

# Emergence and re-emergence of glanders in India: a description of outbreaks from 2006 to 2011

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## Summary

Glanders, a bacterial disease of equines caused by *Burkholderia mallei*, is a fatal infectious disease of equines and has zoonotic significance. The disease has been eradicated from many countries by statutory testing, elimination of infected animals and import restrictions. However, it is still endemic in parts of Africa, Asia, the Middle East and Central and South America. In India, major glanders outbreaks were reported from different parts of the country between 1976 and 1982. Later, sporadic cases of the disease were reported in 1988, 1990 and 1998. The country remained free of glanders for about eight years until the recent outbreaks occurred in eight States from 2006 to 2007. Recurrent episodes have occurred in Himachal Pradesh and Uttar Pradesh, whereas fresh outbreaks occurred in Chhattisgarh from 2009 to 2010. A total of 164 equines were declared positive; a majority of the positive cases ( $n=77$ ) were from Uttar Pradesh, followed by Maharashtra ( $n=23$ ), Uttarakhand ( $n=21$ ) and Andhra Pradesh ( $n=16$ ). Under the provision of Prevention and Control of Infectious and Contagious Disease in Animals Act, 2009, all

the infected animals were euthanised and bio-security measures were implemented to curb the further spread of the disease.

## Keywords

*Burkholderia mallei*, Disease, Control, Equine, Glanders, India, Outbreak.

## Insorgenza e nuova insorgenza della morva in India: una descrizione dei focolai della malattia dal 2006 al 2011

### Riassunto

*La morva, malattia batterica degli equini causata dal Burkholderia mallei, è una patologia infettiva fatale di rilevanza zoonotica. In molti paesi è stato debellata grazie a controlli ed esami prescritti dalla legge, all'abbattimento obbligatorio degli animali infetti e alle restrizioni all'importazione. Continua tuttavia a essere endemica in alcune parti dell'Africa, dell'Asia, del Medio Oriente e dell'America centrale e meridionale. In diverse regioni dell'India, tra il 1976 e il 1982, sono scoppiati focolai di morva. Negli anni successivi (1988, 1990 e 1998) si sono registrati casi sporadici. La malattia non si è più manifestata per circa otto*

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*anni fino ai recenti focolai che hanno colpito otto stati nel 2006 e nel 2007. La morva è poi ricomparsa negli stati di Himachal Pradesh e Uttar Pradesh, mentre nuovi focolai si sono registrati a Chhattisgarh tra il 2009 e il 2010. In totale gli animali positivi alla malattia sono stati 164, per la maggior parte (n=77) stanziati nello stato di Uttar Pradesh, con i rimanenti capi distribuiti negli stati di Maharashtra (n=23), Uttarakhand (n=21) e Andhra Pradesh (n=16). Come previsto dalla Legge sulla prevenzione e sul controllo delle malattie infettive e contagiose negli animali (2009), tutti i capi infetti sono stati eutanizzati adottando misure di biosicurezza per impedire l'ulteriore diffusione della malattia.*

### Parole chiave

*Burkholderia mallei*, Controllo, Equino, Focolaio, India, Malattia, Morva.

## Introduction

Glanders is a fatal and infectious disease of horses, donkeys and mules that is caused by *Burkholderia mallei*, a Gram-negative non-sporulating, non-encapsulated, non-motile bacterium (11). The organism is closely related to the causal agent of melioidosis, *Burkholderia pseudomallei* (3). The natural hosts for *B. mallei* are horses, donkeys and mules (solipeds), but other animals such as mice, hamsters, guinea-pigs, monkeys, lions and dogs are also susceptible to this pathogen (3, 11). Cattle, swine and chickens appear to be resistant to glanders, even after experimental infection (9, 12).

The disease is commonly classified into three types, viz., pulmonary glanders, nasal glanders and cutaneous glanders. The clinical signs observed in these three forms of glanders are described below:

- Pulmonary glanders is characterised by the formation of round, greyish, firm, encapsulated nodules containing yellowish, cheesy pus that is embedded throughout the lung tissue
- Nasal glanders is typified by the formation of nodules in mucous membrane of the nasal cavity, particularly the septum. Many of these nodules rupture and liberate mucopurulent exudates which become

mixed with the serous or mucopurulent exudates that are discharged from the nostrils. This yellowish green exudate is highly infectious. The ruptured nodules form ulcers with irregular, raised and hyperaemic borders. The ulcers heal slowly to form a stellate scar. Regional lymph nodes are very often swollen.

- Cutaneous glanders or farcy is identified by the typical glanders nodules that form along the lymph vessels between affected lymph nodes. These nodules often rupture through the skin to discharge a yellowish pus and form deep ulcers which heal slowly. The affected area gives an appearance of streaks along the hind limbs.

In most outbreaks, these forms are not clearly distinct and may occur simultaneously in an animal. Chronic forms are more common, with the acute form typically progressing to death within about a week. The acute form is more common in donkeys and mules than in horses.

*B. mallei* is an obligate animal pathogen that has not been found free-living in the environment; however, *B. pseudomallei* can be isolated from tropical soil. The organism has an affinity for warm and moist conditions and may survive for up to three months in stable bedding, manure, feed and water troughs (particularly if heated), wastewater and equine transporters (saddlery and harness equipment). Transmission among equids is primarily by oro-nasal mucous membrane exposure, inhalation and mastication (possibly ingestion) of skin exudates and respiratory secretions of infected animals, including those with latent and subclinical infection. Sharing feed and water troughs facilitates such transmission (19, 21).

Humans may become infected with *B. mallei* through contact with infected animals, through laboratory accidents and through inhalation (3). Natural transmission from animals to humans is apparently inefficient, as animal handlers are rarely infected. In contrast, *B. mallei* poses a considerable risk to laboratory workers. Most of the laboratory cases of glanders in humans occurred because of the absence of adequate laboratory facilities required to work with this organism or they

were in breach of practice of standard microbiological techniques (SMTs)/good laboratory practices (GLPs)/biosafety. Untreated human cases of glanders result in an extremely high mortality rate (3). However, early and aggressive treatment with combinations of systemic antibiotics can be curative (18).

*B. mallei* is one of the few pathogens that was used as a biological weapon during World War I (23) and World War II (5). The potential re-emergence of *B. mallei*, as a biological weapon, is of great concern. *B. mallei* is highly infectious via the respiratory route as an aerosol. The incubation period is long, making it difficult to trace the source of an outbreak or attack. The symptoms are non-specific and there is virtually no modern clinical experience to facilitate disease recognition. The illness has a high mortality rate if not recognised and treated. The organism is resistant to multiple antibiotics, making empirical therapy likely to fail (6). No vaccine is presently available against *B. mallei*.

Glanders has been eradicated from many countries through statutory testing, elimination of infected animals and import restrictions. However, the disease is still endemic in parts of Africa, Asia, the Middle East, and the Central and South America (2). Glanders has been a major concern in India. Cases of glanders were reported from different parts of the country during the post-independence era, mostly in northern India (1, 4, 17, 21, 25). A detailed account of the incidence and epidemiology of glanders on military farms in India was reported in 1975 and 1981, indicating high morbidity in the areas of dense populations of horses, donkeys and mules (21). A focal outbreak of the disease was reported from Punjab and Haryana in 1988 and 1990 and again in 1998 (1, 8, 25). A case of cutaneous glanders in 1998 in a mule from the Rohtak District of Haryana was the last confirmed case prior to the 2006-2007 outbreak in India (8). No disease was reported in India for about eight years until 2006 when glanders re-emerged in several parts of the country (10). At this time, outbreaks revealed several clinical cases of typical nasal/cutaneous forms of glanders in different states of India.

Glanders outbreaks have had a significant economic impact and are also of zoonotic importance. The disease is notifiable in India (it is also one of the diseases listed by the World Organisation for Animal Health [*Office International des Épizooties*: OIE]) and affected animals must be eliminated to control the further spread of this dreaded disease. Thus, the elimination of affected and in-contact animals, restricted movements and the postponement of animal fairs and games result in considerable financial losses to equine owners and race courses. This paper describes various glanders outbreaks in India during the periods 2006-2007 and 2009-2010. It also evaluates the dynamics of the disease and discusses issues for efficient control strategies.

## Materials and methods

### Collection of biological samples

Samples of blood, nasal exudate and pus were collected aseptically from animals showing typical clinical signs of glanders and from apparently healthy in-contact animals. These samples were transported to the laboratory on ice.

### Complement fixation test

Serum samples were subjected to the complement fixation test (CFT) in line with standard protocol described by the OIE (24) using commercial kits (Bioveta, a.s., Komenského) for the detection of *B. mallei* antibodies. It is important to mention that serum samples from equines, including donkeys and mules, were initially heat inactivated at 56°C for 30 min. In the event of persistence of anti-complementary activities in donkey and mule serum, sera were inactivated at 62°C for 20 to 30 min. Repeat samples from these animals were treated in the same way in case of persistence of problems. Positive reactors were finally confirmed using the mallein test as described below. A CFT titre of 1:8 and above was considered positive for glanders. Tests were repeated three times before declaring the final results as positive or negative.

## Isolation and identification of bacteria

Nasal swabs containing pus were plated on glycerol-dextrose agar (GDA) which is actually nutrient agar containing 5% glycerol and 1% dextrose following standard protocol (14). In addition, a single colony simulating *B. mallei* was streaked on a fresh plate to obtain pure culture. The organism was identified based on morphological, cultural and biochemical characteristics and the observation of non-motile bacteria in the motility test (19).

### The mallein test

The mallein test is one of the most sensitive, reliable and specific tests for glanders. The test was performed to confirm positive reactors prior to the elimination of the animal after the methods described earlier had been completed (16, 22). The concentrated mallein purified protein derivative (PPD) (obtained from the Indian Veterinary Research Institute in Izatnagar) was injected intradermopalpebral into the lower eyelid of suspected animals at a dose of 0.1 ml. The test was read at 24 h and 48 h post injection (hpi). Horses showing marked oedematous swelling of the eyelid, other clinical signs, such as purulent discharge from the inner canthus or conjunctiva accompanied by photophobia, rise in body temperature, pain and depression within 12-72 hpi were considered positive for glanders infection.

### Polymerase chain reaction

A *B. mallei*-specific polymerase chain reaction (PCR) assay that targets the *fliP* gene was performed to confirm the organism. Oligonucleotide primer pair targeting *fliP* gene included the forward primer 5'-CCCATGGCCCTATCGAAG-3' and the reverse primer 5'-GCCCGACGAGCACCTGATT-3' (20). Two to three pure colonies of *B. mallei* were dissolved in 200 µl nuclease-free water and culture was boiled for 10 min and snap-chilled on ice. After brief micro-centrifugation, 2 µl clear supernatant was used as a template for PCR. The DNA was first denatured at 95°C for 5 min and was then subjected to 30 cycles of amplification which comprised

denaturation at 95°C for 1 min, annealing at 61°C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. PCR products were analysed in 1.2% agarose gel and results were documented using the Gel Documentation System (Alpha Innotech, San Leandro, California) under a UV transilluminator.

## Results

### Pattern of glanders occurrence in India

The timeline and number of animals affected during the glanders outbreaks in states in India between 2006 and 2007 and 2009 and 2010 are presented in Table I. Recovery of *B. mallei* isolates from clinical specimens is summarised in Table II. A total of 18 *B. mallei* isolates were recovered from horses ( $n=7$ ), mules ( $n=8$ ), ponies ( $n=2$ ) and a donkey ( $n=1$ ). The clinical signs, diagnosis, course and dynamics of disease outbreaks, microbial isolation and serological surveillance strategies are discussed.

#### First outbreak in Maharashtra (July 2006)

Preliminary information describing a glanders-like disease in equines was reported by the Western Regional Disease Diagnostic Laboratory (WRDDL) in Pune during the last week of July 2006. Clinical cases showing the typical nasal/cutaneous form of glanders were observed in Pune and Panchgani (Fig. 1). Major clinical signs included nasal ulcers, nodular swellings all over the body, oedematous swelling or wounds on the legs, cough, dyspnoea, reduced appetite and wasting. Three described forms (i.e. cutaneous, nasal and pulmonary) of glanders were not distinct in different animals. Bio-samples including nasal swabs ( $n=2$ ), abscess swabs ( $n=3$ ) from three animals and serum samples ( $n=17$ ) were collected from Pune. Clinical specimens, including nasal swabs ( $n=13$ ), abscess swabs ( $n=2$ ), and sera ( $n=7$ ), were collected from seven clinical cases in Panchgani. Sera ( $n=20$ ) were also collected from in-contact and apparently healthy horses for serological diagnosis.



Table I  
Status of glanders outbreak in various states of India, July 2006-December 2010

Serial No.	States	Date	No. of serum samples	Positive samples*	Species			
					Horses	Ponies	Donkeys	Mules
1	Maharashtra	July-Aug 2006	357	23 (4)	20	3	0	0
2	Uttar Pradesh	Dec 2006-March 2007	548	70 (2)	40	8	16	6
3	Punjab	Feb 2007	723	3 (0)	3	0	0	0
4	Uttarakhand	March 2007	701	21 (1)	0	0	0	21
5	Andhra Pradesh	Sept 2007	1 771	16 (4)	0	0	0	16
6	Himachal Pradesh	Oct 2007	252	6 (1)	0	0	0	6
7	Haryana	Nov 2007	239	1 (0)	1	0	0	0
Re-occurrence of glanders								
8	Chhattisgarh	Oct 2009-Jan 2010	286	13 (2)	12	1	0	0
9	Himachal Pradesh	May 2010	314	4 (1)	0	0	0	4
10	Uttar Pradesh	Dec 2010	121	7 (3)	3	0	0	4
Total			5 312	164 (18)	79	12	16	57

\* figures in brackets indicate number of isolates recovered

Table II  
Distribution of *Burkholderia mallei* isolates recovered from nasal and abscess swabs

Maharashtra	Uttar Pradesh	Punjab	Uttarakhand	Andhra Pradesh	Himachal Pradesh	Chhattisgarh	Total
NS = 15 (H: 3) AS = 5 (P: 1)	NS = 24 (5) (H: 2, M: 1, P: 1, D: 1) AS = 3 (0)	NS = 2 (0)	NS = 9 (M: 1)	NS = 5 (M: 4)	NS = 16 (M: 2) AS = 6 (0)	NS = 15 (H: 2)	NS = 86 (17) (H: 7, M: 8, P: 1, D: 1) AS = 14 (P: 1)

NS nasal swab  
AS abscess swab  
H horse  
M mule  
D donkey  
P pony

Figures in brackets indicate the number of isolates recovered

On laboratory testing of collected samples, one abscess swab of a pony from Pune and three nasal swabs of horses from Panchgani yielded results confirming the presence of *B. mallei*. Out of 357 serum samples (self collection/collected and despatched by local veterinarians), a total of 23 (horses = 20 and ponies = 3) were found positive for glanders using the CFT.

The authorities of the Maharashtra State Animal Husbandry Department and the Department of Animal Husbandry, Dairying and Fisheries (Ministry of Agriculture, Government of India) were informed. Strategies for the prevention of further spread of the disease to other areas and neighbouring

states, as well as action in conformity with the Glanders and Farcy Act, 1899, which has since been repealed and replaced by the Infectious Animal Disease Act, 2009, were also suggested. Accordingly, 23 affected animals were reportedly eliminated by the State authorities. Follow-up of serological surveillance from 2007 to 2010 revealed a negative status of the disease in Maharashtra.

#### Outbreak in Uttar Pradesh (December 2006)

In December 2006, a field veterinarian reported the nasal form of the disease among donkeys at a brick kiln in Anantpur village in the Gautam Budh Nagar District in Uttar Pradesh.

The serum samples were collected from 78 equines, including horses, mules and donkeys from Anantpur and the adjoining village of Mawana in the District of Meerut. Serological examination of serum samples using the CFT yielded 15 positive reactors for glanders.

A. Typical nasal form



B. Typical cutaneous form



Figure 1  
Detection of glanders in Pune, Maharashtra in 2006  
Nasal and cutaneous forms observed in the same animal

Following this incident, active surveillance and follow-up activities conducted by the National Research Centre on Equines (NRCE) and the State Animal Husbandry Department authorities were initiated. In particular, samples collected from animals from Meerut, Baghpat, Ghaziabad and Gautam Budh Nagar District were subjected to serological examination. In all, 70 of a total of 548 samples tested were found positive for glanders until June 2007. Two *B. mallei* isolates were recovered from nasal swabs ( $n = 17$ ) of horses. A total 70 equines, including horses ( $n = 40$ ), donkeys ( $n = 16$ ), mules ( $n = 6$ ) and ponies ( $n = 8$ ), were found positive for glanders. No glanders-positive reactor was found upon subsequent testing of 664 serum samples until March 2008.

#### Report from Punjab (February 2007)

Glanders was suspected in a mare in Gurudwara Dukh Niwaran Sahib in Ludhiana District, Punjab Province in February 2007. A total of 723 serum samples (from horses and ponies) and two nasal swabs from suspected mares were collected for laboratory examination. Three mares tested positive using the CFT; no isolation was made from nasal swabs.

#### Outbreak in Uttarakhand (March 2007)

In March 2007, clinical cases of disease in ponies were reported from the Haldwani area of Nainital District. After isolation of the causative organism from nasal swabs ( $n = 9$ ) from one infected mule, serological surveillance conducted among equines from a nearby area resulted in the detection of 21 seropositive mules out of a total of 701 equines tested. Follow-up surveillance did not reveal any positive case in the State.

#### Outbreak in Andhra Pradesh (September 2007)

In September 2007, glanders was suspected in the Mehboob Nagar District of Andhra Pradesh. Four *B. mallei* isolates were recovered from nasal swabs ( $n = 5$ ) collected from mules that exhibited the typical nasal form of glanders. On the basis of CFT, 16 cases of glanders were confirmed out of a total of 1 771 equines tested.

### **Detection of glanders in Himachal Pradesh (October 2007)**

During October 2007, the disease was reported in mules and donkeys in the Nahan area of the Sirmaur District of Himachal Pradesh. Nasal swabs ( $n = 7$ ), abscess swabs ( $n = 3$ ) and serum samples ( $n = 252$ ) were collected from mules, donkeys and ponies. Investigations of 252 equines revealed 6 confirmed cases of glanders in mules using the CFT. *B. mallei* was isolated from one nasal swab collected from a mule.

### **A case of glanders in Haryana (November 2007)**

A single case of glanders was reported from a horse in the Karnal District of Haryana in November 2007. No other case was found positive out of a total of 239 serum samples tested from horses, donkeys and mules from nearby areas.

### **Glanders outbreak in 2009-2010**

As part of follow-up activities after the first fulminant outbreak in Maharashtra and then in Uttar Pradesh, Punjab and Uttarakhand, the NRCE in Hisar conducted a nationwide surveillance and monitoring. Serum samples were collected from equines in affected states, neighbouring states and in the states situated along animal migration routes, and were tested using the CFT. To ensure quality control in sample collection for to facilitate diagnosis, state veterinarians were trained in sample collection, proper packaging and shipment. This also had a positive effect on the effective implementation of the glanders surveillance and monitoring programme. The status of the glanders outbreak was promptly conveyed to the Department of Animal Husbandry, Dairying and Fisheries, Remount Veterinary Corps (RVC) authorities and to State Governments. After a lull across the country in 2008, glanders emerged in Chhattisgarh state for the first time.

### **Outbreak in Chhattisgarh (October 2009)**

During October 2009, the NRCE received information from Raipur District in Chhattisgarh State about a 'glanders-like' disease condition in indigenous horses. The NRCE team investigated the case promptly.

The cutaneous form of glanders was detected in five horses in Raipur. Serum samples and nasal swabs were collected from infected and in-contact animals, and from healthy animals from an organised farm located 3 km away. Based on clinical signs, agent identification and serological investigation of 63 horses, four animals were found positive using the CFT and *B. mallei* was isolated from nasal swabs ( $n = 15$ ) from two horses. The identity of the organism was also confirmed by PCR, the motility test and biochemical tests. The animals were eliminated by the State authorities, in accordance with the new Infectious Animal Disease Act, 2009. The disease continued to remain on the premises until March 2010, as new animals introduced in the premises became affected. Glanders re-emerged in the Raipur District between January and March 2010 which was revealed during follow-up surveillance activities. During the entire outbreak in this State, 13 animals (horses = 12 and pony = 1) revealed positive titres in CFT from the total of 286 equines tested. Continuous serological monitoring has been conducted on 330 equines throughout the State of Chhattisgarh, since April 2010 to March 2011, using the CFT and has rendered negative results.

### **Additional cases in Himachal Pradesh (May 2010)**

In May 2010, glanders was again reported from the Pandoh area of the Mandi District in Himachal Pradesh. Serum samples ( $n = 314$ ), nasal swabs ( $n = 3$ ) and abscess swabs ( $n = 9$ ) were collected from mules and donkeys. The nasal form of glanders was observed, serum samples from four mules gave positive titre in the CFT. Two mules died within a week, implicating the acute form of glanders. One *B. mallei* isolate was recovered from a nasal swab.

### **Recurrence in Uttar Pradesh (December 2010-January 2011)**

Continuous serological surveillance of the equine population from Uttar Pradesh revealed negative results in 2008 and 2009. However, glanders was reported in mules in December 2010 by the veterinary officer in Chandpur in the Bijnor District. Ponies, mules



and donkeys working in brick kilns were affected by the disease. Nasal swabs ( $n = 7$ ), abscess swabs ( $n = 3$ ) and blood samples were collected from infected animals. Three *B. mallei* isolates (one each from a pony, mule and donkey) were recovered from nasal swabs. One case of glanders in horses was also reported from the village of Hasanpur Nali in the Ghaziabad District. Of 121 equines tested, seven animals (mules = 4, horses = 3) were found positive for glanders using the CFT.

In conclusion, 164 equines out of a total of 5 312 equine samples tested from eight states were found to be seropositive for glanders and 17 *B. mallei* isolates from nasal swabs ( $n = 86$ ) and one *B. mallei* isolate from abscess swabs ( $n = 14$ ) were recovered on GDA media.

#### **Elimination of infected animals and control measures**

The incidence of glanders in the above-mentioned states was brought to the attention of the relevant animal husbandry authorities and measures were taken to restrict movements of animals to and from infected areas. Equine owners were given adequate safety devices, such as gloves, face masks and aprons and were trained to handle infected animals until elimination. All CFT-positive animals ( $n = 164$ ) were euthanised by intravenous injection of sodium-barbiturate in accordance with standard guidelines. Carcasses of the culled animals were buried 1.83 m (6 feet) in soil far from localities and using the necessary protective measures to prevent carnivores from unearthing graves. Compensation decided by the state animal husbandry departments was provided to equine owners who suffered the loss of animals. Infected premises, feeding and husbandry equipment were thoroughly disinfected with sodium hypochlorite (500 ppm available chlorine), lime-water, lyzol and phenyl. Stable walls were white-washed and painted. Equine owners were advised not to introduce new animals in the infected stables for up to three months. State animal husbandry authorities were requested to participate in the collection and despatch of samples for follow up serological surveillance purposes across the entire state.

#### **Dynamics of glanders outbreak**

Glanders was prevalent across India (7) and the most significant outbreaks are believed to have occurred prior to the implementation of the glanders control strategies under the Glanders and Farcy Act, 1899 (Act No. XIII of 1899). This, in fact, is the first animal disease act that was implemented in pre-independent India to control and eradicate the disease. Enforcement of legislative measures, diagnosis and monitoring of glanders by the then Imperial Bacteriological Laboratory in Mukteshwar contributed to a decrease in the number of glanders cases. However, limited documented information regarding the time and space of glanders outbreaks in the pre-independent era is available. Post-independence, the disease erupted as outbreaks during late 1960s (4, 17), when India faced two major wars (Indo-China in 1962 and Indo-Pakistan in 1965 and 1971). This disease eruption may have been due to the increased porosity of borders and establishment of the disease in equines belonging to the army engaged in war-related activities at or near the borders. However, the disease was contained and controlled successfully but the niche tends to remain in north India, where the equine population is high and exposure to infected animals was most likely to occur.

The NRCE which is exclusively dedicated to research on equines, came into existence in 1985. With the combined intervention of the NRCE and veterinary service authorities, glanders incidence again reduced drastically. Two focal outbreaks of glanders in Punjab and Haryana were reported by the NRCE in 1988 and 1990, respectively (1, 25). One case of farcy was reported in Haryana in 1998 (8). At that time, no case of glanders had been detected in India for almost eight years. It appears that infection existed unsuspected and unobserved somewhere in the northern parts of the country and the disease remained unnoticed. Following the outbreak of glanders in Maharashtra in 2007, investigations conducted by the NRCE and information gathered from farmers of affected animals revealed that infected animals were purchased in animal fairs from traders of north India. Similarly,



infected animals found in Andhra Pradesh were actually purchased from Uttarakhand and Uttar Pradesh in north India. Equine owners in Chattisgarh reported that the animals had come from northern states of India. A detailed investigation on the movement of equines indicated that the source of infection still persists in northern areas of India. Thus, this region requires specific attention and intense control/containment measures to eradicate the disease from the country.

## Discussion

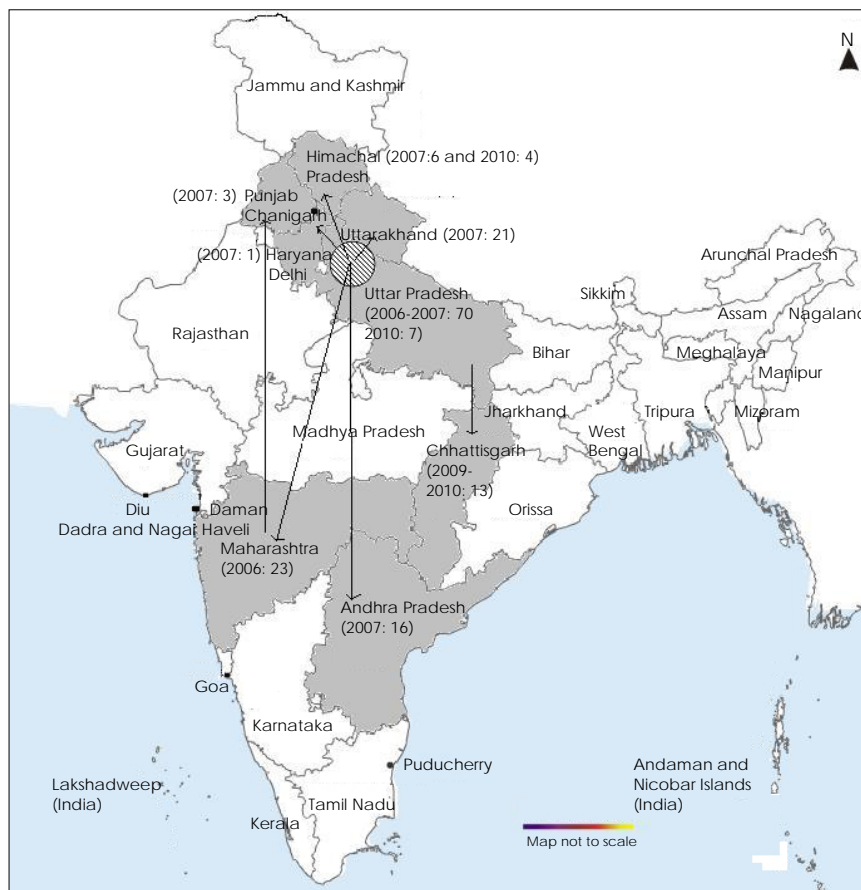
In India, cases of glanders were reported in both the pre-independence and post-independence periods. Although disease incidence did subside for a while, cases of glanders were reported from time to time in various parts of the country. Sporadic incidents and outbreaks in certain pockets of India have been reported by Gangulee *et al.* (4), Mishra *et al.* (13), Ray (15) and Sen *et al.* (17). Verma *et al.* (21) provided a detailed report on the incidence and epidemiology of glanders on military farms in India. In both 1988 and 1990, focal outbreaks of the disease were observed in Punjab and Haryana (1, 25). Kumar *et al.* (8) in 1998 reported a case of cutaneous glanders in a mule from Rohtak in Haryana which was the last confirmed case until the re-emergence of glanders in 2007.

The NRCE continues to actively monitor the disease situation nationwide. Intense follow-up visits are still conducted to ensure active serological surveillance. Follow-up testing performed by the NRCE and physical surveillance by local authorities thereafter has been done on over 3 500 equines. Verma *et al.* (21) reported that many cases of glanders are latent and clinically inapparent, so systematic testing is essential to identify all infected animals in an outbreak. The disease was successfully controlled in the State of Maharashtra after the 2006-2007 outbreaks, thanks mainly to efforts of the NRCE in coordination with WRDDL and the Government of Maharashtra.

After the first detection of glanders in July 2006 in the District of Pune, the disease was detected in other states as a result of heightened awareness, surveillance and follow-up action conducted by the NRCE. The outbreak of glanders started in July 2006 in Pune District, Maharashtra, and gradually extended to six other states in 2006 and 2007 (Fig. 2). The spread and pattern of diseases were elucidated by gathering data of animal movements. An analysis of data on animal movements showed that Uttar Pradesh could be the glanders hotspot. Migration of infected equines from this State could have been responsible for the spread of the disease to Maharashtra and Punjab. Subsequently, animal movements from Punjab led to the spread of glanders to Andhra Pradesh. On account of the long incubation period of the disease and antibiotic treatment of affected animals, the disease was not prominent and animals appeared healthy. Animal traders knowingly or unknowingly sold apparently healthy (but in reality infected) animals at animal fairs, at times at lower prices. Apparently healthy but chronically infected animals constitute the source of infection to other animals.

In rural India, equines are the sole source of income among the poor. Some of the equines are used for pulling the marriage cart locally known as 'booggy', as grooms traditionally ride mares during a marriage to reach the place of the bride to mark the symbol of courage, dedication and sacrifice. Donkeys, mules, and ponies are generally used for labour, mainly carrying bricks in brick kilns or on construction sites, and for carrying construction materials. In the hilly terrain of Uttarakhand and Himachal Pradesh, equines are mainly used for carrying pilgrims to holy religious places and for carrying fruit during the harvesting season. Contractual hiring and equine trading are most common in western Uttar Pradesh.

Glanders is a notifiable disease in India and the Glanders and Farcy Act, 1899, was the first act on animal diseases to be promulgated in India. It has now been substituted by the Infectious Animal Disease Act, 2009, which



Areas shaded in grey: infected states

Figure 2

Infected states of India infected by glanders, 2006-2011

Figures in brackets indicate the number of glanders-positive cases reported from the respective states in the year indicated

Circular zones in Uttar Pradesh state are suspected to be the source of glanders infection

Movement of infected animals and spread of disease in different states are indicated by arrows

was implemented in the country to control the disease as well to save human life by introducing quarantine and the elimination of infected animals. In accordance with the erstwhile Glanders and Farcy Act, 1899, glanders-positive animals had to be destroyed and equine owners received 50 Indian Rupees (equivalent to US\$1) in compensation for the loss of each animal. The compensatory amount was encouraging for the equine owner in the late 1800s. This amount is now very insignificant and in no way encourages the owner to have their animals eliminated in accordance with the provisions of the Act. As a result, owners in affected areas are not willing to get their animals tested for glanders. Worse still, owners sometimes flee with infected animals to avoid the culling. A lack of

awareness among equine owners of the zoonotic nature of the disease, their reluctance to visit a local animal health care department and consult veterinarians, and their preference of indigenous medicine over modern therapy are the main factors that explain the under-reporting or absence of reporting of cases of glanders-positive animals in time. It is only after the disease situation deteriorates and infection spreads to more animals, that glanders is suspected and comes to the attention of the veterinary officers. The compensatory issues, the lack of awareness of animal owners, delays in reporting by veterinary professionals are among the important social reasons that pose a challenge to disease control.

New legislation, namely, the Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009, came in to effect in March 2009 to prevent, control, and eradicate infectious and contagious diseases affecting animals, and also to prevent outbreaks or the spread of such diseases from one state to another, and to meet the international obligations of India for facilitating the importation and exportation of animals and animal products. This new Act repealed and replaced the Glanders and Farcy Act, 1899. It also encompasses other zoonotic diseases. This legislation prohibits, in the event of an outbreak, the movement of all animals of all species, from the place where in which they are kept, to any other place. Markets, fairs, exhibitions, or any other activity which involves grouping or gathering of any species of animals within a controlled area are also prohibited by law.

Thus, glanders has a tremendous psycho-socio-economic impact on rural equine owners which has resulted in a disastrous psychological, financial and social impact on poor equine owners. The State animal husbandry departments have been empowered to decide upon compensation amount of glanders-affected animals. To support equine owners and increase awareness of the disease, some state governments offer reasonable compensation to owners of infected animals before or after euthanasia of the affected animals.

It is concluded that the nidus of the disease remained in a small area of northern India, especially in the States of Uttar Pradesh and

Uttarakhand. Concerted efforts by regional disease diagnostic laboratories, veterinary extension activities to inform farmers on equine diseases, the development and implementation of realistic and implementable policies will be of the utmost importance in the control and eradication of the disease from India in the future.

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## Conflict of interest

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