical signs of the disease which cannot be said to be pathognomonic for the disease [22]. This method of diagnoses would have led to a lot of misdiagnoses and wrong decisions in post exposure prophylaxis (PEP) administration to dog bite victims and consequently, human deaths.

Only 5.9% (1 of 17) cases reported to the four referral hospitals had an up-to-date anti-rabies vaccination history which spells out why rabies is still a tough battle to overcome in south-west Nigeria and indeed in other parts of Nigeria (Table 1). This agrees with the report of Ehimiyein [23] in North -West Nigeria, where it was established that 75% of dogs were not vaccinated against rabies. These figures are short of the target vaccination levels of 70%; and sustained and effective vaccination coverage of 70% in the dog population has been termed sufficient to prevent outbreaks of dog rabies [24]. In the study carried out by Ogunkoya et al. [25] it was observed that the total doses of dog anti-rabies vaccines produced by NVRI, Vom between 1956 and 2006 (51 years production) was 2,137,615 with the highest annual production of 183,600 doses in 1979. This figure represents an average annual production of 43,625 doses per year which is rather too low to ensure annual vaccination (60-70%) of the current estimate of over 8 million dog population in Nigeria but rather revealing that only about 10% of dogs' population ever received anti-rabies immunization.

With 28.8% (19/66) of total specimen obtained from referral hospitals not good enough for the DRIT, it spells out another challenge of laboratory confirmation of specimen collected with inadequacies in preservation and transport facilities. In a prospective study of brains carried out in Bangkok Thailand in 1996, only 1.11% of the specimens were decomposed and not eligible for the rabies antigen detection using FAT which is lower than the amount of decomposed specimen obtained during this study. In Nigeria and many other developing countries in the tropics, at least 10% of brain samples received at laboratories for rabies diagnosis are decomposed because of

inadequacy of transportation and storage facilities [25-27] leading to the misdiagnosis of most of the cases. For efficient transportation,

various methods of preservation and transportation have been experimented [25,27] but are yet to be fully developed for use.

States visited	Osun state		Ekiti state		Oyo state		Ogun state	
Towns	Osogbo	Ilesha	Ado-ekiti	Ijero	Ibadan	Ibadan	Abeokuta	Abeokuta
Name	Ministry of Agric and food security clinic, Osogbo	Ministry of Agric and food security clinic, Ilesha	State veterinary Hospital, Ado-ekiti	State veterinary Hospital, Ijero	State Veterinary Hospital, Mokola	Veterinary Teaching Hospital, UI	State Veterinary Hospital, Abeokuta	Veterinary Teaching Hospital, FUNAAB
No. of cases reported	5	1	1	1	Nil-refer cases	4	2	3
Method of diagnoses	History, c/s, quarantine & observation	History, c/s, quarantine & observation	History, c/s, quarantine & observation	Laboratory confirmation	Nil	History, c/s, quarantine and observation	History, c/s, quarantine & observation	History, c/s, quarantine & observation and laboratory confirmation
Method of Handling					Referred to Veterinary Teaching Hospital, U.I		Referred to Veterinary Teaching Hospital, FUNAAB	

Table 1: Summary of cases reported to the four referral hospitals.

The 12.8% prevalence of rabies antigen in dog brain samples from two referral veterinary hospitals obtained from this study was higher than that obtained by Hambolu et al. [28] in Lagos state who reported 1.58% and 5% reported in Abia State [8], this may be attributed to the fact that they sampled apparently healthy dogs as against this present study that targets suspected cases.

The indeterminate result obtained with the formalin preserved samples could be as a result of damage to the integrity of the brain which is probably the reason behind the introduction of prior trypsinization to improve the immunofluorescent staining of antigens in formalin-fixed, paraffin- embedded tissues by Huang et al. [29].

The effect of the Virus Transport Medium on the integrity of the specimens could not be ascertained in this present study, but the purpose for which it was stored on the medium was not rabies antigen detection. The referral hospital which could not even state the type of Virus Transport Medium used to preserve the specimen or the reason why that choice was made or the diagnostic method the specimen were intended for would not be able to tell the effect or action of the Virus Transport Medium on the integrity of the brain cells.

Reports on rabies to the international community is low, in fact Nigeria gave report on rabies to World Health Organization only once between 1988 and 1992 [30]. At least, 10,000 persons have been estimated to be exposed to rabies annually in Nigeria, apparently underestimated [21] because there is no efficient monitoring and reporting system of the disease.

Conclusions

Rabies is very much endemic in Nigeria as the referral hospitals in the four south-west states visited, saw at least two cases reported in the past five years under review.

Low vaccination coverage which is short of the target anti-rabies vaccination coverage of 70-80% recommended by WHO shows that rabies is not on its way out of the nation unless an active step towards achieving this is embarked upon by the government.

The lack of collection of specimen from cases presented to referral hospitals, proper preservation of specimen when they are collected and submission of these specimen to laboratories for confirmation makes the true incidence of the disease in Nigeria and the extent of havoc it causes are difficult to estimate

Recommendations

Referral hospitals should improve upon record keeping system so as to enable disease reporting and disease trace back which is an important aspect of surveillance. Hospital records should be computerized to reduce the bulkiness of paper work during research and better clerking of patients and clients should be embarked upon in order to have full details that will enable disease surveillance and trace back. Clients should be educated on the importance of giving full details about themselves and their animals.

The government and other stakeholders should intensify awareness programs and carry out sustained vaccinations campaigns in south-west Nigeria and other parts of the nation to be able to achieve the target anti-rabies vaccination coverage of 70-80% recommended by WHO.

Facilities for proper specimen collection, preservation and transport should be made available in referral hospitals as this is key in ensuring that specimen meant for confirmation arrive laboratories in good condition that will enable confirmatory diagnoses to be carried out.

Incentives should be made available for rabies reporting as this will encourage community members and veterinarians or other animal

health worker to participate in the reporting of rabies cases to appropriate authorities

Acknowledgements

The authors wish to express their gratitude to CDC for the provision of the DRIT kit which was used for this study, Prof. Olapade for allowing us use his microscope to view our slides and to the members of staff of CCPZ for their immense contribution to the actualization of this study.

References

1. Zulu GC, Sabeta CT, Nel LH (2009) Molecular epidemiology of rabies focus on domestic dogs (*Canis familiaris*) and black-backed jackals (*Canis mesomelas*) from northern South Africa. *Virus Res* 140: 71-78.
2. Jackson AC (2013) Current and future approaches to the therapy of human rabies. *Antiviral Res* 99: 61-67.
3. Salmón-Mulanovich G, Vásquez A, Albújar C, Guevara C, Laguna-Torres VA, et al. (2009) Human rabies and rabies in vampire and nonvampire bat species, South-eastern Peru, 2007. *Emerg Infect Dis* 15: 1308-1310.
4. World Health Organisation Expert Committee on Rabies (1992) 8th Report. World Health Organisation Technical Report Series No 824: 84.
5. Adeyemi IG, Zessin K (2000) Retrospective dog rabies vaccination evaluation at the University of Ibadan, Nigeria (1988-1992). *Vet arhiv* 70: 223-230.
6. Nawathe DR (1980) Rabies Control in Nigeria. *Bull of Int Epiz* 92: 129-139.
7. Umoh JU, Belino ED (1979) Rabies in Nigeria. A historical review. *Int J Zoonoses* 6: 41-48.
8. Mshelbwala PP, Ogunkoya Ab, Maikai BV (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 Veterinary Science*.
9. Boulger LR, Hardy J (1960) Rabies in Nigeria. *West Afr Med J* 9: 223-234.
10. Jogai S, Radotra BD, Banerjee AK (2000) Immunohistochemical study of human rabies. *Neuropathology* 20: 197-203.
11. Dean DJ, Abelseth MK, Atanasiu P (1996) The fluorescent antibody test. In: Meslin FX, Kaplan MM, Koprowski H (Eds) *Laboratory Technique in Rabies* (4th Edn) WHO, Geneva pp: 88-95.
12. Kotwal S, Narayan KG (1985) Direct immunoperoxidase test in the diagnosis of rabies--an alternative to fluorescent antibody test. *Int J Zoonoses* 12: 80-85.
13. Bourgeon AR, Charlton KM (1987) The demonstration of rabies antigen in paraffin embedded tissues using the peroxidase- antiperoxidase method: a comparative study. *Canadian Journal Veterinary Research* 51: 117-120.
14. Niezgoda M, Rupprecht CE (2006) Standard operating procedure for the direct rapid immunohistochemical test for the detection of rabies virus antigen. National Laboratory Training Network Course. Atlanta: US Department of Health and Human Services, Centers for Disease Control and Prevention pp: 1-16.
15. Lembo T, Niezgoda M, Velasco-Villa A, Cleaveland S, Ernest E, et al. (2006) Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerg Infect Dis* 12: 310-313.
16. Otolorin GR, Aiyedun JO, Mshelbwala PP, Ameh VO, Dzikwi A, et al. (2015) A Review on Human Deaths Associated with Rabies in Nigeria. *Journal of Vaccines & Vaccination* 6: 1.
17. Mshelbwala PP, Abdullahi SU, Maikai BV, Onyiche ET, Ogunkoya AB (2015) Evaluation of Two Rapid Diagnostic Tests for Rabies Diagnosis under Field and Laboratory Conditions in Nigeria. *J Vaccines Vaccin* 6: 272.
18. Kitala PM, McDermott JJ, Kyule MN, Gathuma JM (2000) Community-based active surveillance for rabies in Machakos District, Kenya. *Prev Vet Med* 44: 73-85.
19. Wilde H, Briggs DJ, Meslin FX, Hemachudha T, Sitprija V (2003) Rabies update for travel medicine advisors. *Clin Infect Dis* 37: 96-100.
20. Idachaba SE, Olaleye S, Chukwukere S, Abechi AS, Ehizibolo DO, et al. (2009) Ten year (1998-2007) retrospective evaluation of the status of dog rabies in Plateau State, Nigeria. The proceedings of the 46 Annual Congress of the Nigerian Veterinary Medical Association "Anambra 2009" pp: 73-78.
21. Ehizibolo DO, Ehizibolo PO, Ehizibolo Sugun MY, Idachaba SE (2011) The control of neglected zoonotic diseases in Nigeria through animal intervention. *African Journal of Biomedical Research* 14: 81-88.
22. Fekadu M, Greer PW, Chandler FW, Sanderlin DW (1988) Use of avidin-biotin peroxidase system to detect rabies antigen in formalin-fixed paraffin embedded tissues. *Journal of Virological Methods* 19: 91-96.
23. Ehimiyein AM, Niezgoda M, Orclarri L, Osinubi MOV, Ehimiyein IO, et al. (2014) Efficacy of a Direct Rapid Immunohistochemical Test (DRIT) For Rabies Detection in Nigeria. *African Journal of Biomedical Research* 17: 101- 107.
24. Coleman PG, Dye C (1996) Immunization coverage required to prevent outbreaks of dog rabies. *Vaccine* 14: 185-186.
25. Elsa TA, Ogunkoya AB (1996) Survival of rabies virus in 50% buffered glycerol under different temperatures. *Nigeria Journal of Basic and Applied Sciences* 5: 15-18.
26. Barrat J (1996) Simple technique for the collection and shipment of brain specimens for rabies diagnosis. In: Meslin FX, Kaplan MM, Koprowski H (Eds) *Laboratory Techniques in Rabies*, World Health Organisation, Geneva, Switzerland pp: 425-432.
27. Dürr S, Naïssengar S, Mindekem R, Diguimbye C, Niezgoda M, et al. (2008) Rabies diagnosis for developing countries. *PLoS Negl Trop Dis* 2: e206.
28. Sunday E, Hambolu, Asabe A Dzikwi, Jacob KP, Kwaga, et al. (2014) Rabies and Dog Bites Cases in Lagos State Nigeria: A Prevalence and Retrospective Studies (2006-2011). *Global Journal of Health Science* 6:1.
29. Huang SN, Minassian H, More JI (1976) Application of immunofluorescent staining on paraffin sections improved by trypsin digestion. *Laboratory Investigation* 35: 383-390.
30. Ogunkoy AB (1997) Rabies Basic Concepts, Problems and Prospects of Its Control in Nigeria. (1st Edn) Oreofe Nigeria Ltd pp: 112-124.