CORPUS PUBLISHERS

Corpus Journal of Dairy and Veterinary Science (CJDVS)

Volume 3 Issue 2, 2022

Article Information

Received date : April 19, 2022

Published date: May 09, 2022

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Keywords

Rabies; Non-Canine Species; Sahelian Regions; Cameroon

Research Article

Rabies in Non-Canine Species: A Retrospective Study of Laboratory Detected Cases in the Sahelian Zone of Cameroon from 2014 To 2020

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Abstract

A seven years retro-prospective study (2014–2020) was conducted to determine non-canine rabies cases detected at the LANAVET, in the Northern regions of Cameroon. Information was collected from laboratory registers and reports within the defined period. The main results show that 76/278 (27.3%) non-canine rabies cases have been detected (63/218 in DFAT: 28.9%; 13/60 in PCR: 21.7%). Five groups (Chiroptera: 88.2%; Felines: 6.6%; Primates, Bovidae and Suidae: 5.3%) and ten animal species were concerned. These results highlight the necessity to conduct an active surveillance of rabies in dogs and bats, to minimise other mammal cases.

Introduction

Rabies is a vaccine-preventable zoonotic disease of all warm-blooded animals including human caused by a Lyssavirus [1,2], and is present on all continents and endemic in most African and Asian countries [3]. This fatal viral disease transmitted to humans through contact (bites or scratches) with infected animals, both domestic and wild; 99% of rabies cases are dog-mediated and the burden of disease is disproportionally borne by rural poor populations, with approximately half of cases attributable to children under 15 [2]. The zoonosis is of major medical and economic importance causing an underestimated human mortality of 59,000 deaths per year worldwide [4]. The detection of rabies viral antigens by Direct Fluorescent Antibody Test (DFAT) in clinical specimens, preferably brain tissue (collected post mortem) is the gold standard for rabies diagnosis [5]. There are several interactions between the 3 types of epidemiological cycles of rabies: canine rabies, sylvatic rabies and chiropteran rabies [6]. Thus, transmission is average from canines to other species [6] and this makes rabies cases rare in domestic species other than dogs. In Cameroon, financial losses linked to rabies prevention measures and post-exposure treatments of over US $71, 0687 and 1690.28±4.76 years of life lost between 2004–2013 have been documented in three cities Garoua, Yaoundé and Ngaoundéré [7]. Urban rabies is wildspread and dogs have long been considered as the source of animal and human rabies in Cameroon [8,9] (Kouri 1985, Awah-Ndukum et al, 2003, Awah-Ndukum et al, 2004). Despite the endemicity of dog rabies in Cameroon, there is paucity of data on rabies in other mammals and canids in the country. To bridge this knowledge gap, this current paper was aimed at reporting cases of rabies other than canine rabies diagnosed at the National Veterinary Laboratory (LANAVET) from 2014 to 2020.

Methodology

Data collection

Data were extracted from laboratory registers and archived DFAT and/or PCR rabies-related reports. Information relative to the history of the suspicions and the origin of samples was also collected.

Laboratory analysis

The heads of animals suspected of rabies were sent to the LANAVET by the field public veterinarians for diagnosis. After reception and registration of samples, they were taken to the rabies necropsy room and placed under the biological safety cabinet for removal of the brain. Using a scalpel blade, the skin and flesh were removed and then an incision on the skull was made using an electric saw, hammer and chisel. Once the skulls were opened, the brain tissues were aseptically removed and placed in sterile petri dishes. Dishes with samples were clearly labelled with the processing date, diagnostic number and region/site of origin and then wrapped in aluminium foil prior to laboratory analysis. The remnant heads were incinerated in order to avoid as much as possible any exposure or contamination with the external environment. The detection of rabies antigens in brain samples (hippocampus, medulla oblongata, cerebral cortex or cerebellum) by direct immunofluorescence is the reference method recommended by the OIE and WHO [5]. On fresh samples, the DFAT gives reliable results in more than 95 to 99% of cases [5]. It is carried out by fixing cerebral smears (previously fixed with acetone) using anti-nucleocapsid antibodies (mono or polyclonal) coupled with fluorescein and allowing the detection of all the different species of Rabies virus. A positive response during detection under a fluorescence microscope, yields greenish or yellowish-green inclusions, clearly brilliant with variable shape and size [1]. In the LANAVET, the techniques used are DFAT and conventional Reverse Transcription Polymerase Chain Reaction (RT-PCR).

Direct fluorescent antibody test: The brains collected were analysed by DFAT at the LANAVET in Garoua as recommended by the OIE [5]. For this purpose, a smear of brain tissue (primarily the Ammon horn) was made as follows: a small fragment

from the tissue to be tested was taken and spread on a slide by crushing it with a second slide to obtain a thin layer. After drying, the slides were placed in a slide holder filled with cold acetone (-20 °C) for one hour, then dried again (30 min) and incubated for 30 min with the anti-nucleocapsid conjugate (0.1ml/slide) at 37 °C in a humidified chamber according to the manufacturer's instructions (BIO-RAD® Kit, 357-2112). Washing was done two times for 5 min with PBS (Phosphate Buffer Saline) and a few seconds in distilled water. The Conjugate used was a rabies anti-nucleocapsid conjugate, adsorbed, freeze-dried, consisting of labelled polyclonal antibodies capable of detecting all known Lyssavirus species, regardless of the animal species tested. Each work session was validated by the absence of fluorescence on the negative control slide (brain of dog previously tested negative) and their presence on the positive control slide (brain of dog previously tested positive in LANAVET).

Conventional reverse transcription polymerase chain reaction: Some rabies suspected samples were analysed by RT-PCR. The primers allow the detection of all virus variants as the N gene is a well conserved gene in rabies viruses (Lelièvre et al, 2009). It takes place in 02 main steps: extraction of the genetic material (RNA) using GIAGEN® kit according to the manufacturer's instructions then preparation of the reaction mixture and amplification as described by the OIE Terrestrial Manual [5]. The primers used are indicated in Table 1.

Table 1: Primers for Rabies virus.

Primers	Sequences (5'–3')	Position
RabForPyro	AACACYYCTACAATGGA	59–75
RabRevPyro-biot 1	TCCAATTNGCACACATTTTGTG	662–641
RabRevPyro-biot 2	TCCARTTAGCGCACATYTTATG	662–641
RabRevPyro-biot 3	TCCAGTTGGCRCACATCTTRTG	662–641

Results and Discussion

From 2014 to 2020, rabies antigens have been detected in 76 of 278 tested samples, in 5 species other than dogs, namely Chiroptera (bats), Felines (cats), primates (monkeys), domestic Bovidae (Bovine) and Suidae (pigs) (see Tables 1 & 2 below). Most of rabies cases detected during routine diagnosis were in cats (5/8, 62.5%); those detected in Chiropterans are the result of a study on the prevalence of rabies in bats and the risk of transmission to humans (publication in progress). The clinical signs noted during the collection of anamnestic data, include aggressiveness and attacks, resulting in bites or scratches. Some animals were observed to mimic the dog's barking behaviour. Rabies in cats is difficult to diagnose in the early stages and, of the 14 cats examined by a veterinarian, only 3 were believed to have rabies on initial examination. Major signs of rabies in cats reported by veterinarians included behavioural change, gait abnormality, strange or unusual look in the eyes, and a wound within the preceding 6 months. Owners reported increased frequency of vocalization as an early sign [10]. An estimated 21 476 human deaths occur each year in Africa due to dog-mediated rabies. Africa is estimated to spend the least on PEP and have the highest cost of human mortality [2]. In a study on epidemiologic factors, clinical findings, and vaccination status of rabies in cats and dogs in the United States in 1988, all animals (cats and dogs) had received only a single dose of vaccine in their lifetime and were vaccinated when they were between 3 and 6 months old [11]. This could mean that rabies cases are a consequence of vaccination failures. Besides scientists have shown that once 70% of dogs are vaccinated, rabies can be successfully controlled in an area and human deaths can be prevented [4]. In non-human primates, regional tourism, the wildlife trade and the cultural practice of keeping these animals as pets, particularly in coastal regions, appear to be major risk factors for the increase in human cases [12]. In fact, cases detected at LANAVET where from people who keep these animals at home as pets (Table 3).

Table 2: Analysed samples grouped by diagnostic technique.

Diagnostic Technique	No Tested	% Tested	No of Positive	% Positive
DFTA	218	78,4%	63	28,9%
PCR	60	21,6%	13	21,7%
Overall	278	100%	76	27,3%

Table 3: Summary of data related to the cases detected.

Group	Scientific Name	No Tested	No of Cases	Diagnosis (PCR or DFAT)	Regions	Commemoratives
Felines	*Felix catus*	05	05	DFAT	Adamawa (01), North (04)	Aggression and incessant mewing, attacks on children and adults
Primates	*Cebus capucinus*	02	02	PCR	Adamawa, Far North	Aggression and scratches (attacks on children)
Chiroptera	*Chaerephon chapini*	212	57	DFAT	North	Study of rabies in bats in the northern region of Cameroon
	Chaerephon leucogaster					
	Chaerephon pumilus		10	PCR		
	Eidolon helvum					
	Scotophilus leucogaster					
Bovidae	*Bos indicus*	01	01	DFAT		Aggressiveness and 'barking'
Suidae	*Sus scrofadomesticus*	01	01	PCR	Adamawa	Aggressiveness and 'barking'
Total		278	76			

Conclusion

Rabies continues to ravage animals and the risk of transmission to humans is increasing. Vaccination programmes are most often targeted at dogs and cats, and to a lesser extent at primates. The disease is most commonly transmitted to humans by dogs, cats, bats. This study shows that most of non-canine rabies cases in domestic animals are from cats, and bats are great reservoirs with a serious human exposition risk [13]. These results highlight the necessity to conduct an active surveillance of rabies in dogs, cats and bats, to minimise other mammal cases.

Acknowledgement

This work was carried out under the supervision and the technical support of the National Veterinary Laboratory headquarters-LANAVET (Garoua).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

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