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CLINICAL & BASIC RESEARCH

Prevalence of Rabies in Various Species in Yemen and Risk Factors Contributing to the Spread of the Disease

*Hassan A. Al-Shamahy, Ameera Sunhope, Khaled A. Al-Moyed

معدل انتشار داء الكلب في أنواع الحيوانات المختلفة في اليمن وعوامل الاختطار المساهمة في انتشاره

حسن عبد الوهاب الشماحي، أميرة أحمد سنهوب، خالد عبد الكريم المؤيد

الملخص: **الهدف**: هدفت هذه الدراسة إلى معرفة انتشار فيروس داء الكلب بين مختلف الحيوانات الداجنة والبرية المقدمة إلى المختبر البيطري المركزي من مناطق مختلفة في اليمن، كذلك هدفت هذه الدراسة إلى معرفة العامل المصاحب لخطر الإصابة المؤدية إلى الانتشار بين الحيوانات والعوامل المساهمة لانتقاله للبشر. **الطريقة**: تم الحصول على عينة من الدماغ لكل من ال180 حيوان المقدمة إلى المختبر البيطري المركزي لاختبار فيروس داء الكلب بواسطة اختبار الأجسام المضادة التألقي المباشر. **النتائج**: من بين العدد الإجمالي للحيوانات التي هجمت على البشر، كانت 63.3% منها إيجابي لداء الكلب. كانت الكلاب هي الحيوانات الرئيسية التي شاركت في الهجمات بنسبة 92%، منها 62.7% كانت إيجابية لداء الكلب. وكانت نسبة الذكور 70.6% منها 60.6% إيجابية لداء الكلب ، ونسبة الإناث 29.4% منها 68.9% كانت ايجابية لداء الكلب. شكل الذكور نسبة 69.8% من مجموع البشر الذين تعرضوا للهجوم، منهم 62.9% تعرضوا للهجوم من قبل حيوانات مصابة بداء الكلب. بشكل عام كانت عوامل الخطر التي ساهمت في انتشار داء الكلب هي وجود جثث الدواجن والنفايات الأخرى في المنطقة المجاورة للهجمات (العامل المصاحب لخطر الإصابة = 9.5) مع نسبة 84.8%، يليها الوقت من السنة، وخصوصا العطل المدرسية (العامل المصاحب لخطر الإصابة= 3.8) مع نسبة 78%. **الخلاصة**: يمكن أن نخلص من هذه الدراسة إلى أن داء الكلب متوطن في اليمن مع ارتفاع كبير في نسبة الحيوانات المصابة بداء الكلب التي شاركت في الهجمات على البشر، وخصوصا بين ذكور الكلاب الضالة. يعتبر الأطفال الذكور هم الأكثر عرضة للهجوم بواسطة الحيوانات المصابة بفيروس داء الكلب. كذلك تمت معرفة ان وجود النفايات (ذبائح الدواجن بشكل خاص) وفترات العطل المدرسية لها ارتباط ملحوظ بزيادة خطر تعرض الإنسان لداء الكلب.

مفتاح الكلمات: داء الكلب؛ البشر؛ الحيوانات؛ عوامل الاختطار؛ اليمن.

ABSTRACT: ***Objectives:*** This study aimed to describe for the first time the prevalence of the passively-reported rabies virus among different domestic and wild animals submitted to the Central Veterinary Laboratory from various areas in Yemen, and to study prevalence proportion ratios (PPR) that contributed to the spread of rabies among animals, and its transmission to humans. ***Methods:*** A brain sample was obtained from each of the 180 animals and tested for rabies virus by a direct fluorescent antibody test. ***Results***: Out of the total number of animals involved in attacks on humans, 63.3 % were positive for rabies. Of these, dogs were the main animal involved in attacks with a percentage of 92%, of which 62.7% were positive for rabies. Of animals involved in attacks, 70.6% were males of which 60.6% were positive, and 29.4% were females of which 69.8% were positive. Males comprised 68.9% of the total human individuals attacked, of whom 62.9% were attacked by rabies-positive animals. The significant risk factors that contributed to the spread of rabies in general included the presence of poultry carcasses and other waste in the vicinity of the attacks (PPR = 9.5) with a percentage of 84.8%, followed by the time of year, in particular school vacations (PPR = 3.8) with a percentage of 78%. ***Conclusion***: Rabies is endemic in Yemen with a very high rabies-positive rate for animals involved in attacks, particularly for stray male dogs. Male children were most often involved in attacks by rabies-positive animals. The presence of food waste (particularly poultry carcasses) and school vacation periods were found to correlate significantly with increased risk for human exposure to rabies.

Keywords: Rabies; Humans; Animals; Risk factors; Yemen.

Advances in Knowledge

- *Previously, few studies have focused on rabies in Arab countries. This study provides new information about rabies in Yemen, including the prevalence of passively-reported rabies virus among different domestic and wild animals from different areas in Yemen, and the risk factors that contribute to the spread of rabies among animals and its transmission to humans.*
- *Such information is important in recommending policy for the prevention and control of rabies in Yemen.*
- *Moreover, rabies is likely to be a growing problem in Yemen, in spite of its decrease or disappearance world-wide and particularly in neighbouring countries in the Arabian Peninsula.*

Department of Medical Microbiology & Immunology, Faculty of Medicine, Sana'a University, Sana'a, Yemen
E-mail: shmahe@yemen.net.ye

Application to Patient Care

- *The findings of this research could contribute to the formulation of treatment and control policies for human and animal rabies and, ultimately, to the prevention of its spread.*
- *The findings highlight the deficiency or absence of control programmes in Yemen. The very high rabies-positive rate for animals involved in attacks, particularly for stray dogs, suggests that these animals should be vaccinated or eradicated.*
- *The risk factors identified as being highly correlated with a positive rabies diagnosis are helpful in identifying measures that could help in disease control (e.g. a safer system for the disposal of chicken carcasses).*

Rabies is a zoonotic viral infection of the central nervous system that causes encephalitis, with a fatality rate of nearly 100%. The annual number of human deaths worldwide caused by this disease is estimated to be 55,000, and more than 99% of all human deaths occur in developing countries, mainly in Asia.[1]

Rabies is considered one of the most important public health problems in the World Health Organization's (WHO) Eastern Mediterranean Region (EMR). The majority of human deaths due to rabies during the 1990s occurred in Afghanistan, Egypt, Iran, Iraq, Morocco, Syria, Tunisia and Yemen.[1,2] Yemen is a country in which canine rabies is endemic; the number of people bitten by rabid dogs has increased noticeably since 1990, mostly due to the increase in the population of dogs throughout Yemen's cities and villages, which seriously affects the lives of the inhabitants. There are more than a million dogs in Yemen, of which about 10–20% are owned while the rest are strays, living on food from garbage and spreading diseases among people and other animals.[3] Additionally, until now no official measures have been in place for the control and prevention of rabies in Yemen, and a vaccine has not been available for domestic or wild animals. People bitten by positive animals receive one dose of human rabies immunoglobulin (HRIG) and 4 doses of rabies vaccine over a 14-day period. The first dose of rabies vaccine is given as soon as possible after exposure, with additional doses on days 3, 7, and 14. The vaccine is administered in Rabies Control Units under the supervision of the National Rabies Control Program (NRCP).[3]

Annually, up to 7,000 people are exposed to animal bites in Yemen since records began.[3] In some years, more than 30 persons have died of rabies. However, the official death rates in humans are known to be highly inaccurate and do not represent the actual size of the problem, since only a limited number of people bitten by animals in Yemen go to Rabies Control Units and many are not documented by the NRCP.[3]

The aims of this study were, first, to estimate the prevalence of the rabies virus among different animal species in Yemen; second, to analyse the animal case histories with a view to rabies risk and prevention and, finally, to study the risk factors that contribute to the spread of rabies among animals and humans.

Methods

A cross-sectional analytical study was used to estimate the prevalence of the rabies virus among different animal species, then to analyse the animal case histories with a view to assessing the rabies risk and the means of prevention and, finally, to study the risk factors that contribute to the spread of rabies among animals and humans.

The Central Veterinary Laboratory (CVL) in Sana'a, the capital city of Yemen, is the reference laboratory which receives samples from villages, poultry farms, quarantine stations and veterinary clinics from all the governorates in Yemen. The study was carried out over a period of 7 months, from June to December 2011. The study proposal was approved by the Department of Medical Microbiology & Clinical Immunology at the Faculty of Medicine & Health Sciences at Sana'a University.

A full history was taken for each of the 180 individuals who were attacked and brought in specimens, and the findings were recorded in a predesigned questionnaire. The data collected included personal information on the individuals attacked, the characteristics of the animal which inflicted the bite, the type of contact, predisposing factors, and so on. A consent form was completed by each participant.

Specimens from the animal inflicting the bite were obtained from the person attacked (or from parents or others), and usually consisted of brain

Table 1: Rabies positivity results for the 180 animals suspected of having rabies brought to the Central Veterinary Laboratory in Yemen, June to December 2011, stratified by demographic characteristics

	Total attacking animals		Animals positive for rabies	
	n	%	n	%
Species of animal				
Dog	166	92	104	62.7
Fox	3	1.7	3	100
Donkey	3	1.7	3	100
Cat	3	1.7	1	33.3
Goat	2	1.1	2	100
Hyena	1	0.6	1	100
Cow	1	0.6	0	0
Rat	1	0.6	0	0
Gender of animal				
Male	127	70.6	77	60.6
Female	53	29.4	37	69.8
Ownership status				
Owned	106	58.9	59	55.7
Stray	74	41.1	55	74.3

tissue or a spinal cord swab. Such specimens may be stored at 2–8° C when they are to be tested within 24 hours. If specimens are to be kept for longer periods, they should be stored at -70° C in flame-sealed or taped vials until tested. In each case, the head was removed from the body of the animal at the base of skull, exposing the spinal cord adjacent to the *medulla oblongata*. A sterile cotton wool swab was introduced into the occipital foramen towards the direction of the eye, rotated several times, removed, and used to prepare the slides. Samples were collected from the base of the *cerebellum*, *hippocampus* and *medulla oblongata*. The slides were air dried and fixed in acetone.[4,5]

The rabies virus was detected by a commercially available direct fluorescent antibody test (FAT) (Fujirebio Diagnostics, Inc., Malvern, Pennsylvania, USA). For direct rabies diagnosis, smears prepared from the brain were fixed in cold acetone and then stained with monoclonal anti-rabies conjugated with fluorescein isothiocyanate (FITC). In the presences of the rabies virus, an antigen-antibody complex will form. If the tissue being examined contains no viral antigen, specific complexes will not be formed. Rabies virus anti-rabies antibody complexes are visualised using a fluorescence microscope. Positive reactions demonstrate bright apple-green fluorescence of particles ranging in size and morphology from dust particles to prominent Negri body cytoplasmic inclusions.

Analysis was carried out using a prevalence proportion ratio (PPR) for the association of positive rabies with personal information on individuals attacked, characteristics of the animal inflicting the bite, type of contact and risk factors. The Taylor series 95% confidence intervals (CI) were calculated by analysis of a single table. Furthermore, the chi-square value for statistical significance was calculated using the Yates continuity corrected statistics, but Fisher's exact test was

Table 2: The relationship of rabies-positivity results with animal attack rate and provocation status in 180 rabies-suspicious animals brought to the Central Veterinary Laboratory, Yemen, June to December 2011

Attack rate	Total animals involved in attacks		Rabies-positive animals		Rabies-negative animals		PPR	CI	*P* value
	n	%	n	%	n	%			
1 individual	130	72.2	71	54.6	59	45.4	02	0.07–0.49	0.0001
2–3 individuals	44	24.5	37	84.0	7	16.0	4.1	1.59–10.7	0.001
5–9 individuals	6	3.3	6	100.0	0	0.0	undefined	0.14	
Provocation									
Provoked	40	22.2	25	62.5	15	37.5	0.96	0.44–2.1	0.9
Unprovoked	140	77.8	89	63.6	51	36.4	1.05	0.48–2.3	0.9

PPR = prevalence proportion ratio; CI = 95% confidence interval; PPR >1 = at risk; significant result = P <0.05.

Table 3: Distribution of attacked individuals according to their gender and age in relation to rabies-positive animals

Characteristics of individuals attacked	Total attacked individuals		Rabies-positive animals		PPR	CI	*P* value
	n	%	n	%			
Male	124	68.9	78	62.9	0.94	0.46–1.9	0.85
Female	56	31.1	36	64.3	1.06	0.5–2.6	0.99
≤10 years old	101	56.1	74	73.3	2.67	1.4–5.24	0.003
11–20 years old	47	26.1	26	55.3	0.63	0.3–1.3	0.25
>20 years old	32	17.8	14	43.8	0.37	0.16–0.87	0.019

PPR = prevalence proportion ratio; CI = 95% confidence interval; PPR >1 = at risk; significant result = P <0.05.

used for small cell sizes with a two-tailed *P* value using Epi Info, Version 6 (Centers for Disease Control, Atlanta, Georgia, USA).

Results

Out of the 180 samples tested by FAT, 63.3% were positive for rabies. Dogs were the main species involved in attacks (166/180; 92%) of which 104 (62.7%) were diagnosed as positive for rabies. Foxes, donkeys, cats, goats, and hyenas were also found to be positive for rabies in this study [Table 1]. Of the animals involved in attacks, 70.6% were males, of which 60.6% tested positive for rabies, and 29.4% were females, of which 69.8% were positive. Of the animals involved in attacks, 58.9% were owned, of which 55.7% were positive for rabies, and 41.1% were strays, of which 74.3% tested positive [Table 1].

Table 2 shows the attack rate and provocation status of animals involved in attacks. There was a significant correlation (P <0.05) between high attack rates and the rabies positivity of the animals involved. Males comprised 68.9% of the total individuals attacked, of whom 62.9% were attacked by rabies-positive animals, and females comprised 31.1%, of whom 64.3% were attacked by rabies-positive animals. Of those attacked, 76.5% were bitten by the rabies-positive animals in the head or neck, 67.1% were bitten in the arms or trunk, and 52.2% were bitten in the lower extremities [Tables 3 and 4]. There was a significant association between a category III severity of attack and positivity for rabies, in which the PPR = 4.9, CI = 1.33–19.6, and P = 0.001 [Table 4]. Most attacks occurred in rural areas (86.7%), and the positive rate of rabies was slightly higher in rural than in urban areas (64.1% *versus* 58.3%, respectively) [Table 5].

Regarding the risk factors that contributed to the spread of rabies among susceptible animals

Table 4: Distribution of attacked individuals according to exposed body site and severity

Characteristics	Total attacked individuals		Positive animals		PPR	CI	*P* value
Area of bite	**n**	**%**	**n**	**%**			
Head or neck	34	18.9	26	76.5	2.14	0.85–5.6	0.11
Arms or trunk	79	43.9	53	67.1	1.34	0.6–2.6	0.44
Lower extremities	67	37.2	35	52.2	0.47	0.24–9.2	0.026
Severity of attack							
**Category I	2	1.1	1	50.0	0.58	0.02–21.4	>0.05*
**Category II	12	6.7	3	25.0	0.17	0.09–0.73	0.009
**Category III	166	92.2	110	66.3	4.9	1.33–19.6	0.011

*PPR = prevalence proportion ratio; CI = 95% confidence interval; PPR >1 = at risk; significant result = P <0.05; * = Fisher exact P value.*

***2004 World Health Organization terminology for severity: Category I = touching or licking by the animal on intact skin; Category II = minor scratches or abrasions without bleeding or being licked by the animal on broken skin; Category III = transdermal bites or scratches, or contamination of mucous membranes with saliva.[20]*

Table 5: Distribution of attacked individuals according to residence in relation to the rabies-positive animals

Residence	Total attacked individuals		Rabies-positive animals		PPR	CI	*P* value
	n	%	n	%			
Rural	156	86.7	100	64.1	1.3	0.49–3.3	0.75
Urban	24	13.3	14	58.3	0.78	0.3–2.05	0.58

PPR = prevalence proportion ratio; CI = 95% confidence limits; PPR >1 = at risk; significant result = P <0.05.

and transmission from dogs to humans, the major factor was the presence of poultry carcasses and waste food with a prevalence of 84.8%, PPR = 9.5, CI = 4.4–20.7, *P* >0.001. Next in importance was the time of year, since during school vacations the exposure of children to animal bites increased (PPR = 3.8, CI = 1.9–7.71, *P* >0.001). Another factor was the cultivation of *qat* (a tropical plant whose leaves are commonly used in Yemen as a stimulant), with a percentage of 67%, but this was not statistically significant [Table 6].

Discussion

This study revealed a high percentage of positivity in animals brought to the laboratory for rabies analyses (63.3%). This result agreed with the CVL results, in which two-thirds of the animals examined were positive for rabies. Similar findings have been reported in Iran (66.8%) and Tanzania (68%).[6,7]

Dogs were found to be the main source of infection in Yemen (92%). This agreed with two previous studies in Yemen, and in those done in several other developing countries.[8–13] These similarities were due to the fact that most of these countries have common characteristics and practices, such as poor solid waste disposal and a high dog population co-occurring with the absence of measures to control numbers. Consequently, standard policies should be applied to ensure the proper disposal of poultry carcasses and other waste, as well as to control the dog population. These policies should be implemented in parallel with programs of vaccination against rabies for domestic and wild animals under the supervision of the NRCP, and improved surveillance of rabies among wild and domestic animals in Yemen.

Concerning animal ownership, 74.3% of stray dogs were positive for rabies as opposed to 55.7% of owned dogs [Table 1]; the higher rate in strays might be attributed to their contact with other stray dogs that have been infected, or with infected endemic

Table 6: Risk factors attributed to the spread of rabies in Yemen

Risk factors	Total individuals attacked		Rabies-positive animals		PPR	CI	*P* value
	n	%	n	%			
Presence of wild animals	139	77.2	87	62.6	0.87	0.39–1.9	0.84
School vacations	91	50.6	71	78.0	3.8	1.9–7.7	<0.001
Uncontrolled dog population increase	152	84.4	96	63.2	0.95	0.38–2.37	0.92
Poultry carcasses and waste	99	55.0	84	84.8	9.5	4.4–20.7	<0.001
Solid waste	59	32.8	35	59.3	0.78	0.39–1.5	0.58
Slaughter and markets waste	141	78.3	92	65.2	1	0.48–2.1	0.86
Qat cultivation	146	81.1	98	67.0	0.84	0.31–2.26	0.89
Cultivation of crops other than *qat*	120	66.7	77	64.2	1.1	0.56–2.2	0.86
Dairy farms	10	5.6	6	60.0	0.86	0.2–3.8	0.9
Livestock grazing	164	91.1	105	64.0	1.4	0.44–4.3	0.73

PPR = prevalence proportion ratio; CI = 95% confidence interval; Qat = tropical plant whose leaves are commonly used in Yemen as a stimulant; PPR >1 = at risk; significant result = P <0.05.

wild animals. This latter explanation is supported by the fact that in this study, and previous studies in Yemen, all the foxes and hyenas tested were positive for rabies.[8,9] This was similar to the findings of a WHO report in Oman in 1997.[2]

Of the animals involved in attacks, 77.8% were unprovoked by the individual attacked, and 63.6% of these animals were positive for rabies. This also agreed with a previous study in Chad, in which 81% of the animals involved in attacks were unprovoked and 61.8% of these animals were also positive for rabies.[13] This could be explained by the fact that a normal dog does not attack unless it or its offspring are themselves attacked. Conversely, a rabid dog attacks without any prior provocation. Moreover, a single rabid animal may attack more than one individual, as observed in this study, where 37 of the rabid animals had attacked 2–3 individuals with statistical significance (P <0.001) and another 6 rabid animals had attacked 5–9 individuals separately. This finding agree with studies conducted in Uganda and Alaska.[10–14]

In this study, it was found that males were attacked more than females, with a ratio of 2.2:1; this ratio was similar to that found in a number of reports from Turkey (2:1), Asian countries (1.6:1) and the USA (1.7:1).[15–18] The main reason behind these findings is that males spend more time outside the home than females, and so are more exposed to the possibility of animal bites.

About 56.1% of all bites observed in this study were inflicted on children ≤10 years of age, of whom 73.3% were attacked by rabid animals, giving a significant PPR of nearly twice that of other age groups [Table 3]. This result is similar to those reported from Tanzania and Uganda.[7,10] The finding is also consistent with the rabies mortality annual report of the NRCP in Yemen, in which more than two-thirds of the rabies deaths in 2008 were among children.[3] This could be explained by the fact that children spend more time outdoors, face animals alone, provoke animals and are less able to protect themselves. A further possible factor is that their height is parallel to the heads of animals, which makes children's heads more exposed to severe bites.

Concerning the parts of the body exposed to attack, the arms or trunk were the most frequently bitten, being the objects of attack in 43.9% of the total individuals attacked, of whom 67.1% were attacked by rabies-positive animals [Table 4]. Similarly, an Iranian study documented that the upper extremities of the attacked individuals were the most frequently bitten, with a percentage of 53.8%.[19]

According to the WHO terminology for the severity of such injuries,[20] 92.2% of the individuals attacked in this study had category III injuries, while only 6.7% had category II, and 1.1% had category I injuries [Table 4].[21] These findings are comparable with the findings of studies in Chad, the USA and Thailand.[12–14]

The burden of rabies falls mostly on poor rural communities, since 86.7% of attacked individuals in this study resided in rural areas. This percentage is higher than the findings of other studies conducted in Iran (79.4%), Spain (75%) and Turkey (56%).[15,17,22]

School vacations were a significant risk factor contributing to the contraction of rabies [Table 6]. This finding is consistent with that observed in Thailand and could be explained by the fact that children spend a lot of time playing outdoors during vacations.[12] It also highlights the pressing need for dedicated safe areas for children to play such as parks and clubs. Concerning the presence of poultry carcasses and other waste food in the vicinity of attacks, animals involved in attacks were using the waste as a source of food. The high statistical significance of this factor (P = 0.0001) is nearly 9 times greater than that recorded for other factors involved in the spread of rabies. This risk should be reviewed with particular concern, since the poultry sector is considered to be one of the most important recipients of investment in Yemen and has been increasing over the last decade. However, little attention has been paid to these farms in terms of bio-security and the hygienic handling of poultry carcasses and waste.

Conclusion

Two-thirds of the total number of animals brought to the laboratory were positive for rabies. Dogs were the main animals involved in attacks, and constituted the main reservoir for rabies. The burden of rabies falls mostly on poor rural communities, and particularly on children. The presence of poultry carcasses and other waste was the main predisposing factor contributing to the spread of rabies, followed by the time of year, namely during school vacations.

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