Brief Report

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Epidemiological Study of Rabies Infection in Specimens Sent to Pasteur Institute of Iran in 2015

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ABSTRACT

BACKGROUND AND OBJECTIVE: Rabies is an acute untreatable fatal encephalitis, which is fully preventable. Determination of prevalence and the main source of the virus can help with controlling and eradicating the disease. This study aimed to evaluate the prevalence of rabies in the suspicious specimens submitted to the national reference center for rabies, Pasteur Institute of Iran, during March-August 2015.

METHODS: This cross-sectional study was performed on 195 rabies-suspected specimens of brain tissue. The diagnosis was done based on the determination Negri bodies through direct immunofluorescence method. In addition, rabies infection was evaluated in terms of animal type and geographic distribution.

FINDINGS: The results showed that the prevalence rate of rabies infection was 59.5% (116 specimens). This infection was mostly observed among dogs, so that from 116 cases of positive rabies, 45 cases were canine (39%). In categorizing the specimens into carnivorous and ruminant, it was found that rabies was more prevalent in carnivorous animals (p=0.01). In terms of geographic distribution, the most cases of positive rabies were reported in Khorasan Razavi province with 23 positive cases (24.2%).

CONCLUSION: Rabies infection is highly prevalent in carnivorous animals, especially dogs, and it is still considered as a dangerous zoonotic disease.

KEY WORDS: Encephalitis, Dog, Prevalence, Rabies, Zoonotic.

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Introduction

Rabies is a zoonotic disease, the most common clinical presentation of which is acute fatal encephalitis in humans and other mammals. This disease is caused by neurotropic viruses from the lyssavirus genus. This disease has two types of epidemiological cycles: the urban cycle in which dogs have the most important role, and the wild cycle that is related to wild animals (1, 2). Regarding rabies infection, countries are divided into four categories of highly, moderately, less, and not prevalent. Rabies annually causes 60000 deaths in developing countries, 95% of which occur in Africa and Asia.

Dogs are responsible for more than 95% of human rabies cases. The annual cost of prophylaxis after rabies exposure in Africa and Asia is estimated at 583.5 million dollars (3-7).

Studies suggest that rabies control programs in dogs have a significant role in reducing the transmission of rabies to humans; however, in developing countries, planning and implementing these programs are one of the biggest challenges governments encounter (8,9). Rabies is a native and important zoonotic disease in Iran (8). Studies and laboratory data demonstrate that wild animals in Iran are also infected with rabies virus, and they act as a source of virus in nature. Annually, numerous cases of animal bite are reported in different parts of Iran. The lethal effects of rabies are prevented in most of the cases due to referring to health centers on time and receiving prophylaxis (9-12).

Although a great number of domestic animals receive anti-rabies vaccination every year, this program is not efficient enough to reduce and eventually eliminate this disease. To solve this problem, there needs to be a detailed plan and an extensive vaccination program for animals contributing to rabies transmission to humans to eliminate rabies and reduce its adverse effects such as death and physical and mental damages secondary to animal bite, livestock death, prophylaxis, inter-agency cooperation should be considered. (13,14).

Determination of prevalence and focus of infectious diseases can help with understanding the status of the disease in question, assessing health indices, especially in previous programs, and implementing strategic programs, especially for health care providers, to improve the current situation (15). This study aimed to evaluate the prevalence of rabies in suspicious specimens. This study evaluated contamination of wild and domestic animals with rabies infection in different provinces of Iran. Identification of animal carriers or sources of the disease can help with determining the target population for vaccination programs, which can pave the way for eradication of rabies in Iran.

Methods

In this cross-sectional study, we evaluated 208 brain specimens suspected for rabies, which were submitted to the national reference center for rabies, Pasteur Institute of Iran, from health centers of different provinces of Iran from March to August 2015. The specimens were examined through direct fluorescence antibody test (DFAT), as a reference rabies test, to identify Negri bodies (16).

A small part of the specimens was removed from the tube by a forceps and was put on tongue depressor. Then, a thin layer of the specimens was put on a glass slide. The specimens were kept in cold acetone for one hour at -20°C, and then were stained with the specific fluorescein-conjugated antibody.

The Negri bodies were identified using ultraviolet microscope. If the sample was negative, 10%(W/V) suspension was prepared from brain tissue on isotonic buffer with antibiotics, and was injected to the brain tissue of newborn Syrian mice.

In case the disease manifestations such as weight loss and lower limb paralysis and eventually death were observed after 30 days, DFAT was performed on the brain tissue. If the animal survived four weeks after the injection, diagnosis of rabies was ruled out. Chi-square test was performed using SPSS, version23, and $p<0.05$ was considered significant.

Results

From the 208 suspected specimens of rabies, 13 specimens were excluded from the study due to the insufficiency for preparation of the expansion. From the remainder of the specimens, 116 (59.5%) cases were identified with positive rabies. From the submitted specimens, canine and bovine brain tissue specimens comprised the highest percentage of the samples at (46.1% and 23.1% respectively), respectively (90 and 45 specimens), and the lowest percentage of the specimens belonged to rats (1 specimen; fig 1). The most cases of positive rabies based on animal type were pertinent to jackals and

cows, respectively (100% and 84%, respectively). In carnivorous and ruminant categories, canine and bovine specimens comprised the highest percentage(46.1% and 23.1% respectively) (table 1), and rabies infection was more prevalent in carnivorous animals, as compared to ruminant ones (p=0.01). Regarding the provinces, the most amount of specimens were sent from Khorasan Razavi (38 cases), followed by Azarbayejan Sharghi (28 cases; table 2).

Table 1. Distribution suspected specimens of rabies

Sample	No.	Sample percentage	Positive	Positive percentage
Dog	90	46.1	45	50
Cow	45	23.1	38	85
Sheep	14	7.1	6	43
Wolf	9	4.5	5	55
Jackal	9	4.5	9	100
Cat	6	3.1	0	0
Fox	5	2.6	3	60
Goat	4	2.1	3	75
Calf	2	2.1	1	50
Camel	2	1.1	0	0
Rat	1	0.5	0	0
Donkey	4	1.1	3	75
Human	4	2.1	3	75
Total	195	100	116	59.5

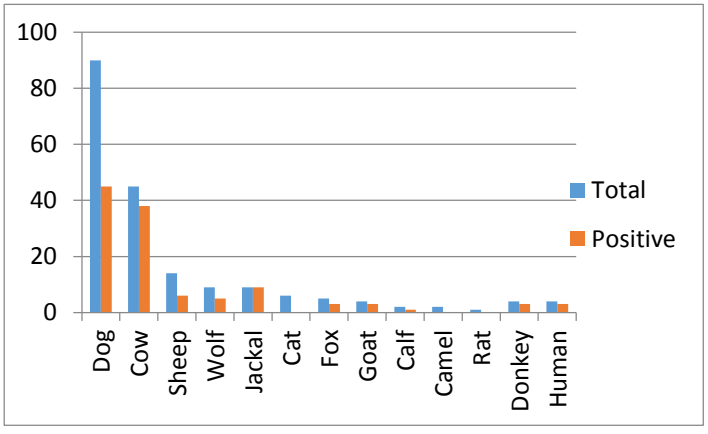

Figure 1. Distribution of rabies infection based on animal type

Table 2. Distribution of the received specimens based on province and the number of positive cases of rabies

Provinces	Total samples	Positive
Azarbayejan Sharghi	28	16
Azarbayejan Gharbi	10	7
Khorasan Razavi	38	23
Isfahan	10	6
Sistan va Balouchestan	4	2
Tehran	5	0
Kordestan	19	14
Hamedan	8	3
Kermanshah	4	3
Hormozgan	1	1
Zanjan	9	4
Yazd	3	0
Golsetan	2	0
Lorsetan	7	6
Ghom	8	6
Chaharmahal va Bakhtiari	4	3
Khorasan Shomali	1	0
Fars	2	0
Ghazvin	2	0
Semnan	2	2
Markazi	1	1
Kerman	18	11
Ilam	1	1
Alborz	2	1
Khoozestan	6	6
Total	195	116

Discussion

According to the results of this study, treadmill exercise at intensity of 18 meter/minute for half an hour per day (five days a week for four weeks) could reduce OAE factor of anxiety-like behaviors caused by ovariectomy in mice. It is noteworthy that estrogen exerted similar effects on these behaviors. On the other hand, combination of estrogen and exercise had a more significant effect on the reduction of anxiety-like behaviors in both OAE and OAT factors compared to estrogen and exercise alone.Reduced anxiety followed by regular exercise could be associated with changes in the hippocampus. As observed in adult rodents, running could increase the number of new stimulating

neurons in gyrus dentatus, while stimulating the production of dendritic branches on these neurons, throughout the hippocampal circuit. In rodents, ventral hippocampus is directly involved in processing behaviors such as stress and anxiety (25).

According to the literature, exercise enhances neurogenesis in gyrus dentatus of hippocampus in mice leading to an increase in growth factors, such as insulin-like growth factor-1 and brain-derived neurotrophic factor, which play a pivotal role in the regulation of anxiety-like processes (26). Recent findings suggest that mice who receive treadmill exercise for five consecutive weeks tend to have reduced levels of adrenocorticotropin (ACTH) and corticosterone, which results in the reduction of anxiety-like behaviors in these animals (27). Exercise enhances the function of hypothalamic-pituitary axis to control subsequent stressors. This process is associated with changes in the release of corticosterone, termination of response to this hormone, or both these parameters together. Changes in the release of corticosterone from the adrenal gland could be caused by altered sensitivity of this gland to ACTH, as well as changes in the release of ACTH from the pituitary gland (28). Previous research has indicated that estrogen could also be effective in reducing anxiety through affecting the amygdala and hippocampus (29, 30). Furthermore, estrogen could decrease anxiety and stress through E2 receptors stimulation of hypothalamic-pituitary-adrenal axis (31). In one study, FULK et al. performed 45 minutes of treadmill exercise on rats at medium intensity (five times per week for ten weeks), and the intervention led to a significant reduction in anxiety-like behaviors of animals (32).

These findings are consistent with the results obtained by the present study. In another research, Uysal et al. reported that treadmill exercise could decrease plasma levels of corticosterone in mice causing a significant reduction in anxiety (33). Exercise is associated with physiological and behavioral effects, including improved learning ability, reduced anxiety-like behaviors, neurogenesis and angiogenesis, increased neurotrophic factors and changes in various signaling molecules. In their study, Salam et al. concluded that running on a wheel in the Rotarod device (two weeks) resulted in the significant reduction of anxiety-like behaviors in male mice (34). Furthermore, Vollert et al. claimed that rats receiving regular exercise had normal corticosterone concentration and reduced anxiety-like behaviors compared to those without exercise (35). Similarly, findings of Haydari et al. suggested that exercise could decrease the level of anxiety in mice (36). Results obtained in the current study indicated that exercise alone could reduce anxiety-like behaviors in ovariectomized mice, which is consistent with the results of the aforementioned studies.

Therefore, considering the harmful effects of synthetic estrogen, exercise could be a beneficial alternative to reduce anxiety in menopausal women. In conclusion, since physical exercise comes in a variety of forms and effects on the brain, it is recommended that further research be conducted as to discover the most efficient types of exercise and their influence on molecular mechanisms involved in the reduction of anxiety. Comparison of these findings with estrogen therapy could yield beneficial results for clinical situations.

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References

1. Lembo T, Hampson K, Haydon DT, Craft M, Dobson A, Dushoff J, et al. Exploring reservoir dynamics: a case study of rabies in the Serengeti ecosystem. J Appl Ecol. 2008;45(4):1246-57.

2. Knobel DL, Cleaveland S, Coleman PG, Fèvre EM, Meltzer MI, Miranda ME, et al. Re-evaluating the burden of rabies in Africa and Asia. Bull World Health Organ. 2005;83(5):360-8.

3. Dodet B, Goswami A, Gunasekera A, de Guzman F, Jamali S, Montalban C, et al. Rabies awareness in eight Asian countries. Vaccine. 2008;26(50):6344-8.

4. Dacheux L, Reynes JM, Buchy P, Sivuth O, Diop BM, Rousset D, et al. A reliable diagnosis of human rabies based on analysis of skin biopsy specimens. Clinical Infectious Diseases. 2008;47(11):1410-7.

5. Malerczyk C, Detora L, Gniel D. Imported human rabies cases in Europe, the United States, and Japan, 1990 to 2010. J Travel Med. 2011;18(6):402-7.

6. Bharti OK, Ramachandran V, Kumar SR, Phull A. Pup vaccination practices in india leave people to the risk of rabies lessons from investigation of rabies deaths due to scratch/bite by pups in remote hilly villages of himachal pradesh, India. World J Vaccines. 2014;4(1):7-10.

7. Davlin SL, VonVille HM. Canine rabies vaccination and domestic dog population characteristics in the developing world: a systematic review. Vaccine. 2012;30(24):3492-502.

8. Fayaz, A, Simani, S, Janani, AR, Farahtaj F, Esfandyari, B, Eslami, N, et al. Epidemiological survey of rabies in mazandaran province during 1996-2006. J Babol Univ Med Sci. 2010;11(5):70-5.[In Persian]

9. Shirzadi M, Pourmozafari J, Shamsipour M. Status of Animal bite and rabies cases in northeast provinces of Iran during 2009-2010. J Zoonoses. 2014;1(1):18-22(

10. Simani S. Rabies situation in Iran. J Veter Res. 2003;58(3):275-8.[In Persian]

11. Fayaz A, Fallahian V, Simani S, Eslamifar A, Mohammadian A, Hazrati M, et al. Epidemiological characteristics of persons exposed to rabies in Tehran referred to Pasteur Institute of Iran during the years of 1993-1994 and 2008-2009. Pejouhesh. 2011;35(3):168-73.[In Persian]

12. Simani S, Gholami A, Farahtaj F, Fayaz A Yousefi-Behzadi M. Epidemiological survey of different rabies virus strains in Iran. J Sci Islamic Rebub Iran. 2001;12(4):315-20.

13. Peters V, Sottiaux M, Appelboom J, Kahn A. Posttraumatic stress disorder after dog bites in children. J Pediatrics. 2004;144(1):121-2.

14. Oehler RL, Velez AP, Mizrachi M, Lamarche J, Gompf S. Bite-related and septic syndromes caused by cats and dogs. Lancet Infect Dis. 2009;9(7):439-47.

15. Allender J, Rector C, Warner K. Community & public health nursing: promoting the public's health: Philadelphia: Lippincott Williams & Wilkins; 2013.

16. Whitfield SG, Fekadu M, Shaddock JH, Niezgoda M ,Warner CK, Messenger SL. A comparative study of the fluorescent antibody test for rabies diagnosis in fresh and formalin-fixed brain tissue specimens. J Virol Methods. 2001;95(1–2):145-51.

17. Gholami A, Fayaz A, Farahtaj F. Rabies in Iran: past, present and future. J Med Microbiol Infec Dis. 2014;2(1):1-10.[In Persian]

18. Faizee N, Hailat N, Ababneh M, Hananeh W, Muhaidat A. Pathological, immunological and molecular diagnosis of rabies in clinically suspected animals of different species using four detection techniques in Jordan. Transbound Emerg Dis. 2012;59(2):154-64.

19. Ata F, Tageldin M, Al-Sumry H, Al-Ismaily S. Rabies in the sultanate of oman. Vet Rec. 1993;132(3):68-9.

20. Johnson N, Un H, Fooks AR, Freuling C, Müller T, Aylan O, et al. Rabies epidemiology and control in Turkey: past and present. Epidemiol Infect. 2010;138(03):305-12.

21. Saitou Y, Kobayashi Y, Hirano S, Mochizuki N, Itou T, Ito FH, et al. A method for simultaneous detection and identification of Brazilian dog-and vampire bat-related rabies virus by reverse transcription loop-mediated isothermal amplification assay. J Virolog Methods. 2010;168(1):13-7.
22. Menezes R. Rabies in India. Canad Med Associat J. 2008;178(5):564-6.
23. Cisterna D, Bonaventura R, Caillou S, Pozo O, Andreau ML, Dalla Fontana L, et al. Antigenic and molecular characterization of rabies virus in Argentina. Virus research. 2005;109(2):139-47.
24. Cleaveland S, Kaare M, Tiringa P, Mlengeya T ,Barrat J. A dog rabies vaccination campaign in rural Africa: impact on the incidence of dog rabies and human dog-bite injuries. Vaccine. 2003;21(17):1965-73.
25. Knobel DN, Lembo T, Morters M, Townsend SE, Cleaveland S, Hampson K. Dog rabies and its control. 2013. Available from: http://www.who.int/rabies/animal/dogs/en/
26. Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, et al. Correction: Estimating the global burden of endemic canine rabies. PLoS Negl Trop Dis. 2015 May 11;9(5):e0003786.
27. Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. Nature. 2007; 447:279-83
28. Pavlin BI, Schloegel LM, Daszak P. Risk of importing zoonotic diseases through wildlife trade, United States. Emerg Infect Dis. 2009;15(11):1721.