GLANDERS: A PUBLIC HEALTH CONCERN

1. INTRODUCTION

Glanders is a highly infectious and often fatal zoonotic disease primarily affecting horses, donkeys and mules. It is a notifiable disease of equines caused by Gram-negative bacterium, *Burkholderia mallei*. It is a rare disease in humans with cases having occurred in veterinarians, other people working closely with horses, and laboratory workers handling the organism. In addition to animals with clinical disease, asymptomatically infected animals also pose risk to humans. Humans are accidental hosts and human to human transmission is rare.

2. ETIOLOGICAL AGENT:

*Burkholderia mallei*, is Gram-negative bacillus and is closely related to *Burkholderia pseudomallei*. *B. mallei* is sensitive to the external environment and destroyed by exposure to direct sunlight within 24 hrs and is killed by most of the common disinfectants such as sodium hypochlorite and other chlorine compounds, potassium permanganate, copper sulphate, formalin, chlorine, 70% ethanol and 2% glutaraldehyde. The organism can remain viable for 3 to 5 weeks in damp environment and decomposing material, may survive for up to 4 weeks in clean water and for about 6 weeks in contaminated stables (stable bedding, manure), water troughs and fodder. This bacterium has been listed as potential agent for biological warfare and bioterrorism under CDC category B.

3. EPIDEMIOLOGY:

3.1 HISTORY:

Glanders was first described by the Greeks in 450-425 BC and again by the Romans in 400-500 AD. Glanders has also been known by other names viz., equinia, malleus, droes and farcy. First human case of glanders was reported in 1793. Its zoonotic potential was not reported until the beginning of the 19th century.

3.2 GLOBAL SITUATION

ANIMALS

Glanders was a worldwide problem in equids for several centuries, but this disease was eradicated from most countries by the mid-1900s. In contrast, the disease is still endemic in developing world. The disease is endemic in parts of Africa, Southern Asia, Middle East and Central and South America. The incidence of *B. mallei* infection in equines, camels and zoo animals has been reported in the Middle East and Southern Asia which...
includes United Arab Emirates, Bahrain, Iran, Iraq, Kuwait, Lebanon, Mongolia, India, Myanmar, Afghanistan, Pakistan and Philippines. In South America, regular occurrence of glanders was reported in Brazil. The disease is also prevalent in few countries such as Eritrea and Ethiopia in Africa.

**HUMANS**

Glanders is a zoonotic disease. In 1913, one authentic case was of a Veterinary Pathologist at Punjab Veterinary College, Lahore in erstwhile India has been reported. Six unrelated cases of glanders occurred in 1945 among laboratory at Maryland, USA. Subsequently in 2000, a microbiologist at U.S. Army Medical Research Institute of Infectious Disease (USAMRIID) was presumptively diagnosed for glanders due to his work history and was successfully treated. Two Japanese laboratory workers accidentally exposed to *B. mallei* and died at the Ping Fan Institute in 1937.

**3.3 INDIAN SCENARIO**

**ANIMALS**

In India, it is believed that glanders was first observed in the cart horses in 1881. Confirmed cases of *B. mallei* infection in Indian equines were documented in 1913. A detailed account of incidence and epidemiology of glanders in military and civilian farms in India had been reported in the early 1980s. A sudden re-emergence of the disease was observed in 2006 and it continued to affect equids in several regions of the country. Subsequently surveillance was intensified which resulted in detection of new sporadic cases all most every year from Uttar Pradesh, Maharashtra, Himachal Pradesh, Uttarakhand, Chhattisgarh, Andhra Pradesh, and Punjab. Continuous surveillance revealed glanders outbreaks at Jammu, Gujrat, Rajasthan, Madhya Pradesh, and Haryana. The reports of glanders in animals in India is shown in Table.1.

**HUMANS**

Despite many reported cases of the disease in equines, no human case of glanders has been reported in India till date.

**3.4 RISK GROUP**

Occupation exposure is the main risk factor to veterinarians, farmers, horse traders/ fanciers, laboratory workers, and other persons who are closely associated with equines or handling the organism.

**3.5 TRANSMISSION:**

**ANIMALS**

Ingestion of the organism is the major route of infection. Experimental evidence suggests that inhalation of the organism is less likely to result in typical cases of the disease. Acquiring the disease through skin or mucous membranes is possible but rare. The transmission is facilitated if animals share feeding or watering facilities. *B. mallei* is readily spreads from fomites. Carnivores usually become infected when they eat contaminated meat.

**HUMANS**

*B. mallei* is transmitted to humans through contact with tissues or body fluids of infected animals. The bacteria enter the body through cuts or abrasions in the skin and through mucosal surfaces such as the conjunctiva and nose. The infection may occur by inhaling infected aerosol dust. Animal-to-human and human-to-human transmission of glanders is rare.
3.6 INCUBATION PERIOD

ANIMALS
The incubation period for glanders in equines ranges from a few days to many months (2 - 6 weeks).

HUMANS
In humans, the acute form of disease has a typical incubation period of 1–14 days, while the chronic form of the disease has an incubation period of up to 12 weeks. A localized cutaneous infection typically follows within one to five days.

4. CLINICAL SYMPTOMS

4.1 ANIMAL GLANDERS
Glanders has traditionally been clinically categorized into nasal, pulmonary and cutaneous forms based on the most commonly affected organ systems in equines. Glanders can have nasal, pulmonary and cutaneous forms.

i) Nasal form: In nasal form, deep ulcers and nodules develop inside the nasal passages resulting into mucopurulent, yellowish often copious discharge. Discharge may be unilateral or bilateral and can become blood tinged. The ulcers may coalesce resulting sometimes into nasal perforation. Healed ulcers become star–shaped scars, which may be found concurrently with nodules and ulcers. Regional (submaxillary) lymph nodes become enlarged bilaterally or unilaterally. They are usually indurated in acute illness and may suppurate and drain. Nasal infections can further spread to involve the lower respiratory tract.

ii) Pulmonary form: Pulmonary involvement is most common clinical form, often occurring in combination with other forms. Affected animals develop nodules and abscesses in the lungs. Pulmonary form may remain inapparent or may be characterized by mild to severe respiratory signs such as coughing and dyspnea with febrile episodes and progressive debilitation.

iii) Cutaneous form: Cutaneous form (Farcy) is characterized by multiple nodules developing in the skin along the lymphatics, which may rupture and ulcerate discharging an oily, thick yellowish exudate. Regional lymphatics and lymph nodes are also enlarged with lymphatics filled with a purulent exudate. Some animals may also reveal joint swelling, painful edema of legs or orchitis. Skin lesions may appear anywhere but are most common on inner thighs, limbs and abdomen. Skin lesions in glanders heal very slowly, often continuing to discharge fluid although dry ulcers may also be seen. Animals with cutaneous glanders may eventually become debilitated and can subsequently die.

Based on clinical progression of the disease, glanders cases may be characterized as acute (or subacute), chronic or latent forms.

a) Acute form: Donkeys and mules often develop acute disease after exposure. Amongst donkeys and mules, the latter appear to be more resistant and course of the illness may be slower in them. Acute disease is characterized mainly by nasal and respiratory involvement with clinical signs such as high fever, decreased appetite, weight loss, depression, bouts of coughing and progressive dyspnea. An initial watery nasal discharge, often unilateral at first, can develop into the classic nasal form. There may also be a purulent ocular discharge as well. Animals with the acute form usually die in a few days to a few weeks from septicemia and respiratory failure.

b) Chronic form: Horses usually develop chronic glanders after exposure. Chronic form develops insidiously and lasts for months to years, with periodic episodes of exacerbation, resulting in slowly
progressive debilitation. Although the initial signs may be mild and easily overlooked such as intermittent low fever and slightly labored breathing, progression of the lesions results in listlessness, generalized weakness and wasting, with an intermittent cough. Some animals become lame, with swelling of the joints in the hindquarters, or have hematuria, polyuria, diarrhea, epistaxis or orchitis. Signs of nasal glanders or skin involvement may also be seen and stressors can result in acute bronchopneumonia. Chronic glanders is eventually fatal in most animals, although a few may recover clinically while remaining carriers. Animals that recover from glanders can also relapse.

c) Latent form: In latent cases of glanders, lesions may occur sporadically in the lungs and other internal organs. The clinical signs are usually minimal and most often consist only of intermittent low fever, nasal discharge and/or occasional labored breathing. Latent glanders is most commonly encountered in horses. As regards clinical signs in animal species other than equines, naturally infected dromedary camels reveal signs similar to equines such as fever, lethargy, emaciation and nodules and ulcers in the nasal passages with severe mucopurulent discharge. In carnivores (cat, dog, tiger) that acquire infection from eating infected meat, nodules and ulcers are found in the nasal passages, conjunctivae and deeper respiratory tract with purulent yellowish nasal discharge. Additional clinical signs may include swelling of lymph nodes and dyspnea.

Differential Diagnosis:
- Strangles (*Streptococcus equi*),
- Melioidosis (*Burkholderia pseudomallei*)
- Ulcerative lymphangitis (*Corynebacterium pseudotuberculosis*)
- Botryomycosis, Sporotrichosis (*Sporothrix schenckii*)
- Pseudotuberculosis (*Yersinia pseudotuberculosis*)
- Epizootic lymphangitis (*Histoplasma farciminosum*)
- Horse pox
- Tuberculosis (*Mycobacterium tuberculosis*)
- Gutteral pouch empyema
- Dermatophilosis
- Dermatomycoses
- Trauma and allergic conditions.

4.2 HUMAN GLANDERS

The clinical symptoms of glanders in humans are non-specific therefore accurate diagnosis and treatment is difficult. The symptoms in humans include low-grade fever and chills, malaise, fatigue, myalgias, backache, headache, rigors, chest pain and lymphadenopathy. Following forms of glanders have been described which are as follows:

i) Mucosal: Involvement of the eye and conjunctiva in *B. mallei* infection presents with excessive lacrimation and photophobia.

Nasal involvement is characterized by inflammation and swelling of the nose, which is common following inhalation of *B. mallei*. This may be followed by copious nasal discharge. Additionally, infection may invade the nasal septum and bony tissues, causing fistulae and tissue destruction.

The face may swell and regional lymphnodes may become inflamed. Infection may also extend to the lower respiratory tract, resulting in bronchitis accompanied with cough and mucopurulent sputum production.

ii) Cutaneous: Cutaneous manifestations include papular lesions that may erupt anywhere on the body with chronic, indolent course of infection. A localized infection with a discharge typically develops at the entry site. The abscess can
ulcerate and drain for a long period. Inflammation may extend along regional lymphatics and cause lymphangitis with numerous foci of suppuration along their course.

**iii) Pulmonary:** A pulmonary infection typically results in pneumonia, pulmonary abscess, pleuritis, and plural effusion. Signs and symptoms of pulmonary infection can include cough, dyspnea, chest pain, and mucopurulent sputum. Non-specific signs and symptoms such as fatigue, fever (often exceeding 102°F), chills, headache, myalgia, lymphangitis, sore throat, pleuritic chest pain, cough, tachypnea, dyspnea, nasal discharge, and gastrointestinal signs often accompany respiratory infections. Many symptoms may take up to 2 to 3 weeks to develop.

**iv) Disseminated:** Dissemination from local cutaneous or mucosal infection results in septicemia and the colonization of internal organs such as the spleen, liver, and lungs with the development of abscesses. These infections are typically associated with septic shock and high mortality. The reported mortality rate for the septicemic form of glanders is as high as 50% with treatment and greater than 95% without treatment.

**Differential Diagnosis:**
Typhoid fever, Tuberculosis, Syphilis, Erysipelas, Lymphangitis, Pyemia, Yaws, Melioidosis etc.

**5. PATHOGENESIS**
*B. mallei* after penetrating the mucosa travels through lymphatics and reaches regional lymph nodes from where it spreads hematogenously after proliferation. Varied necropsy lesions have been observed in equines such as granulomas and ulcers in different tissues and nodules and fulminating ulcers on mucous membranes of nasal passages, larynx and upper lip. Bone lesions have also been observed in mules, humans and experimentally infected animals. In pulmonary glanders, lungs may reveal nodular foci underneath the pleura and diffuse miliary granulomatous nodules with caseonecrotic centre. Edema of lung lobes and severe bronchopneumonia is observed in acute glanders often with serosanguineous fluid in paranasal sinuses, trachea and nasal cavity.

Histological lesions in equines generally include granulomatous or pyogranulomatous inflammation of lung tissues, wherein macrophages and epithelioid cells are the predominant cells. Variable sized foci of hemorrhage, edema and aggregation of interlobular and intra-alveolar fibrin are also observed. Vasculitis and thrombosis of nasal blood vessels with neutrophilic granulocyte infiltration is observed in nasal form.

The main protective mechanism of *B. mallei* to escape phagocytosis includes intracellular localization and presence of capsule and capsular lipopolysaccharide.

**6. LABORATORY DIAGNOSIS:**
The rarity of recent human cases may make glanders a difficult diagnosis even in regions with exceptional medical facilities. For equine glanders, complement fixation test (CFT) is the OIE (World Organization for Animal Health) recommended diagnostic test. There are no specific serologic tests for human glanders diagnosis. However, serologic tests like complement fixation test (CFT) and indirect haemagglutination test were instrumental in diagnosing US laboratory-acquired infections between 1944 and 1953. Besides, enzyme-linked immunosorbent assay (ELISA) may be used for human glanders diagnosis.

**SAMPLE COLLECTION AND TRANSPORTATION**

For serology:
- **Humans/Animal:** Collect 5 ml blood per vial. Preferably, separate the serum following standard biosafety
precautions. Transportation of blood/serum sample is required to be done in triple layer packing in cold chain (2-4°C) to designated laboratories.

The laboratories designated by Ministry of Health and Family Welfare (Government of India) (Vide letter no: 4-Z/2009/PRC Dated 18\textsuperscript{th} October 2011) for diagnosis of the disease in suspected human cases are as following:

- ICAR- National Research Centre on Equines (NRCE) Sirsa Road, Hisar, Haryana-125001 India

- Central Military Veterinary Laboratory (CMVL)Sardhana Road, Meerut Cant, Meerut, Uttar Pradesh- 250001, India

The samples for diagnosis of the disease in suspected human cases can be sent by state to above mentioned designated laboratories under intimation to IDSP, NPO (Email: idsp-npo@nic.in) for information.

7. TREATMENT:

Treatment with sulphonamides (trimethoprim-sulfamethoxazole, TMP-SMX) has been recommended. Recently, Piperacillin/tazobactum as an alternative for currently used drug ceftazidime for the treatment of glanders in view of the emergence of ceftazidime-resistant clinical isolates in Southeast Asia.

8. PREVENTION AND CONTROL:

8.1 GLANDERS CONTROL POLICY IN INDIA

Glanders and Farcy Act, 1899 (Act 13 of 1899) was the first Act on animal disease to be enacted in India for testing and destruction of glanders affected horses and notification of disease outbreak by veterinary authorities. The act has since been replaced by the ‘Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009’ which enunciates provisions for prevention, control and eradication of various infectious and contagious diseases affecting animals in India. The act serves as the prevailing glanders control policy in India.

8.2 ANIMAL SURVEILLANCE

As per prevailing International zoosanitary regulations for prevention and control of glanders, outbreaks upon occurrence are required to be immediately notified to World Organization for Animal Health (Office International des Epizooties (OIE)) and positive animals must be destroyed to stop establishment of \textit{B. mallei} in a niche and to avoid animal to human transfer. Strict veterinary regulations including serological testing of animals prior to transport reduce the risk of importation of glanders into disease free areas. OIE Animal Health Code for Terrestrial Animals (2016) enunciates detailed instructions for import of equines and equine products such as semen or serum from glanders-infected and glanders-free countries. However, currently available diagnostic tests for the disease have limitations and an element of risk of spreading the disease still remains even if all precautions are strictly followed. Therefore, in glanders-endemic areas, reasonable control of disease can only be achieved by a strict ‘testing and culling of positive animals’ policy in combination with adequate re-imbursement of animal owners encouraging owners to voluntarily subject their animals to screening for glanders, thus leading to detection and elimination of carrier animals and eventual eradication of the disease.

National Research Center on Equines (NRCE), Hisar is working as National Reference Laboratory for diagnosis of many equine diseases including glanders. The Centre has National Surveillance and Monitoring Programme for glanders in place as a major part of its ongoing
activities. NRCE is significantly contributing for early detection of cases, notification of the disease and subsequent follow-up surveillance for containment of the disease. NRCE has developed recombinant protein based ELISAs. Presently ELISAs are being validated as per OIE protocols.

Central Military Veterinary Laboratory (CMVL), Meerut is the premier animal disease diagnostic laboratory of the Indian Army. The laboratory has unique distinction of being first and only veterinary diagnostic laboratory in India to be accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL) as per IS/ISO 17025: 2005. CMVL is also contributing for detection of glanders cases.

Note: Both NRCE, Hissar and CMVL, Meerut are designated by Ministry of Health and Family Welfare, Government of India as reference laboratories for diagnosis of glanders in humans.

8.3 CONTROL MEASURES

Strict zoo-sanitary measures must be followed to manage the disease in animals as well as to reduce chances of contracting disease in humans. These comprise of disinfection of infected premises, proper disposal of carcasses as per existing guidelines, treatment of animal products like dung, urine and other secretions and code of conduct of hygiene for animal handlers and attendants. The clothing, shoes, other belongings of animal handlers and those of affected animals are likely sources for spread of infection. Thus, special care of such items is to be taken to avoid any unintentional spread of infection. Personnel in close contact with the diseased animals should follow high standard of personal safety, hygiene and strict antiseptic measures.

8.4 INTERSECTORAL COORDINATION

The infected equines, particularly the asymptomatic horses (carrier animals) are the greatest risk to humans. Therefore, local and regional animal and public health authorities need to pay careful attention and work together in the eventuality of a suspected outbreak to expedite identification and control of human infection. Glanders outbreak in equines should be brought to the notice of local medical officer for testing of human contacts after risk assessment of getting infected.

8.5 IEC

Lack of awareness among horse and donkey owners and the limited availability of veterinary services are key factors responsible for under reporting of the disease. Hence, control of glanders needs strict implementation of ongoing policy comprising education, awareness of owners & veterinarians and continuous veterinary education programs.

8.6 DO’S AND DON’TS

Do’s
- Isolate the animal suspected for glanders
- Keep feeding and watering trough separate for sick and healthy animals.
- Bury the left over feed/ fodder rejected by the sick animals.
- Separate persons should handle sick and healthy animals.
- Bury the dead animal deep underground at the designated place.
- Keep close watch for symptoms like nasal and eye discharges, respiratory distress and skin sores among the animals.
- The Veterinary Officers or personnel involved in the animal handling for sample collection or animal burying should use personal protective equipment (PPEs).
• Wash hands with soap and water every time while handling suspected animals/ feed/ water through etc.
• All sheds be disinfected with suitable disinfectants such as benzalkonium chloride, iodine, mercuric chloride, potassium permanganate, 1% sodium hypochlorite, 70% ethanol and 2% glutaraldehyde etc.
• Clean the contaminated materials with solution of 1 part household bleach (0.5% sodium hypochlorite solution) to 9 parts water.
• Personnel having wounds, scratches and abrasions on hands should never handle sick animals.
• Awareness programmes about glanders to be carried out regularly.

Don’ts
• Do not mix sick and healthy animals.
• Do not share feeding/ watering troughs between healthy and suspected sick animals.
• Do not throw in open, the left over feed and fodder left by the sick animals.
• Immediately move the sick animals to designated isolated place.
• Do not allow the same person to handle sick and healthy animals.
• Do not touch eatables without washing hands properly.
• Do not mix up the recovered ponies with the healthy stock.
• Do not open the carcasses for necropsy in case of death due to glanders.
• Do not keep healthy stock in sheds used by sick animals.
• Do not touch nasal/ wound discharges of infected animals/ human beings. These may contain disease bacterium.
• Do not handle the animals if you have small cuts or wounds on hands & arms.
• Do not reuse bedding and other gear of infected animal.

FOR FURTHER READING:
• OIE. General Disease Information Sheet. Available at http://www.oie.int/doc/ged/D13968.PDF
• Yabuuchi, Eiko, et al. Proposal of Burkholderia gen. nov. and transfer of seven species of the genus Pseudomonas homology group II to the new genus, with the type species Burkholderia cepacia (Palleroni and Holmes 1981) comb. nov. Microbiology and Immunology. 1992;36(12):1251-75.
### Table 1: Animal glanders reported in India

<table>
<thead>
<tr>
<th>Year</th>
<th>State</th>
<th>Places</th>
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<tbody>
<tr>
<td>2006-07</td>
<td>Maharashtra</td>
<td>Pune, Panchgani</td>
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<td>Punjab</td>
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<td>Haryana</td>
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An expert group meeting for preparation of Guidelines for Prevention & Control of Kyasanur Forest Disease & Glanders was held on 12.04.2017 at NCDC Delhi. The following participants were worked on preparation of Guidelines for Prevention & Control of Glanders.

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Name</th>
<th>Designation &amp; Place of posting</th>
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<tbody>
<tr>
<td>1</td>
<td>Dr S Venkatesh</td>
<td>Ex-Director, NCDC, Delhi</td>
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<td>2</td>
<td>Dr H K Muniyellappa</td>
<td>Joint Commissioner (Livestock Health), Department of Animal Husbandry Dairying and Fisheries, Delhi</td>
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<td>3</td>
<td>Dr R K Agarwal</td>
<td>HoD, Division of Bacteriology &amp; Mycology, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh</td>
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<td>4</td>
<td>Dr Col Amit</td>
<td>Commandant, Central Military Veterinary Laboratory (CMVL), Meerut, Uttar Pradesh</td>
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<td>5</td>
<td>Dr Lt Col Vipul Kumar Singh</td>
<td>Lab Officer, Veterinary Laboratory (CMVL), Meerut, Uttar Pradesh</td>
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<td>6</td>
<td>Dr Harishankar Singha</td>
<td>Scientist, National Research Center on Equines (NRCE), Hisar, Haryana</td>
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<td>7</td>
<td>Dr UVS Rana</td>
<td>Retd. Joint Director, NCDC, Delhi</td>
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<td>8</td>
<td>Dr Mala Chhabra</td>
<td>HOD &amp; Additional Director, Zoonosis Division, NCDC, Delhi</td>
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<td>9</td>
<td>Dr Sandy Kabra</td>
<td>Additional Director, NCDC, Delhi</td>
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<td>10</td>
<td>Dr Pradeep Khasnobis</td>
<td>NPO IDSP, NCDC, Delhi</td>
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<td>11</td>
<td>Dr Naveen Gupta</td>
<td>Joint Director, Zoonosis Division, NCDC, Delhi</td>
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<td>12</td>
<td>Dr Sanket V Kulkarni</td>
<td>Deputy Director, CSU (IDSP), NCDC, Delhi</td>
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<td>Dr Ruchi Jain</td>
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<td>14</td>
<td>Dr Simmi Tiwari</td>
<td>Deputy Director, Zoonosis Division, NCDC, Delhi</td>
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<td>15</td>
<td>Dr Monil Singhai</td>
<td>Assistant Director, Zoonosis Division, NCDC, Delhi</td>
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<td>16</td>
<td>Dr Davendra Kumar</td>
<td>EIS Officer, NCDC, Delhi</td>
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<td>17</td>
<td>Dr Mayuri Rani</td>
<td>Specialist (Microbiology), Zoonosis Division, Delhi</td>
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<td>18</td>
<td>Dr Rajkumari Mandakini Devi</td>
<td>Deputy Assistant Director, Zoonosis Division, Delhi</td>
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<td>19</td>
<td>Dr Nataraju S M</td>
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*Experts from various specialities have contributed in relevant areas.

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