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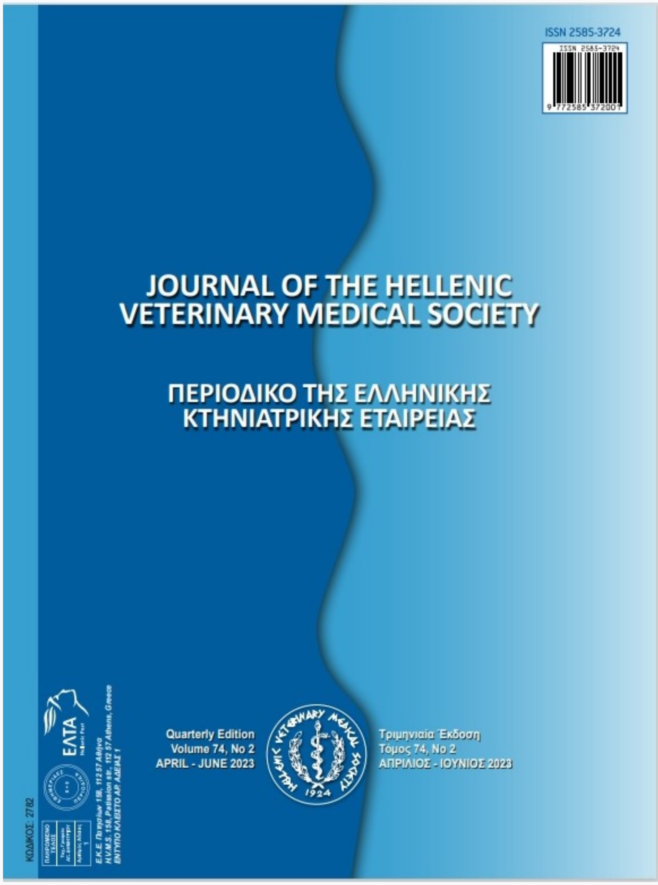

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Case report
Κλινικό περιστατικό

A case of rabies outbreak in a bull-calf from Nigeria

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ABSTRACT: This paper describes a case of rabies outbreak in a bull-calf which led to euthanasia following manifestation of clinical signs of the disease. Infection was confirmed using a rapid immunochromatographic test of the homogenates from brain tissues (the brain stem, hippocampus and cerebellum) sample. Exposure to rabies virus (RABV) had resulted due to an attack by a free-roaming dog (FRD). Mass vaccination campaigns against rabies and improving biosecurity measures to limit access of free-roaming dogs to farms can prevent the occurrence of RABV in dogs, livestock animals and personnel at risk in Nigeria.

Keywords: Rabies; outbreak; dog bite; calf; livestock; Nigeria

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INTRODUCTION

Rabies is a 100% fatal zoonotic disease caused by an RNA virus that belongs to the genus *Lyssavirus* in the *Rhabdoviridae* family, transmitted primarily through the bite of a rabid animal and can be prevented by three effective interventions: public awareness, mass dog vaccination, and post-exposure treatment such as prophylaxis and wound care (Tenzin *et al*., 2020; Chevalier *et al*., 2021). Biting or scratching wounds and licking broken skin and mucous membranes exposed to saliva from a rabid animal are common ways for transmitting the rabies virus (RABV) to humans and other animals (WHO, 2018). Rabies is primarily transmitted to livestock animals such as camels, cattle, sheep, and goats by bites from rabid dogs, but it may also be transmitted through bites from other rabid carnivores (Ahmed *et al*., 2020). Dogs are responsible for up to 99% of human rabies infections, resulting in more than 50,000 deaths globally each year, mainly in the developing countries of Africa and Asia (Tenzin *et al*., 2020; Chevalier *et al*., 2021).

The vicious cycle of lack of reliable epidemiological data, the negligence of the decision-makers and populations, lack of public information and sensitisation on the risk of rabies, and geographic and financial hurdles to accessing rabies vaccinations contribute to the persistence of rabies in Africa (Dodet, 2009). Nonetheless, for poor African countries, implementing a countrywide vaccination campaign is a significant challenge, as they frequently fall short of the needed vaccine coverage (Wobessi *et al*., 2021). Despite the severity of the reported outbreaks and health implications of rabies in Nigeria, control strategies such as mass vaccination and dogs movement restrictions are insufficiently implemented (Ahmad *et al*., 2017). This study reports a case of an outbreak of rabies in a bull-calf in Zamfara, Nigeria.

CASE HISTORY

On August 13, 2020, a nomad brought to the clinic requesting veterinary assistance to investigate the deteriorating health condition of a four-and-a-half-month-old Sokoto Gudali male calf, which manifests dehydration (sunken eyes), respiratory distress, loss of gait, muscular tremor, and jerking. A local veterinary technician treated the calf for limping on August 12, 2020, with intramuscular injections of antibiotics (Penicillin-streptomycin) and diclofenac sodium. The condition of the calf, however, did not improve as a result of these interventions. According to a detailed medical history and physical examination, the calf had been bitten by a stray dog on the lateral abdomen/trunk (Figure 1) 4 weeks before the clinic visit. The bite wound had healed long before the animal began to show clinical signs. On August 13, 2020, the calf was humanely euthanised, and a postmortem examination was performed due to a suspicion of rabies virus (RABV) exposure. There was no detectable gross pathology in the organs or tissues following necropsy

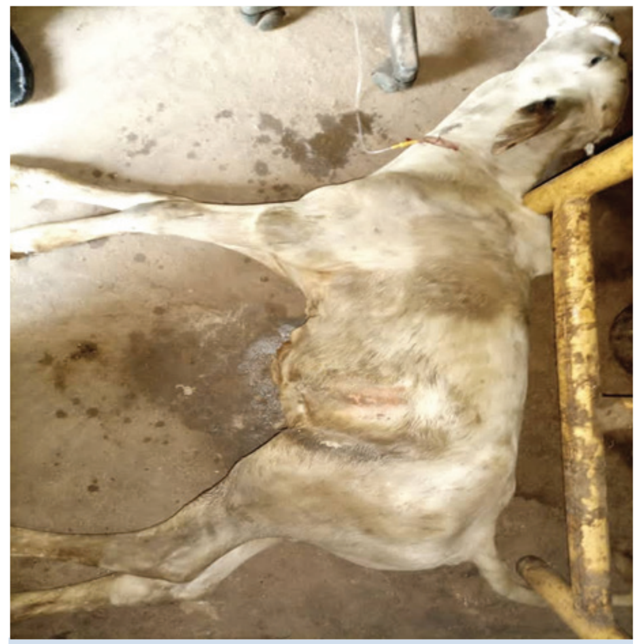

Figure 1 Healed wound (arrowed dark) on the lateral abdomen/trunk of the calf from the bite of a free-roaming dog.

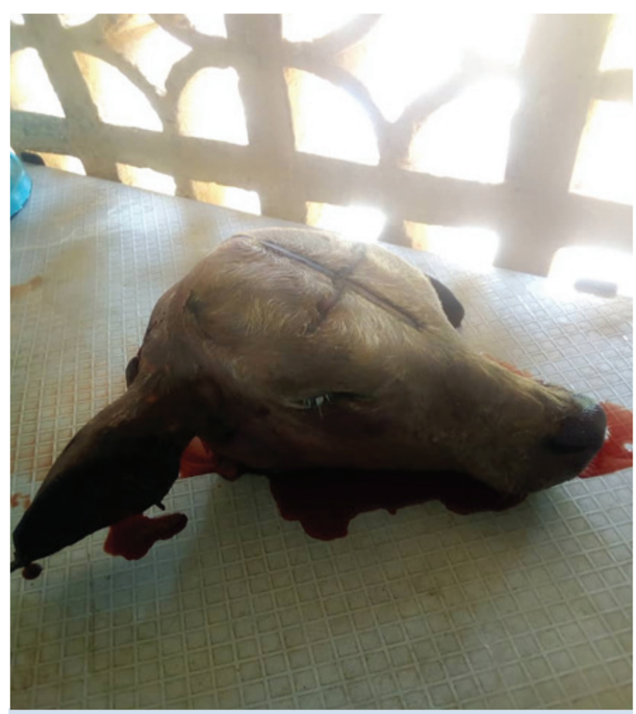

Figure 2 Decapitated head of the humanely euthanized calf.

of the entire carcass. The head was decapitated (Figure 2) and transported on ice to the Rabies Diagnostic Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria, for a rapid immunochromatographic test of a sample from the removed brain tissues (Figure 3). The carcass was incinerated, and the possibly contaminated equipment and clinic floor were disinfected. The rapid immunochromatographic test (lateral flow assay) is appropriate for use in the field, particularly in developing countries with inadequate diagnostic capabilities. It is used to detect rabies virus antigen in brain tissues homogenates and saliva from canine, bovine, and raccoon dogs. Antigen-antibody neutralisation is the basic principle underpinning this test. Antibodies directed against epitopes of the rabies virus nucleoprotein are gold conjugated, and the antigen-antibody complex is then immobilised by a second antibody that is fixed on the test strip (Tenzin *et al*., 2020).

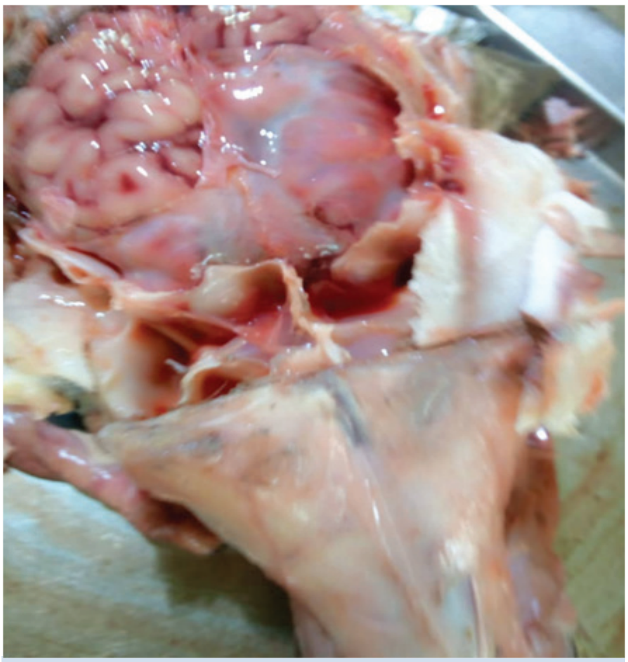

Figure 3 Opened skull of a decapitated calf to harvest brain tissues for a rapid immunochromatographic test.

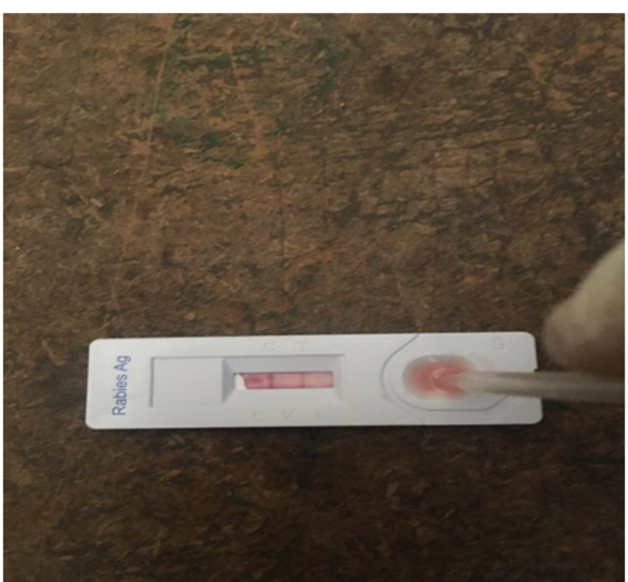

Figure 4 Performing a rapid test on the brain tissues homogenate from a calf bitten by a free-roaming dog. Immunodetection of the RABV antigens by lateral flow technique - RABV was positive, having two purple bands on “C” and “T” lines shown on the result window of a test cassette.

The harvested brain was confirmed to be infected with the rabies virus using a rapid immunochromatographic test (BioNote, Korea) as previously described by Kaltungo *et al*. (2018). Briefly, the brain tissues (brain stem, cerebellum and hippocampus) was swabbed using the cotton swab supplied along with the kit, and then the swab was dipped into the specimen tube containing 1 ml of assay diluent and well swirled to achieve a proper sample extraction. To attain good sample extraction, the swabbing of the brain with a swab and mixing into the assay diluent was repeated 3-5 times. The test cassette was taken out of the foil packet and put on a dry, flat surface. Four drops of the extracted sample were added to the sample well of the cassette using the disposable dropper provided with the kit, and the result was interpreted in 5-10 minutes. After being placed into the sample well, the sample homogenate flows via the gold-labelled monoclonal antibody (Mab) pad, the test zone (“T”), and finally the control zone (“C”). When two purple coloured bands appear within the result window, one on the control line (“C”) and the other on the test line (“T”), the test result is considered positive (Figure 4). A purple band on the test line (“T”) is only visible in the result window if there are enough rabies virus antigens in the sample. A negative result is indicated by the presence of only one purple coloured band (“C”) within the result window, and a result is regarded invalid if no purple coloured band appears.

DISCUSSION

Rabies is widespread in Nigeria, with stray dogs regarded as the principal reservoirs transmitting the virus mainly through bites, enhanced by easy contact between dogs and farm animals/humans (Mschelbwala *et al*., 2013; Atuman *et al*., 2014). The high incidence of infection in rabies-endemic regions is primarily due to free-roaming dogs (FRDs) in human settlements (Tiwari *et al*., 2018). Rabid animals, such as cattle, can also be a potential source of rabies infection to humans, in addition to canines and wildlife reservoirs (Chao *et al*., 2021). Veterinary staff had been reported to contract RABV through damaged skin after inserting bare hands into a rabid cow's mouth for routine examination in observed clinical signs of excessive salivation and difficulty swallowing misdiagnosed as choked throat (Wen *et al*., 2006; Simani *et al*., 2012).

According to the reported observations, there has been an increase in the occurrence of rabies in ruminant livestock such as cattle, sheep, and goats across Nigeria in the last ten years, all as a result of rabid FRDs bites (Mshelbwala *et al*., 2013; Ahmad *et al*., 2017; Ibrahim *et al*., 2017; Kaltungo *et al*., 2018; Dauda *et al*., 2020). This case is no exception, as the affected calf had rabies due to a stray dog bite. Meanwhile, due to varying incubation periods influenced by wound location or depth, the infected cattle developed clinical signs of rabies at different points in time (Chao *et al*., 2021). It has been found that the affected cattle mostly had dog attacks around head and neck regions (47.8%) at the proximity of the CNS, followed by fore and hindquarters (30.4%), and lastly, the truck region (Dar *et al*., 2014). In this case, the calf was bitten by FRD in mid-July, and rabies clinical signs appeared in the middle of August, implying a nearly month-long incubation period. Similarly, this supports previous findings of a 15 to 30 day incubation period in cattle exposed to RABV after being bitten by stray dogs (Jemberu *et al*., 2013; Chao *et al*., 2021).

Many rabies outbreaks in Nigeria are attributed to a lack of rabies control in pet dogs (with access to veterinary services) and free-roaming dogs (FRDs), the majority of which are not registered and are not kept at home in rural and urban areas (Al-Mustapha *et al*., 2021). As previously reported, direct contact with rabid FRDs increases the risk of rabies transmission to livestock and humans (Tu *et al*., 2018; Chao *et al*., 2021). The lack of biosecurity measures to prevent FRDs from accessing animal farms might have been a factor in RABV transmission into cattle herds, typical of extensive livestock production in Nigeria. Due to the existing dog ecology and extensively practised animal production, Nigeria's most viable and efficient strategy is to vaccinate livestock animals such as cattle, sheep, and goats. For cattle in rabies-endemic areas, such as Nigeria, pre-exposure prophylaxis against rabies is recommended (OIE, 2014). Cattle respond well to available rabies vaccines, producing long-lasting serum antibody levels indicative of protection after at least two vaccinations, with a priming dose given at or after six months of age and a subsequent booster given as late as three years after the initial one (Anderson *et al*., 2014; Gilbert *et al*., 2015; Yakobson *et al*., 2015). Furthermore, health promotion for preventing dog bites, dog vaccination, and human post-exposure prophylaxis is regarded as foundations of robust rabies control programmes worldwide (Okeme *et al*., 2020). This report is limited in that the rapid immunochromatographic test used to detect RABV infection was not backed up with either of the additional tests required by the standard for further confirmation, such as fluorescence antibody test (FAT), histological examination, molecular test, virus isolation in cell culture, or mouse inoculation experiment. Using the fluorescent antibody test as the reference standard, the rapid immunochromatographic test has shown a sensitivity of 100% and a specificity of 100% when diagnosing rabies in the field and the laboratory (Lembo *et al*., 2006).

This report presents a case of RABV infection in a bull-calf resulted from a stray dog bite. Biosecurity measures such as preventing entry of dogs into farms and timely prophylactic vaccination of livestock animals should be adopted in all regions across Nigeria. More so, timely anti-rabies vaccination of dogs and personnel (e.g., veterinarians, hospital staff, and farm workers) at the risk of exposure is recommended.

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