

The report of rabies in red fox (*Vulpes vulpes*) in south-west Iran

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Abstract

Rabies, as an endemic fatal zoonotic disease, is transmitted from infected animals to humans commonly through contact (scratches and bites). The present paper provides the first documented evidence of rabies in the red fox (*Vulpes vulpes*). Signs of rabies disease were found in one red fox in Chaharmahal and Bakhtiari province. The red fox had aggressive behavior and attacked humans. The animal died after a while. Soon after the death, the samples were taken from the fox's brain (hypothalamus, cerebellum, and hippocampus). In this report, laboratory evidences of rabies were confirmed by the fluorescent antibody (FAT) and RT-PCR tests in foxes. To prevent and control this deadly disease, suspected cases of rabies in humans and animals should be monitored regularly with a sensitive monitoring system and advanced reporting. This includes the history of exposure, symptoms, clinical examinations, and laboratory results. Rabies can be controlled by vaccination and fast diagnostic tests.

Key words: FAT, RT-PCR, Rabies, Fox, Shahrekord

Introduction

Rabies is a fatal disease causing preventable death, which occurs mainly in Africa and Asia, with poorly available animal control, effective postexposure prophylaxis for humans, and vaccination programs. Rabies is primarily found in wild animal hosts in developed countries. In such areas, rabies directly passes from wild animals such as foxes, raccoons, bats, and skunks to humans rather than domestic animals (Blanton et al., 2011; Kilic et al., 2006). Despite extensive vaccination programs for domestic pets, rabies is still a global hazard. Generally, the disease is fatal. Thus, it is essential to consider rabies postexposure prophylaxis (RPEP) for all victims of unprovoked cat and dog bites. Ten-day quarantine is recommended for cats, dogs, and ferrets. By the appropriate

animal quarantine, victim prevention may not be essential; however, it should be triggered at the first clinical symptoms of the disease in the quarantined animal (Ruskin et al., 1993). When the animal is an escaped dog or a cat, the local epidemiology of rabies should be considered for RPEP initiation. The risk of rabies to humans can be potentially posed by bites from any wild animal based on the rabies transmission level in local wildlife populations. Immediate RPEP should be considered when it is impossible to test wild animals for rabies (Goldstein, 1994). It is essential to take into account two other conditions of tetanus infections and local wound infections and rabies in domestic pet or wild animal bites, while not neglecting prophylaxis. In such injuries, antibiotic

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prophylaxis is essential. There is a higher risk for infections by all deep dog bite puncture wounds, human, cat, or monkey bites, bites in immunosuppressed patients, foot or hand wounds, and any injury enduring surgical repair. Moreover, prophylactic antibiotics are required for these wounds. Wild animal attacks on humans are infrequent. Studies revealed three underlying reasons for wolf attacks, including the wolf may be rabid, and it might regard human beings as prey. Third, the attack may be provoked by a human (Linnell et al., 2002). In the wolf strangled to death, rabies was assessed by our patient while attacking, and the animal was proved as rabid. Human beings, foxes, and wolves rarely interact. However tragic incidents occasionally occur. In developing countries, rabies continues to provide a considerable public health problem. As with all attacks of uncontrolled mammals, wild animal attacks continue to bear a rabies risk in these countries (Türkmen et al., 2012). Rabies is endemic in the wildlife in Iran, where infection of domestic livestock is frequent (Esfandiari et al., 2010). Sever rabies suspicions should be mainly raised by unexpected attacks by wild animals. It is essential to vaccinate domesticated animals to prevent infection and transition of rabies to humans. Wild animals need to be non-handled while particularly avoiding wildlife with abnormal behavior. Bats should be kept out of public buildings and houses.

The first case of rabies is reported in the present paper in the red fox in Shahrekord (south-west Iran).

Materials and Methods

Case history

A report was received by the environment department of Chaharmahal and Bakhtiari province On 15 November 2021 indicating several cases of fox bites in humans, recognized a rabid red fox (*Vulpes vulpes*) in the municipality of Shehrekord (Province of Chaharmahal and Bakhtiari, Southwest of Iran) [1]. The fox was an adult and male biting a 60-year-old man on the

ankle on 15 November. The first aid assistance and complete postexposure treatment were provided by the victim at the local health unit. The exposed person is presently under active health surveillance. The fox had a fear of dogs and humans. Depression was represented by lethargy. The fox died after a while.

Clinical Samples

Soon after the death, the brain samples (hypothalamus, cerebellum, and hippocampus) were taken from the fox (Fig.1). The sample was transported fresh on ice to the laboratory.

Laboratory investigations

FAT

To examine impressions of tissue samples from the brain for rabies infection, FAT was used. Applying FAT examination on the fresh specimens, it was explained by WHO & OIE (Dean DJ, 1996; OIE, 2008) with Iran Veterinary Organization.

RT-PCR

RNA extraction and cDNA synthesis

RNA was extracted using CinnaPure-RNA (Sinaclone, Iran) according to the manufacturer's protocol. According to the product manual, cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Canada). The cDNA was stored at -20 °C until subsequent use.

Molecular Detection of Rabies

To amplify 730 bp products of glycoprotein genes, the modified primer sets GH3 (5' -CTA ACC ACG ATT ACA CCA TTT G-3') and GH4 (5' -CAA AAT CCT CAG CCT CGT C-3') were used (Kissi et al., 1999). The RT-PCR reaction was carried out in a volume of 50 µl. The reaction mixture consisted of 5× PCR buffer, 10 mM dNTP mix, 20 nM of each primer, the RT-PCR enzyme mix and 4 µl of eluted RNA. A thermo-cycling profile of 1 cycle of 30 s at 50 °C, 1 cycle of 15 min at 95 °C, 40 cycles of 30 s at 94 °C, 30 s at

56 °C, and 1 min at 72 °C was employed followed by a final incubation at 72 °C for 10 min. The amplified products was analyzed by gel electrophoresis on 1.8% low electroendosmosis agarose in 1% TBE buffer for 80 mins. The gel was visualized on a UV transilluminator.

Results

Rabies was found in the samples by FAT. Also, rabies genome was detected in the brain samples with RT-PCR (Fig.2).



Fig. 1. The sampling of death rabid fox

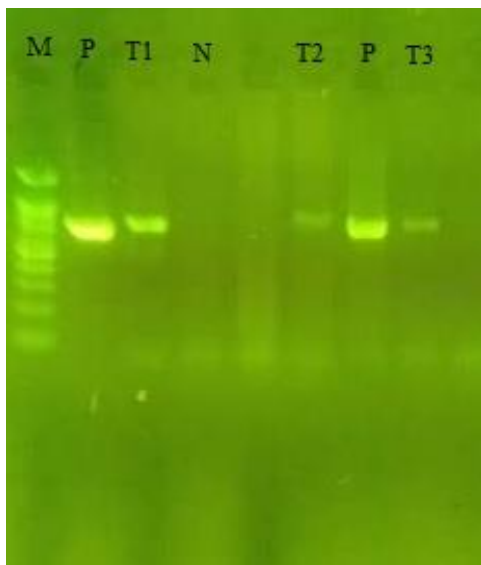


Fig. 2. Agarose gel electrophoresis of RT-PCR products. The lanes illustrate molecular weight marker 100 bp (M), positive control (P), positive samples (T1: hippocampus, T2: cerebellum, and T3: hypothalamus) with the 730 bp band, and negative control (N)

Discussion

In a study by Karimi et al., blood samples were taken from 193 foxes. 26 (13.5%) of the foxes had neutralizing antibody in their blood. This study confirms that during a rabies episode, the Vulpin population may develop a non-fatal disease and produce neutralizing antibodies. Therefore, foxes have a real place in the epidemiology of rabies in Iran (Y Karimi, 1975). Studies of rabies sources in different parts of Iran indicated fox, dog, and jackal as the most common origin of the disease in northern regions. However, wolves are the predominant ones in northwestern and western areas (Simani, 2003). There are various species of animals in every region. Stray dogs and jackals are the most important rabid animals in the Caspian littoral, while foxes and wolves have a crucial role in the mountainous areas of the central plateau (Bokaei et al., 2009). Another report on animal rabies in Kerman province within 1993-2003 presented a considerable seasonal variation in the number of confirmed and suspected rabid animals, peaks in autumn and winter. In most habitats of Iran, the red fox is found, from desert areas to dense forests. It is relatively rare, though it is more abundant in the northwestern and western regions of Iran. Ghorani et al., reported of rabies in a grey wolf (*Canis lupus*) in Chaharmahal and Bakhtiari Province (Iran) (Ghorani et al., 2022). The red fox is on the IUCN (International Union for Conservation of Nature) Red List of Low Concerned Species or LCs.

Wolf, domestic dog, and Chinese ferret badger in Asia are the principal sources of rabies worldwide. The bite of a rabid animal is the only risk of rabies virus transmission (D. E. J. Maclachlan N. James, 2016). More importantly, no definitive clinical presentation exists for rabies. Furthermore, rabies should be included as a differential etiologic diagnosis in all cases of behavioral change, until excluded definitively. Live-attenuated, inactivated,

and recombinant vaccines have been established for the parenteral immunization of humans and animals against rabies. Live-attenuated or recombinant vaccines have been used for successful oral vaccination against rabies in baits to target wildlife species. In numerous countries of Africa, Asia, and Latin America with considerable domestic animal and human mortality, enzootic dog rabies are continually a severe problem. Several doses of rabies vaccines are utilized in these countries, though expensive to maintain and institute. Thus, comprehensive rabies control programs are essential.

In the present study, the red fox had aggressive behavior and followed humans. Less concern was paid for this species conservation status by the International Union for Conservation of Nature (IUCN). Wild animals come to the cities for food in the cold seasons, increasing the possibility of a human bite.

It is also complicated to control rabies in enzootic regions by the existence of wildlife sources in the Caribbean islands as well as several species of canids and wild felids in Africa. In North America and Europe,

agencies for controlling public supported rabies act in the following areas: (1) removal of stray cats and dogs and control of the pets' movement (quarantine is utilized in epizootic conditions, but rarely); (2) immunizing the cats and dogs with suitable vaccines for breaking the virus transmission chain; (3) institution of programs for preventing and controlling rabies in wildlife, which reflect the important regional reservoir animal host(s); (4) laboratory diagnosis for confirming clinical observations and obtaining precise incidence data; (5) surveillance for measuring all control measures effectiveness; (6) public education programs for assuring cooperation (D. E. J. Maclachlan N. James, 2016).

A sensitive surveillance system is required to prevent and control this fatal disease to follow suspected human thoroughly and animal rabies cases through the enhanced reporting system. Such a system includes the history of exposure, symptoms, clinical examinations, and laboratory results. Accurate and rapid diagnosis of rabies is vital owing to its public and zoonotic health.

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Conflict of Interests

The authors declare that there is no conflict of interest.

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گزارش هاری در روباه قرمز (*Vulpes vulpes*) در جنوب غرب ایرانمحمدرضا قرآنی^{۱*} و فهیمه اسلامی^۲^۱ استادیار گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه تبریز، تبریز، ایران^۲ اداره کل حفاظت محیط زیست استان چهارمحال و بختیاری، شهرکرد، ایران

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چکیده

هاری به عنوان یک بیماری اندمیک کشنده مشترک بین انسان و دام، معمولاً از طریق تماس (خراش و گزش) از حیوانات آلوده به انسان منتقل می‌شود. مقاله‌ی حاضر اولین گزارش مستند از هاری در روباه قرمز (*Vulpes vulpes*) را ارائه می‌دهد. علائم هاری در یک قلاده روباه قرمز در استان چهارمحال و بختیاری مشاهده گردید. روباه رفتار تهاجمی داشت و به انسان‌ها حمله می‌کرد. حیوان پس از مدتی تلف شد. بلافاصله پس از مرگ، نمونه‌ها از مغز روباه (هیپوتالاموس، مخچه و هیپوکامپ) گرفته شد. در این گزارش، اثبات آزمایشگاهی هاری از طریق آزمایش‌های آنتی‌بادی فلورسنت (FAT) و واکنش زنجیره‌ای پلی‌مران رونویسی معکوس (RT-PCR) در روباه تأیید شد. برای پیشگیری و کنترل این بیماری کشنده، موارد مشکوک به هاری در انسان و حیوان بایستی با یک سیستم نظارتی حساس و گزارش‌دهی پیشرفته به طور مرتب پایش شود. این موارد شامل تاریخچه مواجهه، علائم، معاینات بالینی و نتایج آزمایشگاهی است. هاری با واکسیناسیون و آزمایش‌های تشخیصی سریع قابل کنترل است.

کلمات کلیدی: FAT، RT-PCR، هاری، روباه، شهرکرد

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