

Original Research Article

Incidence of human rabies following bite or exposure to laboratory confirmed rabid animals

Kanwarpreet S. Sandhu^{1*}, Pahul K. Bawa², Bhupinder S. Sandhu³

¹Department of Public Health, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

²Sri Guru Ramdas University of Health Sciences, Amritsar, Punjab, India

³Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

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*Correspondence:

Dr. Kanwarpreet S. Sandhu,

E-mail: kanwarsandhu08@gmail.com

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ABSTRACT

Background: Rabies is one of the important endemic fatal zoonotic viral disease afflicting humans and animals in Punjab, India. The present study investigated the incidence of rabies in humans bitten/exposed to laboratory confirmed rabid animals, as well as incidence after use of vaccine or rabies immunoglobulin (RIG) and clinico-epidemiological studies.

Methods: A study was conducted during August 2021 to September 2022 on forty (40) rabies suspected animals presented to diagnose rabies by direct fluorescent antibody test (dFAT) at rabies diagnostic laboratory (RDL), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Punjab, India. A detailed questionnaire was prepared for obtaining information about exposure/bite of humans by rabid animals, death of humans, demographic and epidemiological information of victims.

Results: Out of total forty (40) suspected rabies cases, 30(75%) were found positive for rabies by dFAT. Laboratory confirmed rabies (LCR) incidence was 60.80% and 21.73% in stray and pet dogs, respectively. All pet dogs were vaccinated but no stray dog was vaccinated. The LCR incidence in buffaloes and cattle was 77.77% and 100%, respectively. Further in humans exposed to rabid animals (59), males were at more risk than females. The human rabies incidence was 3.38% (2/59). Highest incidence of dog bites in adult males on lower limb was observed from urban stray dogs (60.80%) followed by children. Post exposure vaccination was given to 98.3% humans exposed to rabid animals. Human's rabies in two cases was due to no vaccination or RIG.

Conclusions: Vaccination is an important step in controlling rabies in India. There is a need for integrated and comprehensive management of street dogs and bite management.

Keywords: Epidemiology, Dog bite, dFAT, Incidence, India, Rabies

INTRODUCTION

Rabies is one of the important fatal zoonotic viral disease afflicting humans and animals. Till date, the disease is under-reported in many developing countries and still continues to cause significant human mortality in Asian, South American, African, and Eurasian countries. In the

United States (US), rabies is enzootic in various species of bats and terrestrial reservoir hosts such as raccoons, skunks, foxes and the small Indian mongoose.¹ It is caused by Lyssavirus which is a highly neurotropic negative sense single stranded RNA (ssRNA) virus, belonging to family Rhabdoviridae of the Order Mononegavirales.² Rabies is

endemic in India and prevalent throughout the country except Andaman and Nicobar and Lakshadweep Islands.³

In the world approximately 59,000 people die after being bitten by rabid animals, mostly in Asia and Africa, 40% of whom are children under 15 years of age.³ This disease is transmitted through bites/licks/scratches from rabid animals. In Asian countries, dogs are the main vectors of transmission while bats and other wild animals in America, Africa, and Eurasia.⁴ Rabies transmission to humans is contributed by dogs (99%) (World Health Organization, 2017), 5 to 10% by cats and 2 to 3% by humans and rodents.⁵ Due to the close association between dogs and people, an increased incidence of rabies in dogs could result in an increase in exposure of human beings to rabies. The disease is also of economic importance due to cost of prevention and treatment measures and about 17.4 million undergo post bite immunization.^{6,7} The estimated annual cost of rabies post bite prophylaxis in Asia and Africa is US \$583.5 million, whereas, in America it is US \$300 million. Globally rabies causes an estimated cost of US\$ 8.6 billion per year.⁸

In India, the National Rabies Control Programme has initiated the plan for elimination of rabies by 2030, however it is in the early phase. Although, the main vector of transmission is dog in India, but disease may also be transmitted by other rabid domestic animals to humans by consumption of milk of rabid animal, by giving medicine in mouth to animals by bare hands, milking, by touching secretions and excretions of rabid animals.

In the endemic developing countries, rarely the laboratory confirmation of suspected rabies is carried out either due to high cost, lack of proper training, and resources and time consuming making it impractical for clinical use.⁹ With all these challenges, post-exposure prophylaxis remains the most efficient approach at preventing human rabies deaths post exposure. The patients exposed or bitten by rabid animals are treated by vaccination and rabies immunoglobulin (RIG), but poor patients ignore it and rarely use RIG.

Further, no systematic study has been conducted to observe the occurrence of rabies in humans after exposure to confirmed rabid animals in India. Thus, the present study was conducted to investigate the incidence of rabies in humans bitten/exposed to laboratory confirmed rabid animals, as well as incidence after use of vaccine or RIG and clinico-epidemiological studies.

METHODS

Study area

The incidence study was conducted from August 2021 to September 2022 in the Centre for One Health, School of Public Health and Zoonoses (SPHZ) in collaboration with the Rabies Diagnostic Laboratory (RDL), Department of Veterinary Pathology, located at Guru Angad Dev

Veterinary and Animal Sciences University (GADVASU) Ludhiana, Punjab, India. Punjab is an agricultural state located in north-west part of India, with 27.74 million humans and 0.47 million domestic dogs' population.¹⁰

Inclusion criteria

All human being bitten/exposed to rabies confirmed animals and died of rabies were included in the study.

Exclusion criteria

All humans touching rabid domestic animal utensils/licking unexposed skin were excluded from the study.

Questionnaire design and information collection

A detailed questionnaire was prepared for obtaining information about exposure/bite of human being by rabies suspected animals, demography of victims, circumstances of bite incidences, body parts injured, ownership of biting animal, age, sex and number of human bitten/exposed, vaccination status, treatment given at home and death of human after treatment (Tables 3 and 4). The information was collected from the owner. The exposure history of humans from suspected rabies cases was also recorded. The data regarding death of humans was confirmed telephonically from persons, who brought rabies suspected sample to RDL up to one year of exposure.

Laboratory testing

During this study, 40 brain samples were collected from rabies suspected cases received for post mortem diagnosis of rabies in RDL, Department of Veterinary Pathology, College of Veterinary Science, GADVASU, Ludhiana. Samples were handled by vaccinated person with proper protective clothing. Rabies was diagnosed by using the direct fluorescent antibody technique (dFAT) on impression smears, histopathology and immunohistochemistry from brain tissues mainly cerebellum, hippocampus and brain stem in the RDL at GADVASU.¹¹⁻¹³ Laboratory data was recorded and compiled with the relevant case history.

Statistical analysis

The data were recorded in tabular form and described analysing frequency and proportion for categorical variables.

RESULTS

One-year study was conducted at Centre for One Health in collaboration with RDL located in the Department of Veterinary Pathology, GADVASU, Ludhiana, Punjab, India. During this period, 40 rabies suspected animals were presented at RDL and out of these 30 (75%) animals were found to be confirmed positive by FAT 75% (30/40), IHC

70% (28/40) and histopathology 52.5% (21/40) including 82.6% (19/23) pet and stray dogs, 77.7% (7/9) buffaloes, and cattle 100% (4/4), respectively (Figures 1-3). Out of these none of horse and wild animals were found positive (Table 1). Among the 75% (30/40) rabid animals, highest incidence of exposure/injuries to humans was found to be from dog bite followed by buffaloes and cattle respectively. No exposure was observed from equine and wild animals. Among the dog's highest incidence of exposure/bite among humans was found from dogs 82.60% (from stray dogs 60.86% and 21.73% from pet dogs). Majority of the pet dogs (100%) were vaccinated against rabies vaccine, but none of the stray dog was found to be vaccinated.

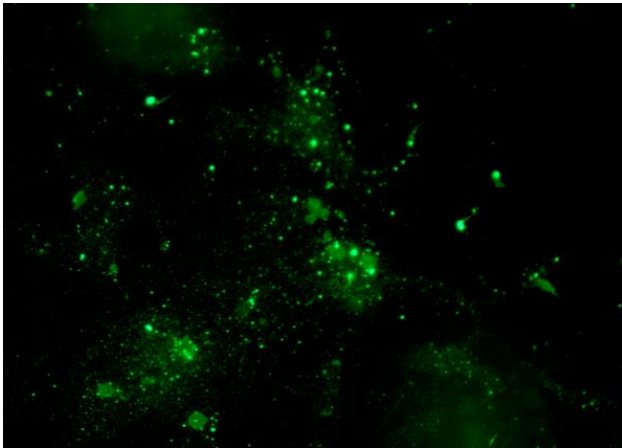


Figure 1: dFAT stained brain impression smear; dog rabies antigen, specific bright apple green fluorescence (particulate and aggregates) seen in abundance.

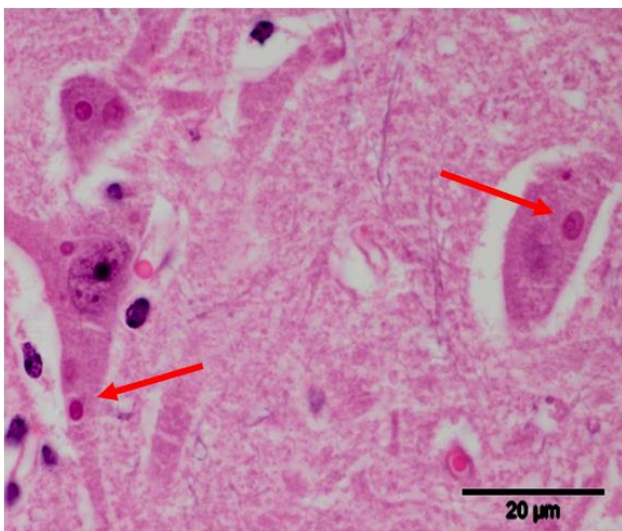


Figure 2: Hippocampus of a rabid buffalo showing intracytoplasmic eosinophilic inclusions (Negri bodies) in the princely, H&E, bar=20 µm.

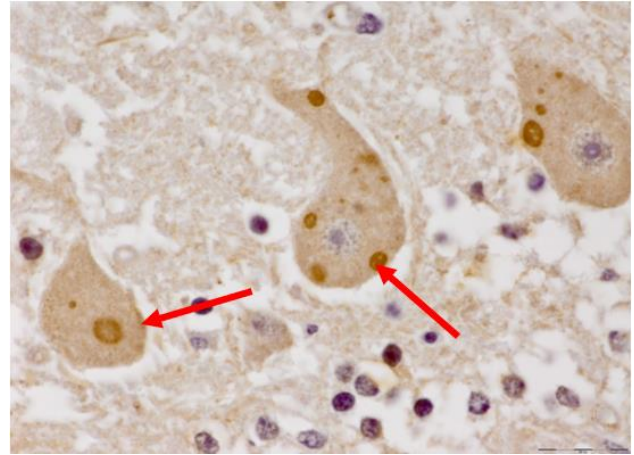


Figure 3: Cerebellum (buffalo) showing several brown, variable size and shape inclusion bodies (viral antigen) within the cytoplasm of Purkinje cell. IHC with DAB substrate, counterstained with Gill's hematoxylin x100x.

The exposure of 59 humans to dogs was mainly by biting at various locations such as arms and hand 13.5% (8/59), head and face 6.7% (4/59), lower limb 23.7% (14/59), thigh 1.6% (1/59) and multiple bites 13.5% (8/59) respectively (Table 3). However, a majority of the bites were single bite injuries 77% (27/35) with highest incidence of exposure to confirmed rabid dogs was on lower limbs 23.7% (14/59).

Similarly, exposure of humans to rabid cattle and buffalo was by touching 8.4% (5/59), putting hand during medication 15.2% (9/59), milk/curd consumption of unboiled milk 10.1% (6/59) and licking 5.0% (3/59) respectively. The greater risk (10.1%) of exposure of rabid animal to humans was by putting bare hand in the mouth of rabid cattle and buffalo (Table 3).

The majority of humans (37/59, 62.7%) exposed to rabid animals belong to urban area and constitutes 38.9% adults, (15-40-year old) followed by children, 22.0% (5 to 14 year old) (Table 3). The greater exposure of humans to rabid animals was observed in males 83.3% (49/59), whereas, 16.9% females were bitten/exposed to rabid animals. To observe the season wise incidents, the dog bite incidents was higher during the summer months (June to August) 42.3% (25/59) followed by winter (December to February) 25.4% (15/59); and spring (March to May) 23% (13/59) respectively. However, lowest incidents were observed during the autumn months (September to November) 10.1% (6/59) (Table 4).

Total number of humans exposed to rabies confirmed animals were 59 and out of them highest number 66.1% (39/59) were bitten by rabid dogs and followed by 20.3% (12/59) to buffaloes and 13.5% (8/59) to cattle respectively (Table 5).

Table 1: Incidence of rabies in animals (n=40).

S. no.	Species	Ownership of animal				Vaccine status		Rabies confirmation			Confirmed positive animals	
		Pet	Stray dog	Domestic livestock	Wild	Vaccinated	Unvaccinated	FAT	Histopathology	IHC	Positive	Negative
1	Dog	9	14	0	0	9	14	19	11	17	19	4
2	Buffalo	0	0	9	0	0	0	7	6	7	7	2
3	Cattle	0	0	4	0	0	0	4	4	4	4	0
4	Horse	0	0	1		0	0	0	0	0	0	1
5	Wild	0	0	0	3	0	0	0	0	0	0	3
Total	40	9	14	14	3	9	31	30	21	28	30	10
%		22.5	35	35	7.5	22.5	77.5	75	52.5	70	75	25

Table 2: Clinical details of the rabies suspected animals (n=40).

Species	Rabies suspected animals/human	Number positive for rabies		Number negative for rabies		% positive	
		Pet/domestic	Stray	Pet/domestic	Stray	Pet/domestic	Stray
Dog	23	5	14	4	0	21.73	60.80
Buffalo	9	7	0	2	0	77.77	0
Cattle	4	4	0	0	0	100	0
Horse	1	0	0	1	0	0	0
Wild animals	3	0	0	0	3	0	0

Table 3: Demographic profile of human rabies (n=59) following bite/exposure by laboratory confirmed rabid animals (n=30).

Rabies confirmed animals (buffalo/dog/cattle)	Age in years and number of human bitten/exposed (n=59)					Location of bite			Type of exposure						
	>5	5-14	15-40	41-60	>60	Arm/hand	Head/face	Lower limb	Thigh	Multiple	Scratch	Licking	Putting hand in mouth for medication	Touching	Consumption of milk
n=30	12	13	23	3	8	8	4	14	1	8	1	3	9	5	6
%	20.3	22.0	38.9	5.0	13.5	13.5	6.7	23.7	1.6	13.5	1.6	5.0	15.2	8.4	10.1

Table 4: Demographic characteristics regarding incidence of rabies in humans (n=59) after exposure to laboratory confirmed rabid animals (n=30).

Rabies confirmed animals (buffalo/dog/cattle)	Treatment given at home (wound washing)		Post bite treatment given to humans		Death of human after treatment	Gender of human victims		Season of bite/exposure				Area	
	Yes	No	Vaccination	RIG		Male	Female	Spring (March-May)	Winter (December-February)	Autumn (September-November)	Summer (June-August)	Rural	Urban
30	32	27	58	6	2	49	10	13	15	6	25	22	37
%	54.2	45.7	98.3	10.1	3.3	83.0	16.9	23.0	25.4	10.1	42.3	37.2	62.7

Table: 5 Incidence of rabies in humans exposed to confirmed rabid animals.

Species	Dog	Buffalo	Cattle	Horse and wild animals	Total human exposed	Total human dead
Number of humans exposed	39	12	8	0	59	2
%	66.10	20.30	13.55	0	100	3.38

The majority of humans 98.3% (58/59), exposed to rabid animals were treated by administration of recommended therapeutic doses of rabies vaccine or by both vaccination and rabies immunoglobulin (RIG). The RIG was administered only to very negligible number of humans 10.1% (6/59), those were severely bitten by dogs on face/ having multiple bite injuries. Death of two humans confirmed telephonically from the persons, who brought brain sample/animal for confirmation of rabies.

The death occurred within 10 to 30 days of exposure after humans were bitten by confirmed stray rabid dogs. However, medical autopsy was not undertaken in any of these cases and hence no laboratory confirmation was possible. Out of these two humans, one was vaccinated only and other ignored treatment and suffered from hydrophobia, aerophobia, salivation and furious form of rabies followed by death. In one victim who died, was having multiple bites by rabid dog and no home treatment was given and vaccination was started after one week of bite and death occurred within 10 days before completion of vaccine schedule whereas, in case of other victim, no vaccination/home treatment or RIG was administered and died within 20 days of bite near the head. In 54.2% humans bitten by rabid dogs or exposed to confirmed rabid animals was given home treatment (wound washing with soap and water before presentation to hospital) along with vaccination (Table 4).

DISCUSSION

The present study revealed that animal-bite injuries, remain an important cause of morbidity and is the main mode of rabies transmission in human in Punjab, India.^{3,14,15} This study also reported high risk groups for animal-bite, animals that are most likely to cause bites and exposure. This study adds to the evidence that animal bites are important public health concern in India. However, no other report of nationwide systematic study has been conducted to study the incidence of human rabies exposed to laboratory confirmed rabid animals, both vaccinated and stray unvaccinated animals. Hence, for the first time in Punjab, India this kind of study was undertaken.

This is the first study on incidence of canine and livestock rabies in Punjab, India leading to human exposure to the confirmed rabid animals. The incidence of rabies was 82.6% in pet and stray dogs that caused bite injuries to humans. There are no systematic data available on the incidence of human rabies caused by dog bite in India. Lower incidence of rabies in canines has been reported from Kerala.¹⁶ Similarly, canine rabies incidences out of

10,000 populations was 41.28 in Ethiopia, 14 in Chad, and 2.71 in Punjab have been reported.^{10,17,18} In this study, higher incidence of rabies was in stray dogs as compare to pet dogs. This could be due to cultural diversities, pet dogs are properly vaccinated and always confined in urban areas of Punjab and rarely interact with stray dogs. Similarly, a study from Shimla reported higher bites by stray dogs.¹⁹ Rabies incidence differ significantly between stray and pet dogs in this study.¹⁸ Due to their overlapping home range, both stray dogs and stray cattle are associated with the human population for food sources.¹⁰ In Punjab, cattle or buffaloes are kept in open areas and thus are more prone to bite by stray dogs.²⁰

As per this study, the annual incidence of rabies in Punjab includes 7 buffaloes, 4 domestic cattle, and 5 pet dogs, with 14 stray dogs being important contributors. Passive disease surveillance data has reported, rabies in 130 dogs, 95 buffalo 78 cattle and 13 equine cases during a 10-year study period in Punjab, India.²⁰ This suggests a need for strengthening of proper rabies monitoring and surveillance programs in India.²¹

Human rabies continued to be an endemic disease in India and in this study, majority of human victims were exposed/bitten by both pet and stray rabid dogs (82.6%) and majority of human rabies victims were adult men (38.9%) followed by children. This may be due to more contact of adults to rabid stray dogs and roaming animals during working hours. Further, higher chances of exposure to rabid animals while feeding the cattle and buffaloes. Higher incidences of children exposure to rabid dogs could be due to their shorter height, playing habit with stray dogs and lower strength to scare away the dogs. This may result in extensive bites that need operative care and thus higher morbidity and their treatment. Similar findings have been reported by other workers.²² Majority of cases of exposure to rabid animals were reported from urban areas because the samples of rabies suspected animals were mainly submitted for rabies diagnosis from urban areas, due to approachability of diagnostic laboratory. The present study findings are consistent with other studies.¹⁹

Out of the total of 59 human victims exposed to rabies confirmed animals, 2 developed the disease and died. The rest 57 cases remained normal throughout the follow up one-year period. This could probably be due to minor degree of exposure. It is also possible that the rabid exposing animal might not shed the virus at the time of exposure (due to intermittent shedding). Majority of dog bite/exposure cases were recorded in the urban area. This was because from urban area more stray dogs were presented to diagnostic laboratory. The two fatal cases

observed were due to stray dog bite, confirming dog as the important reservoir and source of infection for livestock and humans. This is consistent with previous reports, where 95% fatal cases of human rabies were associated with dog bites.²³ However, in developed countries where dog rabies is controlled, the main rabies cycle is associated with wild carnivores.²⁴ Consequently, the key to human rabies prevention and control in India lies in the successful control of canine rabies and the stray dog population.²² In the area of this study, the majority of pet dogs were vaccinated. All the victims exposed to rabid animals in our study were properly treated using recommended doses of cell culture vaccine and wound washing with clean water and soap (54.2%). Except for the two cases, where the death might have been due to improper treatment or no vaccination and RIG of the victim. The use of RIG was negligible (10.1) as revealed by this study. This reflects gross negligence on the part of both the bite victims as well as the healthcare system. In spite of significant improvement in overall healthcare in this country, the prevention and control of rabies needs to be given a priority. There will be decline of human rabies with the increased awareness and use of post exposure vaccinations to the rural population. Simultaneously, extensive public education, particularly for the rural and urban community, is equally important for reducing rabies mortality.²²

The dog bite injuries were mainly reported from lower limb (23.7%) followed by bites to arms, hand and head or face. This is due to the fact that dogs mostly bite from back side suddenly without provocation and humans try to defend with legs and hands.^{25,26} Similarly, exposure of humans to rabid cattle and buffalo was by touching (8.4%), followed by putting hand in mouth while medication (15.2%), milk/curd consumption of un-boiled milk and licking. Cattle can transmit rabies to humans as well as other animals. In Iran, a case of human rabies due to contact with the saliva of rabid cattle was reported.²⁷ Rabies virus was detected in 9/24 (37.50%) saliva samples, 2/17 (11.76%) milk samples and 6/21 (28.57%) urine samples.²⁸ In the present study, humans were exposed to confirmed rabies cattle and buffalo by consuming milk, touching and milking rabid cattle and buffalo, but no human suffered from rabies up to one year of exposure. Moreover, all persons exposed to rabid cattle/buffalo were vaccinated with recommended doses of anti-rabies vaccine. It is recommended that all dog bite/exposed victims be vaccinated with life-saving modern rabies immunization free of cost by government agencies. Incomplete treatment had resulted in human deaths. It is very important to educate peoples about rabies and need for full course of vaccination and use of RIG in multiple bite injuries and bite on face near the brain.

Dog bite incidents were reported throughout the year with more bite incidents during the summer months (June to August) (42.3%). It is difficult to correlate factors that might explain this peak during this period in Punjab. However, higher incident of dog biting during summer season are due to their gregarious wandering and furious nature especially during breeding season and the rabid

dogs bite each other and transmit disease to fellow dogs causing rabies to a greater number of dogs.²⁹ In developed countries, most dog bite incidents occur during the summer months.^{30,31} Such observations have been explained by behavioural changes: more interaction between pets and children during the warmer months having school holidays with less parental supervision, thus increasing the risk of bite incidents.³¹ In Thailand, reports of dog bite incidents in children increased during the months of March-May and October, the period of school vacation.³²

The current study has some limitations. Reporting of a suspected rabies case was dependent on contact between farmers and veterinary doctors and pharmacists belonging to surrounding districts to diagnostic laboratory. Therefore, there is more likely under-reporting of disease incidence. Similarly, it was very difficult to convince owners of animals or those persons who brought stray dogs to report back the death of humans. Most importantly performance of autopsy was not allowed in rabid humans thus there was no laboratory confirmation.

CONCLUSION

To conclude, the occurrence of large numbers of stray dogs and the bite from suspected rabies animal are a public health concern in Punjab, India, which require urgent attention to prevent human rabies. Our study showed that vaccination is important to control human rabies in India. Also, our data clearly indicated that human deaths had occurred due to ignorance, not seeking post-exposure prophylaxis (PEP) and also due to the incomplete treatment course. For 100% protection, vaccination and RIG must be administered to severely bitten victims after proper first aids. Stray dogs remain the main source of infection to humans and livestock. The local epidemiological data of rabies in animals may be shared with the concerned authorities, veterinarians and clinicians to make the right choice in treatment rabies with PEP. Management of dog population through various government programme, enforcement of regulations for licensing of dogs, animal birth control programs and stray dog vaccination is a must in Punjab, lessons on dog behaviour, the risk of dog bites, bite wound management (e.g. washing with soap and water) and rabies can also be integrated into the elementary school curriculum to educate children on the public health hazard of dog bites. Finally, a one health approach is needed for the sustainability of the rabies elimination programme involving all the stake holders from various fields.

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