Case Report

A case report of Rabies in a horse in Tabriz, Iran

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Summary

Rabies is a fatal zoonotic disease with an approximately global distribution and wide host range. Rabies is caused by a Lyssavirus (Rhabdoviridae) that infects the nervous system and salivary glands. In this study, an occurrence of rabies in a horse in Iran is reported. In the spring of 2014 a *Kurd* stallion with severe abnormal behavior was examined in the suburb of Tabriz. The history was taken and the clinical signs that were obvious consisted of: changes in behaviors such as severe aggressive mood and anxiety, ataxia, circling, severe drooling and salivation, muscle tremors, photophobia, and abnormal biting; fusion mode and hypersensitivity were also seen. Body temperature was 39 ^{o}C. To confirm the diagnosis of disease, brain sample was taken by the special rabies sampling device and sent to the rabies laboratory. Because rabies is suspected, to identify the rabies virus the sample was examined using Fluorescence Antibody Test (FAT) in which the observation of the oval apple-green Negri-bodies is diagnostic of the disease. Due to the aggressive behavior and the severity of furious type of the disease and regarding to biting and even killing some sheep by the horse, the stallion was fasten by chains and finally euthanized with presumptive diagnosis of rabies. At the end the case was reported to the provincial Veterinary Organization office. Although the incidence of rabies is low (about %5) in horses and it is relatively uncommon disease in this species, the potential for human exposure highlights the importance of the disease to be investigated in horses.

Keywords: Horse, Rabies, Fluorescence Antibody Test (FAT), Tabriz.

Introduction

Rabies is a fatal zoonotic disease, with an approximately global distribution and wide host range (Daniel and Horton, 2012) and it is one of the oldest infectious disease that known to medical science (Kaplan, 1986; The Hindu, 2016). Rabies is originally derived from the Latin word "*rabere"* which means "*madness*" (Frederik and Murphy, 1999). Rabies ranks 12th on the World Health Organization (WHO) list of major fatal diseases and is an OIE[1] list B disease (Panichabhongse, 2001). Endemic rabies was first confirmed in 1928 following the death of two children bitten by a yellow mongoose in the Wolmaransstad district of the North West Province (Neitz and Marais, 1932). In 2003, wild animals accounted for

[1] World Organization for Animal Health

more than 91% of all cases of rabies reported to the Centers for Disease Control and Prevention (CDC). According to WHO report (WHO, 2000), ten millions of people who were bitten by animals around the world were considered for prevention and treatment against rabies. About 40000 to 70000 rabies-related deaths were reported worldwide each year (Frederik and Murphy, 1999).

Rabies is caused by a neurotropic Lyssavirus (Rhabdoviridae family) bullet-shaped viruses, which have a single-stranded RNA genome and includes six genotypes that infect the nervous system and salivary glands (Radostits, 2000; Sellon and Long, 2014). An animal can become infected if the saliva from an infected mammal contaminates an open wound or a mucous membrane and any form of skin injuries (bite, scratch, or cut). Time between bite and the appearance of clinical signs can be long (usually 2-9 weeks) depending on the wound severity, wound site and its distance from the brain, amount and strain of virus, however, it can be longer than 6 months so the incubation period can vary from several days to many years (Bishop et al., 2003). The pathogenesis of disease is commenced by the entrance of viruses to the nerve cells and spreading out among the whole body. It reaches the salivary glands and nasal secretions after passaging down through appropriate cranial nerves (Abolfazl et al., 2006). These signs range from poor racing performance to unusual behavior and may include spinal cord-, cerebral-, and cranial nerve-related signs; evident lameness; gastrointestinal and genitourinary signs (Sellon and Long, 2014).

All warm-blooded animals are vulnerable to infection by rabies virus, however the degree of species susceptibility varies considerably (Radostits, 2000). Dogs have long been recognized as the main transmitters of the disease to humans and consisted of 90% of the total number of laboratory-proven animal rabies cases (King and Turner, 1993).

Rabies among wildlife of Iran is endemic and the infection frequently occurs in domestic animals (Eke et al, 2015). Rabies has a special place in the history of medical research in this country. In Iran, the most cases of animal rabies have been reported from the north, northeast and northwest of the country (Simani, 2003; Sharifian, 2006; Zeynali et al., 1999).

In horses, as in other warm blooded animals, rabies is a severe and rapidly progressive neurological disease (Wilkins and Del Piero, 2007). Although rabies in horses is low (5 percent) and relatively uncommon, the potential for human exposure makes it important to be considered (Fernanda, 1914). This study reports the occurrence of rabies in a horse in Iran and the discussion of its different epidemiological, clinical and diagnostic aspects.

Materials and Methods

Case History

In the spring of 2014 a *Kurd* Stallion with severe abnormal behavior was examined in the suburb of Tabriz, Iran. The history was taken. Clinical examinations including general observation and some specific examinations such as rectal

temperature, vision, and behavior were performed.

Sampling

Rabies sampling Kit, PAS-78 (Pateur Institute, Iran) was used to carry out Immunofluorescent (IF) test on brain samples. The sampling procedures consisted of the fallowing stages: Small part of the brain withdrawn by pressing the plastic straw into the skull through the hole of the occipital (Occipital Foramen). The number, the date and the sender details were marked on the sample vial. The vial was covered by cotton and was placed inside the plastic-zipped bag. The identification was written on the sample box and was sent to the laboratory.

During all above procedures, gloves were used and any contact with infected secretions of animal was avoided.

Immunofluorescence assay

The Kit 4 ×q.s. 3 ml vial, Lyophilized, absorbed anti- rabies nucleocapsid conjugate (code 357-2112) is used in this technique to detect antigen in specimens using specific labeled antibody. Each vial contains a quantity equivalent to 3 ml of adsorbed fluorochrome labelled immunoglobulins. In this way the smears were fixed in acetone at -20˚C for 30 minutes then a sufficient quantity of clarified conjugate (i.e. 0.1 ml) was added on each smear. They were incubated at 37˚C for 30 minutes in a moist chamber then washed in two successive phosphate buffer (PBS) baths for 5 minutes to remove unbound excess labeled antibody. Finally, a few drops of glycerin buffer were added. Visualization was performed under fluorescent microscope.

Result

History

There was no significant information in the horse background except that the horse was previously grazing in the pasture.

Clinical signs

The observed clinical signs in the infected horse were as fallowed: changes in behaviors such as severe aggressive mood and anxiety, ataxia, circling, severe drooling and salivation, muscle tremors, photophobia, and abnormal biting of stocks bars resulting in breaking of his teeth and bleeding from the mouth, and even killing some sheep. Also fusion mode and hypersensitivity were seen. Body temperature was 39ºC. The stallion was fasten by chains and finally euthanized due to sever and frenzy form of the clinical signs and with presumptive diagnosis of rabies.

Laboratory Diagnosis

In the dark field of the smear viewed under fluorescent microscope, oval-shaped apple-green Negri bodies were observed. These bodies are indicator of the attachment of antibodies to the virus antgens which is the confirmation of the disease (Fig. 1).

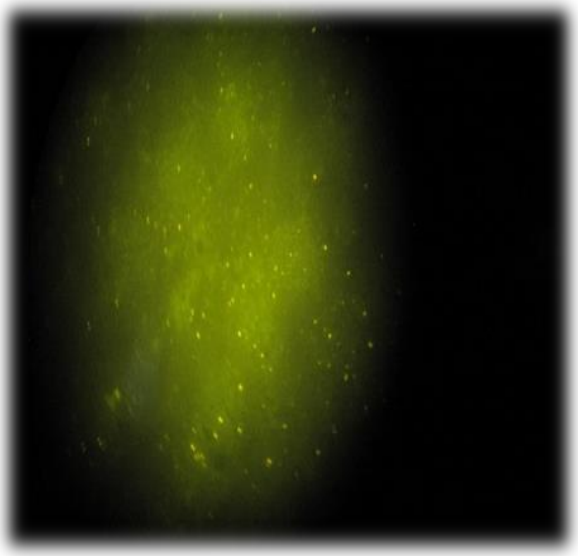

Fig 1. Brain material, stained with antirabies fluorescein conjugate, containing large numbers of oval shaped, apple-green Negri bodies

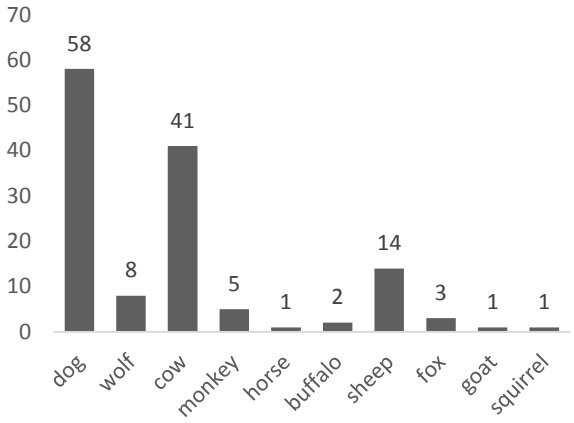

Fig 2. Positive cases of rabies in different species in East Azerbaijan province during 2004-2009.

Discussion

Since the rabies is always fatal, there is no good outcome for a horse that contracts the disease. Many horse owners do not consider the risk to their outdoor equine partners, while household pets are routinely vaccinated for rabies. The potential risk of human exposure from even one infected horse is significant. According to the 33rd World Survey of Rabies for the year 1997, the highest incidence and mortality cases of human rabies were reported from the underdeveloped countries of Asia such as India, Pakistan, and Bangladesh which have high populations and have no specific strategies for controlling rabies (WHO, 2001).

Human rabies is rare in the United States. In the late 1980s, an average of 300 cases of rabid livestock (including cattle, sheep, goats, and horses) were reported each year. In 2001 there were 51 equine and one infected human with rabies reported to the CDC. From 2005-2006, rabies cases in horses and mules increased 12.8%, but decreased by 20% in 2007. Since 2006, 8 horses in Florida have died from rabies. The 37 rabid horses and mules reported during 2010 represented a 9.8% decrease, compared with the number reported during 2009 (Blanton et al., 2011). Mexico reported 357 rabid animals during 2010, nearly 15 of reported rabid animals were horse (Blanton et al., 2011). The number of rabid horses in Ontario was zero from 1997 until the year 2000, when three horses with rabies were diagnosed. From 2000 to 2004, there were 13 rabid horses reported in Ontario. One case of horse rabies occurred in Dufferin County (Bob and Jocelyn, 2001).

Historically, in 1919, an Iranian delegation, dispatched in the peace treaty after World War I, visited the Pasteur Institute in Paris and made arrangements for the establishment of the Pasteur Institute for rabies prophylaxis in Iran. Rabies has been detected in many provinces and has a significant role in economic loss and social

disruption (Simani, 2003; Zeynali et al., 1999; Eslamifar et al, 2008). In Iran, the number of reported animal bites has increased dramatically from 66370 in 1987 to 183000 in 1997. The increase might in part be due to the improved reporting system due to the expansion of health centers to almost all rural areas. In 2002, 350 positive cases of animal rabies have been confirmed in all parts of country, which most of the positive cases have been due to dogs and ruminants especially the increasing number of stray dogs in different provinces of Iran that has raised the number of exposed persons nationwide (Rezaeinasab, 2007; Simani, 2003). Occurrence of rabies in horses in Iran is rare. Rabies can involves all breeds of horses. To date, there is not any previous report about the involvement of Kurdish horses in East Azerbaijan. East Azerbaijan province in which the distribution of rabies infection is severe due to the diverse climatic conditions, with the neighboring country of Azerbaijan (among the countries with high risk and lack of control) and assign a significant area of its territory (Fig. 2).

Rabies approval is difficult because of the wide range of its clinical signs (Fernanda, 1914). Rabies has classically been divided into three major stages; prodromal, furious and paralytic or dumb. All clinical signs are raised from the nervus system involvement and include initially paresthesia and pain at the wound site. Later fever, headache malaise and apprehension are noted. Progression of disease results in changes in mental status. Physical changes are also raised from nervous system involvement and include; difficulty in swallowing, hyper salivation, priapism, tenesmus (painful spasm of the anal sphincter), and ultimately paralysis, ataxia, head pressing and circling, lameness, dilated pupils and photophobia, abnormal chewing and biting. Some may go off feed or refuse to drink, while others continue to eat and drink until shortly before death (thehorse.com, 2009; Fernanda, 1914; Radostits et al., 2000; Bishop et al., and 2003). During 1970 to 1990 the records of 21 horses with rabies were reviewed. Clinical signs of disease at the time of initial examination included ataxia and paresis of the hindquarters, lameness, recumbency, pharyngeal paralysis, and colic. The major clinical signs observed over the course of hospitalization included recumbency (21/21; 100%), hyperesthesia (17/21; 81%), loss of tail and anal sphincter tone (12/21; 57%), fever (11/21; 52%), and ataxia and paresis of the hindquarters (11/21; 52%). Supportive treatment had no effect on survival time and did not correlate with the detection of Negri bodies at necropsy. Cerebrospinal fluid (CSF) was obtained from 6 horses and was determined to be abnormal in 5. The most common abnormality was a slightly high total cell count, with a predominance of lymphocytes (Green et al., 1992).

Most horses become recumbent and convulsed prior to death. The death occurs in three to five days due to the respiratory failure following the onset of clinical signs (Blood-Horse Publications, 2009). At present, there is no effective treatment for horses (and other animals, vaccinated or unvaccinated) with clinical rabies. With horses, generally, rabies infection is not

known until clinical signs appear (Bishop et al., 2003; Fernanda, 1914).

Confirmation of a rabies diagnosis cannot be made by gross pathology or histology and is only diagnosed at postmortem by submitting CNS samples, primarily brainstem, cerebellum, hippocampus and medulla to a local public health laboratory. Direct fluorescent antibody testing (FAT) of fixed brain smears will detect viral antigen, and confirmation of diagnosis can be made by inoculation of tissue culture, inoculation of mice or by molecular tests. A positive test means treatment should be started for anyone who has come in contact with the rabid animal. The most recent developments of real-time quantitative polymerase chain reaction (real-time PCR) not only allow rapid diagnosis but also differentiation between lyssavirus species (thehorse.com, 2009; Fernanda, 1914; Daniel and Horton, 2012). Some reports have shown that leukocytes have slightly increased on laboratory diagnosis but may also be normal or higher than 30000. Moreover, in few patients CSF was abnormal and the number of lymphocytes was increased along with a brief increase in protein content (less than 100 mg / dl) (Mandell et al., 2000).

Other diseases which present clinical symptoms similar to rabies must be considered for differential diagnosis. The best prevention for rabies is vaccination. Core vaccines have clearly demonstrated efficacy and safety (Sellon and Long, 2014). The unvaccinated horse should receive a primary set of three doses and annual boosters (Fernanda, 2010). The American Association of Equine Practitioners (AAEP) recommends annual vaccination for all horses according to the instruction (Wright and Greene, 2016). It is important that contaminated animals are traced, identified, safely confined and reported to the State Veterinary Organization without delay.

Rabies represents an economic burden for both developed and developing countries due to the costs of human post exposure treatment, diagnosis, surveillance and immunization of domestic animals and wildlife, calling for more attention to the control of the disease and research on its different aspects. As a consequence, management of this problem is one of the most important priorities of all countries as well as the Health Ministry of Iran.

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