Original Research Article

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RABIES IN A STREET DOG IN NEW VALLEY GOVERNORATE, EGYPT.

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ABSTRACT: This work directs the attention toward a public health hazard in New Valley Province that may be caused by rabies infection which is known as a fatal viral zoonosis caused by lyssavirus a member of the family Rhabdoviridae, order Mononegavirales. Both warm blooded animal and humans are susceptible to the virus infection mainly through bites of infected animals. Detection of rabies virus was confirmed in a saliva sample obtained from a street dog found showing rolling salivation, off food, general weakness and paralysis of the hind limbs followed by death. Virus detection was confirmed through application of Rabies virus antigen kit; mice inoculation test; cell culture assay, virus neutralization test and direct fluorescent antibody technique. Finally, this result spots the light on the importance of application of rabies control programs in free roaming dogs in order to protect humans and animal populations in New Valley Province. In addition, further studies are in need to know more about the prevalence of rabies and the suitable available methods to control and eradicate it.

KEYWORDS: Rabies; Fluorescent antibody technique; virus neutralization.

1. INTRODUCTION

Rabies is one of the neglected tropical diseases that predominantly affect already marginalized, poor and vulnerable populations. Rabies is present in all continents except Antarctica, with over 95% of human deaths occurring in Asia and Africa. Every year, more than 29 million people worldwide receive post exposure prophylactic vaccine of rabies that is succeeded to prevent hundreds of thousands of rabies deaths annually. Globally, the economic burden of dog-mediated rabies is estimated at US$ 8.6 billion per year, in addition to uncalculated psychological trauma for individuals and communities [1]. Rabies is a preventable viral disease most often transmitted through the bite of a rabid animal. It endangered wildlife species, dogs and cats 2. Rabies virus, the cause of the disease, is a member of the genus lyssavirus in the family Rhabdoviridae, order Mononegavirales. It has conical or bullet shape like other members of family Rhabdoviridae. Its genome is non-segmented, negative stranded RNA genome that is tightly encapsulated into ribonucleocapsid structures similar to other members of the mononegaviruses [3]. Lyssa virus genus is initially referred to rabies –related virus group as (lyssa: from Greek "rage, rabies" that is composed of six serotypes: serotype 1 contains some of classis rabies viruses isolated from a variety of wild and domestic animals around the world; serotype 2 that has been isolated from lagosbat virus; serotype 3 the Mokola viruses and serotype 4 the Duvenhage viruses and other two distinct European bat viruses EBL-1 and EBL-2, Both viruses were responsible for human rabies [4]. The disease affects CNS when virus reaches the brain leading to nervous manifestations as signs of mania, difficult in swallowing, paralysis that started from the hind limbs then directed to forward (trunk and fore limbs), recumbence and ends with death [5]. Reports concerned about the occurrence of rabies in Egypt between 1997 and 1999 clarified that 48 brain samples were collected from suspected rabid animals of different species and they were examined for detection of rabies virus antigen using direct immune fluorescence (DIF) technique. It was recorded that 34 out

of 48 samples (70.8%) were tested positive. Moreover, Nigri bodies were detected in 29 out of 48 brain samples (60.4%) using Seller's stain [6]. So, the present work aims to direct the attention toward the public health hazard of free roaming dogs that may harbor rabies virus especially those live in or near deserts an in case of New Valley Province where there is great opportunity for these dogs to come in contact with naturally infected wild animals.

2. MATERIALS AND METHODS

2.1. Ethical approval

Care and use of the animals were approved by the Medical and Veterinary Research Ethics Committee at the National Research Centre in Egypt (No., 20/053).

2.2. Infected Street dog

A street dog was found in New Valley Governorate, Egypt, showing rolling salivation, off food, general weakness and paralysis of the hind limbs. It was transferred under complete hygienic measures to a well isolated place where it dead after 3 days after observation of its presence. After sample collection, the dog carcass was hygienic disposed using incinerator under strict hygienic measures.

2.3. Sample

Saliva sample was collected from the dog under complete strict hygienic measures and antibiotics were added (100 microgram of streptomycin and 100 IU of Penicillin-G sodium/ml). The sample was kept at -20° C till subjected to trials of virus detection, serological and molecular characterization.

2.4. Rabies virus antigen detection kit

Rabies virus antigen kit was supplied by Bio Note, Inc. (Hwaseong-si, Korea) and used for rapid detection of rabies virus in saliva sample following the manufacturer's instructions.

2.5. Mice

Thirty Weaned Swiss Albino mice (4 weeks old) were used in trials to confirm the presence of rabies virus through the intramuscular mouse inoculation test (MIT) conducted as described by [7] following 3 successive passages where in each passage, 5 mice were inoculated and other 5 mice were kept without inoculation.

2.6. Cell culture

Baby Hamster Kidney (BHK21) cell line was cell lines propagated with Minimum Essential Medium supplemented with 10% new born calf serum and 100 microgram of streptomycin and 100 IU of penicillin-G sodium/ml were added to all cell culture media. The cell line was used for demonstration of the cytopathic effect (CPE) induced by the collected saliva sample and in virus neutralization test. For such purpose, BHK21 cell culture was prepared in 25 ml tissue culture flasks and infected with saliva sample leaving uninfected flasks without infection as control. Virus propagation was carried out through 3 successive passages according to [8].

2.7. Antisera

Rabies antisera unconjugated and conjugated with fluorescein isothiocyanate were supplied by the Department of Pet Animal Vaccine Research (DPAVR), Vet. Ser. Vac. Res. Inst. And used for serological identification of rabies virus

2.8. Detection of rabies virus

2.8.1. Direct Fluorescent Antibody Technique (DFAT):

DFAT was carried out on smears prepared of the brain of dead experimentally infected mice according to [9].

2.8.2. Virus neutralization test (VNT):

VNT was carried out on the 3rd virus passage in BHK cell line using specific anti-rabies serum according to [10].

NVVJ., Vol. 5, Issue (1), 2025

3. RESULTS and DISCUSSION

The described disease signs on the sick dog direct the attention toward rabies infection at the late stage of virus infection, in accordance with what described by [5] who mentioned that paralysis started from the hind limbs then directed to forward (trunk and fore limbs), recumbence and ends with death. Rapid detection of rabies virus in saliva sample using the rapid detection kit revealed clear positive result as shown in Fig. 1. In this respect, it was concluded that the application of a rapid diagnostic tests in detection of rabies antigen can greatly enhance disease surveillance and diagnostic activities, especially in resource poor settings. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of a rapid antigen detection test kit were evaluated relative to a FAT for its fit-for-purpose for confirmation of clinical cases of rabies for early response and enhancing rabies surveillance [9]. It was also found that the rapid test is sensitive and specific which agrees with findings from other studies conducted by [11, 12]. In addition, it is well known that Rabies occurs in saliva where infection usually occurs through infected saliva reaching a bite wound or skin scratches, and breached mucous membranes [13]. Mice inoculation test with saliva sample resulted in and showed paralysis of the hind limbs Fig. 2 by the 4th day and died by the 6th day after experimental infection coming in agreement with [14, 15] Application of DFAT on brain impression smears prepared of dead mice experimentally infected with the dog infected saliva showed strong positive results represented by apple green spots confirming the presence of rabies virus as shown inFig. 3a. In this respect, it was concluded that direct Fluorescent Antibody (DFA) test is the gold standard for routine sensitive and specific lyssavirus post-mortem diagnosis using fluorescein isothiocyanate (FITC)-labeled antibodies to rabies virus (RABV) and viewed using epifluorescence microscopy [16]. In addition, the FA test is now the most widely used method for diagnosing rabies infection in animals and humans where it is accurate and its results can often be obtained within 30 minutes of receipt of the specimen [17].

Inoculation of BHK-21 cell line with the infected saliva showed specific CPE of rabies virus by the 2nd day post infection characterized by cell rounding followed by cell lysis and detachment from the culture surface as shown in Fig. 4b . Similar findings were recorded by [6, 18]

In conclusion, although it is only one positive case of rabies in a street dog, but it constitutes a public health hazard facing human population in the study area. So, it is recommended to use rabies vaccine oral baits to control rabies as it is critically important to prevent human deaths and alleviate its burden in animal species on local and national economies.

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(a) Negative rapid test

(b) Positive rapid test showing clear line as the test control

Figure 1: Rapid detection of rabies virus in saliva sample using the rapid detection kit

Figure 2: Experimentally infected mouse with the obtained saliva sample showing paralysis of the hind limbs

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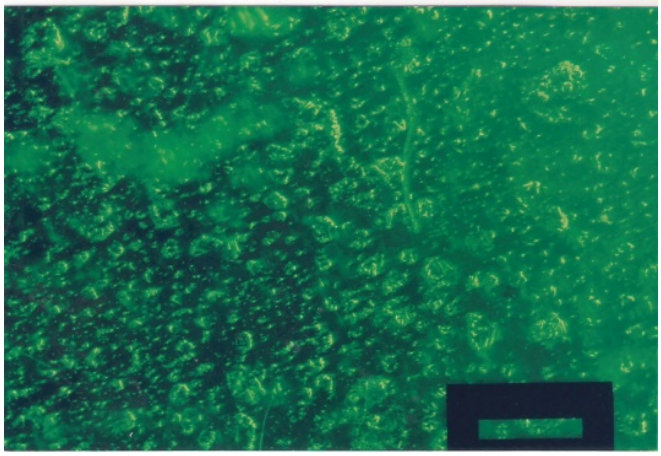
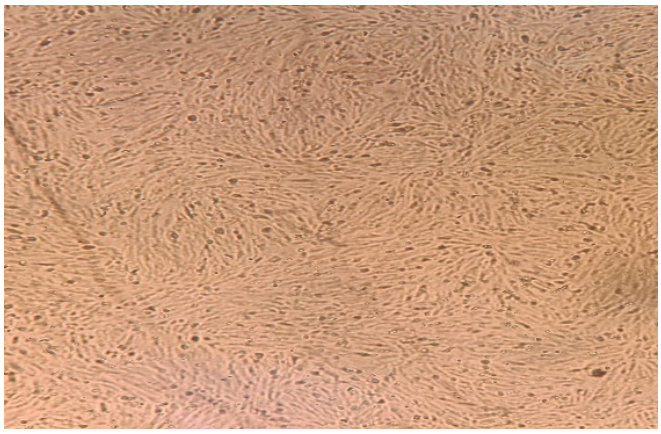
NVVJ., Vol. 5, Issue (1), 2025



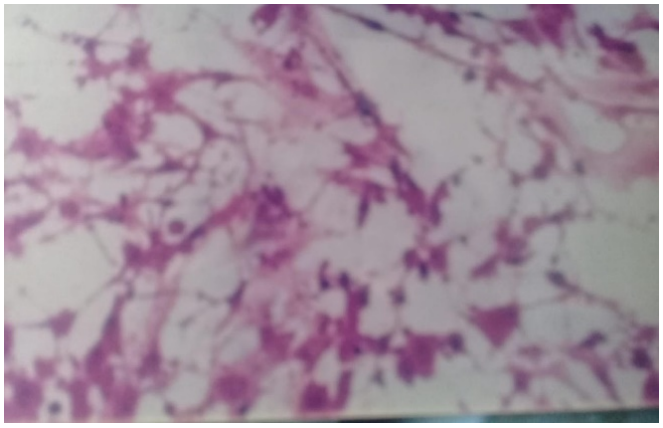
(a) Positive direct FAT carried out on the infected mouse brain smear showing apple green spots

(b) Negative direct FAT

Figure 3: Brain impression smears prepared of dead mice experimentally infected with the dog



(a) Normal BHK cell culture (100Xs)



(b) Infected BHK cell culture with the obtained rabies virus 3 days post cell infection

Figure 4: Cell line with the infected saliva showed specific CPE of rabies virus by the 2nd day post infection