



National Action Plan for Control and Eradication of Glanders in India

(Revised 2025)



Department of Animal Husbandry & Dairying
Ministry of Fisheries, Animal Husbandry & Dairying
Government of India

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Acronyms

CFT	:	Complement Fixation Test
NADRS	:	National Animal Disease Reporting System
ELISA	:	Enzyme Linked Immunosorbent Assay
ICAR-NRCE	:	Indian Council of Agriculture Research – National Research Centre on Equines
NRL	:	National Referral Laboratory
CMVL	:	Central Military Veterinary Laboratory
RDDL	:	Regional Disease Diagnostic Laboratory
CDDL	:	Central Disease Diagnostic Laboratory
DAHD	:	Department of Animal Husbandry & Dairying
DGRVS	:	Director General Remount Veterinary Services

Introduction

Glanders is a contagious and fatal disease of equines, viz., horses, donkeys and mules, caused by infection with the bacterium *Burkholderia mallei* (*B. mallei*). Control of Glanders requires testing of suspect clinical cases, screening of apparently normal equids, and elimination of reactors. *B. Mallei* has zoonotic potential and has been considered as a potential biological warfare or bioterrorism agent as it can cause highly fatal disease in humans.

Clinical manifestation of *B. mallei* infection greatly varies depending on the route of the entry, the virulence of the strain and immunological status of the host. In equines, glanders manifests in three forms: nasal, pulmonary, and cutaneous, and these forms may be acute, subacute, or chronic in nature. In donkeys, the disease is acute and is highly fatal in nature. In horses, infection with *B. mallei* typically takes a chronic course, with animals potentially surviving for months or even years without showing obvious symptoms. These asymptomatic carriers can serve as a source of infection for susceptible animals. In mules, disease may develop either the acute or chronic form of the disease.

- (i) **Nasal glanders:** It is characterized by bouts of coughing, respiratory distress, nasal discharge from one or both nostrils, formation of nodules in the mucous membrane of nasal cavity, particularly of septum and turbinate bones. These nodules gradually enlarge, rupture and ulcerate, and liberate yellowish green mucopurulent exudates. Consistency of the nasal discharge varies from watery to serosanguinous and copious thick nasal discharge, which may obstruct nasal passages. Sub maxillary lymph node enlargement and indurations can be seen in the infected animals. Regional lymphadenopathy and lymphangitis most often accompany nasal signs. Infected animals may die within few days to weak due to respiratory failure and septicemia.
- (ii) **Cutaneous glanders or Farcy:** Typical nodules are present on the flank, inner thigh, belly and extremities especially in hind limbs. Gradually nodular abscesses become ulcerated, and regional lymphatic pathways become thickened and indurated and ooze typical yellow-green gelatinous pus. In severe cases, nodules and ulceration may be seen throughout the skin.
- (iii) **Pulmonary form:** It takes several months to manifest this form of disease. Dyspnoea intermittent fever, paroxysmal coughing or a persistent dry cough accompanied by laboured breathing are clinical signs associated with pulmonary glanders. As the disease progresses,

animal become listless, emaciated, swelling of hind quarter joints and testicles, lameness, epistaxis and haematuria are other signs observed in pulmonary form. In lungs, small light-coloured haemorrhagic nodules are found, which progress to caseous or calcified state and eventually discharge their contents, thus spreading disease to the upper respiratory tract. Other non-specific symptoms are often the first to manifest including thirst, fever (low-grade to high), shivering, drooping of the head, tachycardia, tachypnea, weight loss, prostration and reluctance to move. Sometime, infected animal may exhibit all the clinical forms simultaneously.

The population of equines (horses/pony, donkey and mules) in India is 5.44 lakh (20th Livestock Census, 2019), which is spread almost all over the country. The utility of equines is manifold in comparison with any other domesticated animals. Therefore, the strata of people utilizing them for various purposes vary from the poorest of the poor to the richest of the rich. This result in different types of management and husbandry practices followed in India, which can be broadly classified into organized and unorganized sectors. The organized sector mainly holds sub-population of Thoroughbred or other exotic breeds or their crosses and indigenous equids in presumably bio-secured premises like those of Army, NCC, Race/Turf Clubs, Polo Clubs, Private and Government Stud farms and Riding Schools, Pharmaceuticals, etc. These establishments/premises/installations follow definite management and husbandry practices, which practically separate the equids epidemiologically from other non-descript population of equids. On the other hand, in the unorganized sector, equids (all three species) are reared by people for earning their livelihoods and used in transport of goods and people locally, at construction sites, brick kilns, tourist and pilgrim places, marriages, etc., without following a definite system of management and husbandry practices.

Glanders caused by *B. mallei*, is a notifiable disease in India since 1899, by an Act of Parliament. The disease has been occurring sporadically in a few states but surveillance of the disease during the last decade revealed that *B. mallei* infection has spread to a few more states due to movement of asymptomatic carrier equids.

Development of better diagnostic tests e.g., Complement Fixation Test (CFT) and recombinant ELISA (Enzyme Linked Immunosorbent Assay) in conjunction with physical examination has enhanced the feasibility of mass surveillance. The "mallein" test has been discontinued by most countries and surveillance of Glanders has now become easier than before. The WOA

Terrestrial Manual 2024, in Chapter 1.4, has provided guiding principles for animal health surveillance for control and eradication of any infectious/contagious disease in a country or zone for the benefit of Member Countries.

Being a notifiable disease, MOA&FW issued a set of guidelines (vide letter no F. No. K-50/1/2017/LH dated 18/01/2017) that was binding on the States. To control the outbreak of Glanders in the infected states and prevent spread of the disease to non- infected states/zones, the existing guidelines have now been updated.

This National Action Plan has been framed for the entire population of equids reared in different management and animal husbandry practices in India and the biology, pathogenesis and epidemiology of *B. mallei* / Glanders under the overall conceptual framework of the WOAH Terrestrial Code and the WOAH Terrestrial Manual 2024, (*Chapters 1.4, 4.3, 12.10 and 3.6.11 respectively*). The overall objective is surveillance, control and eradication of Glanders in equines from India.

Clause 1.

Legislative provision

Glanders is a notifiable disease under the Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009 (the Infectious Diseases Act 2009) and hence, the State / UT Governments are required to take necessary control measures as per the provisions of the Act.

- 1.1. All States have framed rules under Section 43 of the Act for Quarantine Camps and check-posts, manner of inspection etc., enabling them to examine all animals entering into their boundaries. States shall adopt combat measures using their resources and shall accordingly notify veterinarians to exercise and perform duties within their jurisdictions as per powers conferred upon them under the Act.
- 1.2. The State/ UT Animal Husbandry Department shall also report any suspected or confirmed cases of Glanders through the National Animal Disease Reporting Systems (NADRS) or any other system that is in place.

Clause 2.

Case definition, diagnostic test, designated laboratory and National Reference Laboratory

- 2.1 The following defines the occurrence of infection with *B. mallei* as per the guidelines of in WOAH Terrestrial Animal Health code (Chapter 12.10) and WOAH Terrestrial Manual 2024.
- (i) ***B. mallei* have been isolated** from a sample from an equid;
OR
 - (ii) **Antigen or genetic material specific to *B. mallei*** has been identified in a sample from an **equid showing clinical or pathological signs** consistent with glanders, or **epidemiologically linked to a confirmed or suspected case** of infection with *B. mallei*, or giving cause for suspicion of previous contact with *B. mallei*; OR
 - (iii) **Antibodies specific to *B. mallei* have been identified** by a testing regime appropriate to the species in a sample from an equid **showing clinical or pathological signs consistent with glanders**, or **epidemiologically linked to a confirmed or suspected case** of infection with *B. mallei*, or giving cause for suspicion of previous contact with *B. mallei*.
- 2.2 Recombinant Hcp-1 ELISA and Complement Fixation Test (CFT), Bacterial isolation, antigen and/or genome demonstration **should be** conducted for confirmation of *B. mallei* infection as per case definition guidelines in WOAH Terrestrial Animal Health code (Chapter 12.10) and WOAH Terrestrial Manual 2024.
- 2.3 Indian Council of Agriculture Research –National Research Centre on Equines (ICAR–NRCE) shall be the National Referral Laboratory (NRL) in the country.
- 2.4 **Declaration of Glanders Positive Cases:** Glanders positive cases should be declared by considering the following situation as indicated in the table below -

Sample type	Hcp-1 ELISA test	CFT titre 1:5 or above	qPCR (in swabs, wound and nodular aspirate samples)	Isolation (in swabs, wound and nodular aspirate samples)	Clinical Presentation(s) + Epidemiological linkage	Results
Serum	Positive	Positive	-	-	Present + epidemiological linkage with <i>B. mallei</i> infected equines.	Positive
Serum	Positive	Positive	-	-	Absent + epidemiological linkage with <i>B. mallei</i> infected equines.	Positive if repeat sample becomes positive in both ELISA and CFT
Serum*	Positive	Negative	-	-	Absent + No epidemiological linkage.	Required repeat sample
Serum**	Positive	Positive	-	-	Absent + No epidemiological linkage.	Required repeat sample
Serum	Positive	Suspicious	-	-	Present + Epidemiological linkage with <i>B. mallei</i> infected equines.	Positive
Serum + Swab	Positive	Positive	Negative	Negative	Present	Positive
Serum + Swab	Positive	Positive	Positive	Negative	Present + No epidemiological linkage with <i>B. mallei</i> infected equines.	Positive
Serum +Swab	Positive	Positive	Positive	Positive	Present + No epidemiological linkage with <i>B. mallei</i> infected equines.	Positive

* If the serum test status is same as in table even after two repeat testing with negative isolation/PCR in nasal swabs/ any other tissues the animal will be declared negative.

** If the test status remains same after first repeat sample as mentioned in table above in both serum as well as nasal swab/any other tissue, the second repeat sample shall be collected by ICAR-NRCE team for testing. If the equines (except mules and donkeys) in such cases still remain negative as above, shall be declared positive looking into the fact that such animals at times remain inapparent carrier for glanders.

- 2.5 Collection of repeat sample for further confirmation: Repeat samples are required for the confirmation of glanders according to the above table. Serum samples, nasal swab/lesions swab are to be collected from the suspected animals at 21 days interval for two times for laboratory diagnosis and reporting the result.
- 2.6. Central Military Veterinary Laboratory (CMVL), Meerut shall be the recognized laboratory for testing of equids from the defense services and those coming in contact with their animals. This data will be shared with NRL and the department (DAHD).
- 2.7. Notification of recognized/designated laboratories shall be made by DAHD on the recommendations of a Committee and technical validation by NRL.
- 2.8. All the Regional Disease Diagnostic Laboratories (RDDLs) and the Central Disease Diagnostic Laboratory (CDDL), Izatnagar, State Diagnostic Laboratories (notified/ nominated by DAHD, GOI) shall also be designated laboratories for Glanders screening/testing subject to recommendations as in para2.5 above
- 2.9. Competency development in terms of capacity building of scientific personnel and supply of reagents to the designated laboratories shall be the responsibility of ICAR-NRCE which is the National Referral laboratory (NRL) for Glanders.
- 2.10. The recognized/designated laboratories shall be verified for repeatability and reproducibility of their test results by the NRL as and when required by the DAHD following a mechanism recommended by ICAR-NRCE, to assure quality results.
- 2.11. All positive cases tested by the designated laboratories (DLs) shall be confirmed by the NRL. Samples tested positive by CMVL on animals other than defence services shall also be referred to NRL for confirmation.
- 2.12. Director, ICAR-NRCE shall communicate the results to the Director/ Joint Director of State/UT Animal Husbandry Department concerned, the Director General Remount Veterinary Services (DGRVS), and Animal Husbandry Commissioner, Government of India, Ministry of Fisheries, Animal Husbandry and Dairying (MoFAH&D) as well as other authorities as decided by DAHD.

Clause 3.

Disease surveillance, control and eradication

- 3.1. Glanders is a notifiable disease in India and it is the responsibility of the State Veterinary Authority to report suspected cases of Glanders. On subsequent confirmation by the NRL, elimination & disposal of carcass will be carried out by the State Veterinary Authorities according to the Infectious Diseases Act, 2009. For defense animals, the Army shall be responsible for these actions.
- 3.2. Glanders surveillance programme which shall include clinical/physical, pathological and serological surveillance as per the extant WOAH guidelines and the guidelines issued by DAHD shall be the responsibility of the State Veterinary Authority concerned with technical support from ICAR-NRCE, Hisar. This will ensure detection of clinical cases and cases where there is infection without exhibition of clinical signs, with the ultimate aim of eradicating the disease and obtaining disease free status for India. Sero-surveillance of equines migrating International borders to prevent cross-border transmission have to be undertaken. Each animal crossing border must be screened using a recombinant ELISA kit. Hotspot areas should be identified, mapped, and monitored for equine movement. Implementation of tracking systems such as ear tagging or microchipping is recommended for improved disease control. Border centric surveillance protocol considering international boundaries shared with Nepal , Myanmar, Bhutan, Bangladesh also China bordering Arunachal Pradesh. Random sampling from the areas of concentrated horse population and all the major Horse fairs to be undertaken.
- 3.3. Appropriate budgetary provision shall be made for National Glanders Eradication Program for all stakeholders.
- 3.4. It shall be the responsibility of State Veterinary Authority to create awareness about the disease according to the policy guidelines of DAHD and technical know how from ICAR-NRCE amongst all stakeholders. The support of NGOs shall also be taken in this effort.
- 3.5. Captive Wildlife surveillance–As infection with *B. mallei* has never been reported in the country in wild equids and other susceptible animal species, they are historically considered free from the disease by virtue of adhering to husbandry and management practices related with relevant bio-security. National Zoos/Zoological gardens/ National parks, etc., shall submit biological samples from all susceptible captive wildlife species (as per standard sampling & transport method) to ICAR-

NRCE, Hisar for testing at least once in a year. In cases of suspected clinical signs & pathology, the technical guidance of ICAR-NRCE shall be taken.

- 3.6. Free ranging Wildlife surveillance– In case of any clinical signs/pathological lesions detected in any susceptible free ranging wildlife, the cases and samples will be referred to ICAR-NRCE through respective wildlife/forest authorities.
- 3.7. Surveillance of glanders in non-equids and premises – biological samples from glanders in-contact small ruminants (sheep, goat) and camels and environmental samples (soil, water, feed) from infected premises should be collected and sent to ICAR-NRCE for glanders testing. At least 5% of the non-equid population in the affected area in addition to all in-contact animals (regardless of species) to ensure comprehensive coverage and early detection.
- 3.8. All designated laboratories and the NRL shall be responsible for testing samples in the National Glanders Eradication Programme run by the Department of Animal Husbandry and Dairying, MoFAH&D, GOI through the respective state authorities.

Clause 4.

Responsibilities of State / UT Animal Husbandry Department in the event of incidence of Disease (Glanders)

- 4.1. State Veterinary/administrative authority shall notify the disease with intimation of quarantine of the infected and in-contact animals, euthanasia of positive cases, proper disposal of carcasses and disinfection of the infected premises, enforcing zoo-sanitary measures under the supervision of the local veterinary authority following the Standard Operating Procedure (Appendix – A).
- 4.2. The area to be notified by the State Veterinary Authority shall be geographically or administratively demarcated (village/colony/ward/panchayat, etc.), which includes 2 Km. around the nucleus / focus of infection and/or the possible epidemiological link to a confirmed or suspected case of Glanders. The interim handling, isolation, and movement restrictions of suspected equines during the period between sample collection and laboratory confirmation of *Burkholderia mallei* infection

- 4.3. Restrictions shall be imposed for movement of equines in and out of the notified area, except on conditions which have been defined in clause 7.1.
- 4.4. 100% equids of the notified area shall be screened within 3 weeks of the incidence of the first case and repeated twice within the next 2 months with samples taken 21-30 days apart.
- 4.5. 25% equids shall be screened beyond the notified area of up to **10 Km (2-10 km)** thrice over a period of 6 months.
- 4.6. Beyond **10 kms**, the Veterinary Authority shall undertake physical / sero- surveillance based on the risk assessment and movement of equines.
- 4.7. The State Veterinary Authority shall share their Gazette notification with the neighbouring States as well as with the other stakeholders in a manner that is deemed fit to control and contain the disease. Creation of specialized mobile task forces under each Regional Disease Diagnostic Laboratory (RDDLs) is advisable to assist states during outbreaks in surveillance, disinfection, awareness, and outbreak containment.
- 4.8. If any organized establishment or compartment falls in or near the area of Glanders notification, having followed the defined animal husbandry and management practices & bio-security measures with regular testing as per clause 7 shall continue its routine activity. Such compartments excluding the defence services, shall inform the State Veterinary Authority about the movement of equines.
- 4.9. Due to lack of global vaccines, treatment regimens and considering zoonotic nature of *B. mallei* infection. Test, track and destroy approach shall be adopted for the elimination of glanders from the country.
- 4.10. Procedure for destruction of positive cases and disinfection of notified areas may be carried out as per specified (Appendix – A) and Animal Welfare Board of India guidelines. Re-introduction of animals to disinfected vacated premises shall be done only after two months of elimination of last reactor.
- 4.11. The attendant/veterinarian/para-vets and health workers in close contact with the diseased animal should follow high standards of personal hygiene and strict antiseptic measures. Proper personal protective equipment including appropriate masks, full body aprons, long shoes and rubber disposable gloves shall be used while collecting samples in a notified area or during handling or disposal of positive cases.

Clause 5.

De-notification

- 5.1. De-notification shall be done provided the post-outbreak surveillance has been followed as per the clause 4 (iv) & 4 (vi).
- 5.2. State Animal Husbandry Department shall send post-outbreak surveillance report to the DAHD, and ICAR-NRCE till the post-outbreak surveillance is over for data recording.
- 5.3. If post-outbreak surveillance (three tests in the first three months, at minimum 21 days apart) yields negative results, the disease in the area shall be de-notified. Accordingly, regulated equine movement shall be withdrawn and equine activities shall be restored. However, surveillance shall continue for another 9 months, with another sample being taken within 3 months of denotification.
- 5.4. It shall be the responsibility of State Veterinary Authority to de-notify the area.
- 5.5. De-notification of the notified area shall be shared with the adjoining states and the public at large.

Clause 6.1

Glanders free State / Zone / Compartment

- 6.1.1. The term “infected” shall be defined as the infection of *B. mallei* confirmed either by bacterial isolation or demonstration of antigen/genomic DNA or antibody to *B. mallei* demonstration.
- 6.2.2. Glanders free states/zone/compartments shall be one where there has never been an occurrence of the disease (historically free) or Glanders/*B. mallei* infection has ceased to occur for at least 10 years or eradication has been achieved by active surveillance in the past 3 years and infection is not known to be established in wildlife within the state or zone.
- 6.3.3. The attainment and maintenance of Glanders-free states/zone/compartments shall require providing sufficient Glanders / *B.mallei* infection surveillance documents for at least past 3 years as well as appropriate bio-security and sanitary measures within the states and its borders.

Clause 6.2

Zones historically free from glanders

Certain zones and regions in India are historically free from Glanders/*B. mallei* infection due to geographical separation and meagre equine population. These zones/regions shall be protected by following the guidelines given below.

- 6.2.1. All equids in the zone/region shall have identification system, movement records and health card.
- 6.2.2. Surveillance of Glanders / *B.mallei* infection shall be carried out on randomised samples collected and despatched as per standard procedures (Appendix – B) and tested by WOAH recommended test as per clause 2 of this document.
- 6.2.3. Surveillance of equids and related species for Glanders / *B.mallei* infection in captive wildlife in national zoos/gardens, etc.
- 6.2.4. Bio-security of the free zone/regions shall be ensured.

Clause 7.1

Criteria for defining/auditing compartment

This is in accordance with WOAH Terrestrial Animal Health Code (Chapter 4.3 zoning and compartmentalization). Following may qualify as compartments: Defense establishments of Army, Navy, Airforce, paramilitary forces, police, other government organizations and Turf Authority of India, all of which maintain equids under regular veterinary supervision. The existence of an animal identification system, bio-security and surveillance system are prerequisite to assess the integrity of the zone or compartment.

Such establishments mentioned above shall be mandatorily certified by the attending veterinarians or the Chief Veterinary Officer of the district, or the Director General, RVC as the case may be, to fulfillment of criteria under Clause 7.1 to be recognized as a compartment. The same shall be reviewed every 5 years by an expert team of veterinarians constituted by DAHD.

Private establishments shall be considered a compartment for a period of 5 years on certification of an expert team of veterinarians constituted by

the DAHD based on the following criteria. Naturally or artificially separated premises/areas with restricted entry of man, materials and animal (log book).

- 7.1.1. Management of animals/stud under the supervision of qualified veterinarians.
- 7.1.2. Proper documentation of health cards of every animal, including records of treatment, vaccination, testing for various diseases etc.
- 7.1.3. Movement record of all animals along with date, place and type of transport system used. Mixing of animals of other compartments having similar management and husbandry practices related to bio security and immune status only shall be permitted.
- 7.1.4. Restricted contact with personnel having attended equids of different immune status and management and husbandry practices.
- 7.1.5. Production records, sources of feed, water and bedding, morbidity and mortality history, visitor's logbook, etc., shall be maintained for evaluation of risk management. All records shall be maintained in a readily accessible form.
- 7.1.6. Anything that could be useful in epidemiological separation of the compartment from other equids of unorganized sector and all factors preventing risk of *B. mallei* infection shall be considered.
- 7.1.7. The private establishments shall be registered with DAHD, Government of India. The State Veterinary Authority shall carry out documented periodic inspection of facilities, biosecurity, records and surveillance procedure.
- 7.1.8. All compartments shall seek notification by DAHD.
- 7.1.9. All defense establishments shall be considered for grant of the disease-free status following the stipulated guidelines and/or based on certification by DG RVS.

Clause 7.2

Criteria for attaining Glanders-free compartment

Over and above the defined criteria of compartment as mentioned above, following conditions shall be met for the attainment and maintenance of Glanders free compartment.

- 7.2.1. Evidence of absence of Glanders / *B.mallei* infection either historically or based on past and ongoing Glanders surveillance as indicated in Clause 6.1.

- 7.2.2. Equids in the compartment have not shown clinical signs & pathology consistent with Glanders during last 10 years.
- 7.2.3. Newly inducted equids shall be quarantined (21 days minimum) and tested during this period before mixing them in the subpopulation.
- 7.2.4. Appropriate biosecurity and sanitary measures are in place (as indicated in clause 7.1 above).
- 7.2.5. All equids have been physically screened.
- 7.2.6. A testing program by WOAH recommended test for demonstration of infection (bacterial culture) or DNA / antibody to *B. mallei* at least during last three years has been established.
- 7.2.7. So long as an ongoing surveillance demonstrates absence of Glanders and principles determined for its definition and establishment are respected, the compartment maintains its free status.
- 7.2.8. Finding evidence of *B. mallei* infection of any magnitude in the Glanders free compartment automatically invalidates its free status.
- 7.2.9. To gain free status in an infected compartment, or regain free status following an outbreak in a previously free compartment shall follow recommendation as described in Clause 5 and 7.2.

Clause 7.3

Movement of horses/equines between compartments for various activities

- 7.3.1. Movement of equines shall be permitted between various Glanders-free compartments meeting the requirement of Clause 7.1 in the notified area.
- 7.3.2. Any equestrian event including horse shows, race, polo, etc., can be organized for participating horses from disease free compartments; even if it falls in the notified areas, following all biosecurity measures.
- 7.3.3. Movement of equids from compartment in the infected zones or notified area to another compartment in any area or vice-versa shall be regulated strictly by contained transport system enforcing epidemiological separation from any risk route.
- 7.3.4. Equids have been tested negative within 30 days of moving out of the compartment for any event in similar subpopulation/compartment and have been tested negative again within 21 days of the return to the original compartment.
- 7.3.5. At destination compartment, strict bio-security measures shall be observed.

Clause 8.

Equine fairs/congregation/events/shows in un-organized sector

- 8.1. Equine fairs, congregation, shows, or any equestrian events in which equids from unorganized sector take part shall not be permitted to be held in **10 Km** of radius of the notified area/focus of infection.
- 8.2. State / UT Animal Husbandry Department and DAHD shall provide the list of notified areas in district, group of contiguous districts, zone or states for reference. Notification shall be uploaded on official websites also.
- 8.3. Fair congregation, shows, any equestrian events shall be permitted in the notified area provided:
 - » There has been no case of Glanders in the notified area during the **past six months** and ongoing surveillance is in place.
 - » The fair is managed by State Veterinary Authority/local municipal authority or by any registered society/body, etc.
 - » The organizer/controlling authority of the event/fair/show shall advertise about the regulation and requirements for participation in the fair through print and media in advance.
 - » Equines from the notified area which fall within 10 km radius from the infection source shall not be permitted to participate in any events organized in the de-notified or Glanders free area.
 - » To become eligible for participation in equine fairs, congregation, shows or any equestrian events, the **owners shall produce a certificate of a Glanders' test with negative results carried out on samples drawn within 30 days**
 - » Every participating equid in the event shall have the health card as per enclosed proforma (Appendix -C). Chief Veterinary Officer of the District and the local veterinarians shall have authority to regulate entrance of the animals in the *Mela* (Fairs)/event ground only after physical examination of every animal. Equines showing any clinical sign and pathology of Glanders shall not be allowed to enter in the event venue.
 - » In equine fairs, blood samples from at least 30% population of participating equines shall be collected randomly following standard operating procedures and be submitted to the designated laboratory or NRL for surveillance of Glanders / *B. mallei* infection.

- » In fairs, congregation, shows, any equestrian events, an animal shall have its own feeding and watering arrangement. Common watering and feeding shall not be permitted.
- » Equine owners shall be encouraged to get their animals physically and serologically examined within one month of return from the fair/event.

Clause 9.

Guidelines for pharmaceuticals, animals house, experimentation facilities, etc. holding equines

- 9.1 Any facility maintaining equines for production of immuno-biologicals, conduction of experiments and other purposes must be free from Glanders/ *B. mallei* infection. For this the equines should be tested twice every year for Glanders and other equine diseases from ICAR-NRCE.
- 9.2 For any new incumbent in these groups the animal should be quarantined and tested. Their new stock are introduced in the facility only after having been tested negative once, if introduced from non-infected states/zones, twice within three months with second test carried out within 21 days before entrance into the facility and quarantined again for at least 21 days for observing clinical signs & pathology, if any, consistent with Glanders.
- 9.3 A team constituted by DAHD and headed by ICAR-NRCE may inspect these facilities for compliance of the guidelines.

Clause 10.

Inter-state movement of equids

- 10.1 Inter-state movement /migration of equids shall be regulated by the state Veterinary Authority in the following manner.
 - 10.1.1 Equids with prescribed "Health Card" shall only be permitted to move between states.
 - 10.1.2 Animals from infected states (having incidence of Glanders during the last 3 years) shall be permitted to enter in non-infected states by

- the State Veterinary Authority upon verification of the certificate of a Glanders" test carried out within 30 days before entry by the animal owner and that equines do not show signs and pathology consistent with Glanders on the day of entry.
- 10.1.3. Movement between non-infected states or historically Glanders-free states shall be permitted with one Glanders test result carried out within 30 days before the date of inter-state migration and that the equids are healthy and do not show any clinical and pathological signs of Glanders on the day of crossing the border.
- 10.1.4. Movement of equids from non-infected states bordering infected states to non- infected states shall be regulated by the terms mentioned above at 10.1.1 and 10.1.2.

Clause 11.

Human Surveillance under One Health Approach

- 11.1. Following confirmation of Glanders in equines, Director ICAR-NRCE, while communicating results to state veterinary authority and the DAHD shall also intimate human health authorities to collect blood samples from all in-contact personnel including the owners and send to ICAR-NRCE for testing. In case of any positive test, ICAR-NRCE shall communicate results to all concerned for necessary action. Similarly, in case if need arises, CMVL will undertake testing of human samples specifically from defense service personnel, who have been handling defence /service horses.

Clause 12.

Compensation

- 12.1. State / UT Veterinary Authority shall be responsible for paying compensation to *bonafide* owners for eliminating Glanders/*B. mallei* infected animals as soon as possible in accordance with notification No. 5-57/2006-LDT (LH) Pt.Vol. II dated 18 August, 2015 and to be paid under ASCAD on a 50:50 basis or in accordance with any modified. notification issued by DAHD.

- 12.2. Compensation shall also be paid to a *bonafide* animal owner whose animal is suspected to be infected with Glanders but dies before receipt of glanders positive test report.
- 12.3. To support immediate economic losses and sustenance of poor equine keeper's profession, immediate release of compensation to beneficiaries preferably within a month is utmost importance.

Clause 13.

Research Priorities

Uniform validation and accreditation system for the ongoing Complement Fixation Test and other WOAHA accepted tests shall be carried out. Availability of Glanders" antigen and uniform standard serum shall be ensured by designated laboratories in consultation with ICAR-NRCE. The reagents for WOAHA -accepted recombinant protein- based ELISA shall be made available to all designated laboratories by ICAR-NRCE and/or commercial sources along with the required training to conduct the test. Efforts shall be made by the ICAR-NRCE, Hisar to develop improved diagnostic methods / diagnostic kits for field and laboratory use. Evaluating the antimicrobial sensitivity and resistance profiles of *B. mallei* isolates maintained at ICAR-NRCE and obtained from out-breaks are important for assessing the extent of drug resistance. This will pave an opportunity for identification of suitable antimicrobial component for effective treatments regimen against *B. mallei* infection. Indigenous rapid pen-side (point-of-care) diagnostic tests for *B. mallei* should be developed by ICAR-NRCE.

Clause 14.

Training and Capacity Building

Continuing Education Programmes on Glanders for the field veterinarians, medical officers, wildlife professionals and laboratory personnel shall be carried out. ICAR-NRCE shall help in capacity building of the Regional Disease Diagnostic Laboratories (RDDLs) and also other Designated State / UT Laboratories for undertaking testing for Glanders.

Clause 15.

Public awareness

Due to the zoonotic nature of *B. mallei*, public awareness shall be carried out by public, private and government institutions concerned, and NGOs to sensitize all stakeholders about significance of the disease with respect to equines and humans. Regular awareness campaigns shall also be undertaken among the pilgrimage/tourist places where equines are used as a means for transportation.

Appendix- A

I. Destruction of the Infected Equines/animals:

If the animals are found positive, all control and containment action should be followed as required under the Act. The positive animal should be traced immediately and if animal is already moved to other place, then further movement should be stopped. All in-contact animals with the positive animal(s) at previous location and the migrated location should also be covered under intensive surveillance. These shall include other species of animals which are also susceptible to this disease including canines, felines, camels etc.

1. Infected animal should be eliminated immediately. In this regard, the Prevention and Control of Infectious and Contagious Disease in Animals (form of vaccination certificate manner of post mortem examination and disposal of carcass) Rules 2010, in vogue need to be followed.
2. In case absolutely essential, the positive animal may be transported to appropriate area for destruction and further disposal in closed vehicles. All the zoo-sanitary measures should be followed at the time of culling and disposal of carcasses.
3. Protective clothing including full body aprons, face masks, rubber/ latex disposable gloves and long shoes to be used by persons in close contact with the diseased animal.
4. The euthanasia is to be performed by a committee of local veterinarians by the use of Pentobarbital combination. The standard dose of thiopental sodium is 1 gram/100 kg body weight. The same could be increased or decreased depending upon the susceptibility of the horses.
5. Guidelines constituted by Animal Welfare Board of India circulated by Ministry of Environment, Forests and Climate Control, Government of India for euthanasia of equine (Pt No 5 of AWBI guidelines) shall be followed.
6. Other permitted drugs may also be used as long as they facilitate humane destruction of animals. Carcass of horses may be disposed of either by immediate burial or burning to prevent the spread of the disease.
7. Burning is preferred, but method of burial could also be adopted. For burial, a suitable site away from streams, river, canals or other water

supply is to be selected. A pit of minimum 8 ft. deep is to be made. The area requirement is about 3 sq. yards.

8. The dead animal is put into the pit with feet upwards which are normally folded. The carcass is covered with quick lime followed by filling of the pit. The burial area is fenced so that stray dogs do not scavenge.

II. Disinfection of the premises:

1. Vacate the affected stables and standings soon after the detection of first Glanders case. However, in-contact animals will be restricted to the notified area.
2. All affected areas including stables, water and feeding troughs, other fittings are disinfected by use of blow lamps or by burning soiled hay and all contaminated disposable equipment and other materials.
3. The suitable disinfectants sodium hypochlorite (500 ppm), 70% ethanol, 2% glutaraldehyde, benzalkonium chloride (1/2000), mercuric chloride in alcohol, potassium permanganate to be used.
4. Organism is less susceptible to phenolic disinfectants. The equipment / vehicles used either for transport of infected animals or used for burial will also be adequately disinfected.
5. Personnel in close contact with the diseased animal should follow high standards of personal hygiene and strict antiseptic measures.

Disinfectants to kill *B. mallei*

B. mallei is susceptible to sodium hypochlorite (500 ppm), 70% ethanol, 2% glutaraldehyde, iodine, benzalkonium chloride (1/2000), mercuric chloride in alcohol and potassium permanganate. It is less susceptible to phenolic disinfectants. This organism can be destroyed by heating to 55°C (131°F) for 10 minutes, or exposure to ultraviolet irradiation. In the environment, *B. mallei* organisms are susceptible to drying and sunlight. Some of the commercially available disinfectants such as **AlkaSept™ Active**, **PowerCull™ Extra**, **CombiSept**, **Bactrex Plus**, **Germitol**, **Germisol**, **Potassium permanganate** (1- 2 grams / litre of water) and **Lysol** (500 ml of Lysol in 9.5 lit of water) can also be used on the premises to sanitize the premises.

Appendix – B

SOP for serum samples collection from equines for Glanders surveillance

I. Materials required:

1. Vacutainer blood collection tubes **containing clot activator** or 10 mL disposable syringes. **Do not collect in Vacutainer containing any anti-coagulant like Heparin, EDTA, sodium citrate, etc.** Use of vacutainer blood collection tubes containing a clot activator with gel (yellow cap) is recommended to prevent hemolysis of samples during transportation over long -- distances
2. Vacutainer needles are needed if the samples are collected in vacutainer tubes
3. 15 mL plastic centrifuge tubes (from Tarson or any other companies)
4. 18 gauge needles
5. Disposable gloves (Nitrile gloves)
6. Face mask
7. Ethanol (70%)
8. Absorbable cotton soaked in 70% ethanol
9. Rack stands to keep blood samples after collection (15 mL centrifuge stands)
10. Cryovials
11. Sharp needle disposal containers
12. Biohazard bags to collect the wastes
13. Bench top centrifuge
14. Marker pen, Diary/Note, Pen
15. Personnel protection kit
16. Soap (Dettol/ Lifebuoy)
17. Towels
18. Hand sanitizer

II. Procedure for the blood sample collection for serum samples

1. First, wear the personnel protection material (Shoes, disposable lab coats, face mask, gloves, safety goggles)
2. Restrain the animal, locate the jugular vein and sterilize the blood

- collection area with 70% ethanol soaked in absorbable cotton
3. Draw the blood with Vacutainer needle if you use the Vacutainer tubes for blood collection or use 18 gauge needle and 10 mL disposable plastic syringes to draw 10 mL of blood samples. **Tubes should be labelled properly and clearly.** This can be done before collection or immediately after sample collection.
 4. After collection of blood into the Vacutainer, invert the Vacutainer for several times to **mix the blood with clot activator** for the proper clotting of the blood sample to get good quality serum for laboratory testing.
 5. If samples are collected in the disposable syringes, discard the needle into sharp disposal container, carefully transfer blood into 15 mL centrifuge tubes and close the tubes tightly. **Tubes should be labelled properly and clearly.** This can be done before collection or immediately after collection of the samples.
 6. Keep them in slanting position (45° angle) for 30 min on the collection site itself for proper clotting of blood and serum separation. This step will avoid haemolysis while onward transmission to the laboratory.
 7. After 30 minutes, keep the tubes in centrifuge stands or keep them in upright position.
 8. **Transfer the samples on ice** (preferable)
 9. Wash hands properly with soap and apply hand sanitizer
 10. After reaching the laboratory, break the clot with a sterile glass rod by ring out the blood clot gently. (*Note: Excessive and forceful clot braking may lead to haemolysis*).
 11. Keep the samples in refrigerator overnight for separation of serum samples.
 12. Centrifuge the tubes for 15 minutes at 2000 RCF
 13. Transfer the serum samples into **1.8 mL screw capped cryovials** with the help of disposable sterile plastic pasture pipettes, close the cryovials and make sure that there won't be any leakages. A **minimum of 2 vials/ animal is required.**
 14. **Label the cryovials properly** Samples should be along with proper details collected at the time of sampling to state designated laboratory or NRCE, Hisar on cooling conditions though messenger or through courier services.

(Note: Do not leave any needles, syringes, gloves etc., at the site of collection, collect them in a biohazard bag, close tightly, and bring it to laboratory, autoclave and dispose as per the standard protocols.)

For any queries, please contact the laboratory personnel in the Glanders testing laboratory, NRCE, Hisar through phone or email.

III. Collection of nasal swab samples for bacterial isolation from Glanders' suspected equines

1. First, wear personnel protection material (*Shoes, disposable lab coats, face mask, gloves, safety goggles*)
2. Clean the external nares with 70% ethanol cotton material
3. Use **transport swab only (Transport swab w/ Amies Medium w/ Charcoal in polypropylene tubes.** (*This can be purchased from HIMEDIA or any other manufacturer*)
4. Do not send the samples in 15 mL centrifuge tubes filled with normal saline or phosphate buffered saline
5. After collection, label it properly and transport to NRCE under cool conditions for bacterial isolation and PCR.

IV. Collection of aspirates from soft nodules for bacterial isolation from Glanders' suspected equines

1. Firstly, wear personnel protection kit
2. Collect aspirates from soft and unopened nodules and do not collect it from opened nodules
3. Use 10 ml disposable syringes fitted with 23 gauge needles
4. Apply 70% ethanol cotton swab on the soft nodules to sterilize the surface area
5. Gently pierce the nodules with 23-gauge needles fitted with 10 ml disposable syringes
6. Collect 1 to 2 ml of pus samples
7. Remove the syringes and discard the needle into sharp disposal container. Do not push the plunger after collection of pus to remove any air from the syringes. This will lead to **aerosolization** of the bacteria and will lead to further spread the disease
8. Transfer the pus sample from syringe to sterile screw cap tube. Discard the syringes in bio-hazard bags.
9. Label the tube with a permanent pen marker and transport to NRCE under cooling conditions for the bacterial isolation and PCR.

V. Interim handling, isolation, and movement restrictions of suspected equines during the period between sample collection and laboratory confirmation of *Burkholderia mallei* infection

1. Notify the veterinary services, about the suspected case, movement of the suspect equine within or out of the premises should be restricted until the diagnosis is confirmed or ruled out.
2. If the State quarantine camp and check-post are available within the vicinity, suspected equines should be quarantined from healthy equines as per the guidelines mentioned in "The prevention and control of infectious and contagious diseases in animals act, 2009" page no. 5, point No. 14.
3. If there such quarantine facility is not available nearby to the suspected case, the animal should be kept in quarantine to the equine keeper's premises.
4. There should be a physical barrier at least 20 meters between suspected equines and healthy equines or other animals in the quarantine camp to prevent further spread of the disease.
5. Husbandry equipment (feeding trough, watering arrangement, rope) should be a separated and dedicated for suspected equines. Do not use the contaminated objects for healthy equines or other animals in the quarantine camp to prevent further spread of the disease.
6. Restrict access to the isolation area to essential personnel (Veterinary officers, Assistant, animal keepers) only, who should adhere to strict biosecurity protocols, including appropriate personal protective equipment
7. Closely monitor the suspected animal for any changes in clinical signs, documenting any new developments by the attending veterinarian.
8. If the animal is confirmed by laboratory diagnosis, positive equines should be euthanized and infected premises should be thoroughly disinfected as per Appendix A page no. 21 and 22 of these documents.
9. The suspected glanders has a potential zoonotic risk. Therefore, in-contact persons should be sampled and tested for glanders and implement appropriate measures to protect human health.

VI. Collection of serum samples before euthanizing confirmed cases of equines having Glanders

This procedure is similar to that of serum sample collection mentioned earlier in this SOP. But serum should be collected in large volume (100 mL) for serum repository. Use 50 ml syringes (2 to 3 syringes/animal). Send 30-40 mL of serum for serum repository. Use 15 mL centrifuge tubes for sending of samples to NRCE, instead of 2 mL cryovials.

(Note: *Do not leave any needles, syringes, gloves etc., at the site of collection, collect them in a biohazard bag, close tightly, and bring it to laboratory, autoclave and dispose as per the standard protocols.*)

Sample submission format:

Owner details	Animal details	Clinical signs, if any	Epidemiological information	Sample type	Fresh/ Repeat sample	Date of Collection	Vety. Hospital
Name, Complete address, Mob No.	(Species- Horse/ pony/ mule/ donkey, age, sex)	Nasal discharge, nodules, respiratory distress, gradual emaciation	Previous outbreak/ purchase/ migration history	Serum, nasal swab, Pus, lesion swab etc.			

Email ID/ Mobile No. of the concerned officer should be mentioned in the cover letter

For any queries, please contact the laboratory personnel in the Glanders testing laboratory, NRCE, Hisar by phone or emails

Appendix- C



GOVERNMENT OF *Respective State*
DEPARTMENT OF ANIMAL HUSBANDRY
& VETERINARY

EQUINE HEALTH CARD ASHWA

SWASTHYA PATRA

PASTE COLOUR
 PHOTOGRAPH OF ANIMAL
 (5X4 inch)

Cross Seal and signature of
 Official Veterinarian

ANIMAL DETAILS

10. EQUID SPECIES

(Horse/Pony/Mule/Donkey)

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2. REGISTRATION NO.

(20 Digit State specific) (Including existing ID if any)

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3. BREED

--	--	--	--	--	--	--	--	--	--

4. SEX

--	--

5. DOB / AGE

--	--	--	--	--	--	--	--	--	--

6. COLOUR

BODY																			
MANE																			
TAIL																			

7. MARKING

FACE																			
MUZZLE																			
BODY																			
FORE LEG																			
HINDLEG																			

GUIDELINES FOR ASHWA SWASTHYA PATRA

1. A Block Veterinary Officer will ensure physical surveillance of equines, and maintain registration and issuance of *Ashwa Swasthya Patra*.
2. The *Ashwa Swasthya Patra* to be issued for Yearling.
3. On sale of animal the *Ashwa Swasthya Patra* is transferable to new owner with existing Registration No.
4. On death of animal, the *Ashwa Swasthya Patra* is submitted with the nearest Veterinary officials who in turn redirect the same to original Issuing Official for deletion of registration details.
5. A veterinary examination and a health certificate is compulsory within 30 days of interstate travel.
6. Equines that are shown or transported regularly may require more frequent testing.
7. All states to ensure that equines entering or being transported across their lines have a negative Glanders and Coggins Test no more than three months old.
8. All equines participating in equine fares, at congregation in tourist and pilgrimage site should hold valid Equine Health Cards.

20 digits code Registration Details as per the *Local Government (LG) Directory, Government of India* in the order as under:

1. Initial two digits (1-2): State Code
2. Next three digit (3-5): District Code
3. Next four digit (6-9): Block/ Panchayat Code
4. Next six digit (10-15): Village Code
5. Final five digit (16-20): Animal register enrollment No. maintained at Block Veterinary Hospital

For e.g. Registration of horse

1. State-**UTTAR PRADESH (09)**;
2. District- **BAREILLY (130)**;
3. Block / Panchayat- **AALAMPUR JAFARABAD (1541)**;
4. Village- **AASPUR (129999)** and
5. Block Animal Enrollment Register No. for 102nd animal- **00102**

Registration No.

0	9	1	3	0	1	5	4	1	1	2	9	9	9	9	0	0	1	0	2
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Appendix – D

GLANDERS' SURVEILLANCE PLAN

1. Introduction

Surveillance is the ongoing systematic collection, storage, analysis, and interpretation of outcome specific data for use in planning, implementing, and evaluating public health policies and practices. Infectious disease surveillance system serves two functions, early warning of potential threats to public health (Livestock/Human) and program monitoring functions which may be disease specific or multi disease in Nature.

The functions of surveillance system are detection and confirmation of case, reporting and data analysis and interpretation and public health response. This includes the reports and feedback from the system to the data providers, stake holders and decision-makers. The quality of surveillance system is defined by the attributes such as completeness, timeliness, usefulness, sensitivity, specificity, representativeness, simplicity, flexibility, acceptability and reliability.

Representativeness refers to the degree to which the reported cases reflect the occurrence and distribution of all the cases in the population under surveillance. Geographical representativeness is important in an early warning system to ensure detection of outbreaks of infectious diseases. For achieving the quality of surveillance system, the proper sampling plan for collections of samples which is random as well as representative to be designed to meet the targeted objectives. The probability sampling methods are appropriate for achieving the surveillance objectives,

A framework for early warning system (EWS) for epidemic preparedness is a system of data collection to monitor livestock exposure to pathogen, in order to provide the timely notice when a crisis of outbreak and thus elicit the appropriate responses. There are three components of early warning system are -

1. Routine surveillance of the targeted disease
2. Modelling the disease risk based on historical surveillance and contemporary environmental data
3. Forecasting future risk through the use of predictive models and

continued epidemiological and environmental surveillance.

The disease data collected may be utilized for developing hotspots maps , is one of the earliest method to identify the risk areas for epidemiology and , risk maps using GIS with modelling of related variables on environmental, remote sensing, agricultural activities, water types, soil types etc.

2. Glanders in India

Given below is the equine population of the country as per the 20th Livestock Census 2019.

S. No.	States/UTs	Horses and Ponies	Mules	Donkeys	Total
1	A & N Islands	0	0	2	2
2	Andhra Pradesh	1884	240	4678	6802
3	Arunachal Pradesh	3051	0	0	3051
4	Assam	12783	724	900	14407
5	Bihar	32176	1491	11264	44931
6	Chandigarh	237	0	0	237
7	Chhattisgarh	675	21	142	838
8	D&N Haveli	39	0	0	39
9	Daman & Diu	15	0	0	15
10	Delhi	2694	136	1087	3917
11	Goa	15	1	0	16
12	Gujarat	21811	5	11286	33102
13	Haryana	9683	2499	800	12982
14	Himachal Pradesh	8851	20415	4797	34063
15	Jammu And Kashmir	63335	16722	9563	89620
16	Jharkhand	1378	73	400	1851
17	Karnataka	7018	51	8790	15859
18	Kerala	560	0	65	625
19	Lakshadweep	0	0	0	0
20	Madhya Pradesh	13260	2543	8135	23938
21	Maharashtra	18892	681	17572	37145
22	Manipur	1083	0	2	1085
23	Meghalaya	273	0	0	273
24	Mizoram	159	8	0	167
25	Nagaland	70	0	2	72
26	Odisha	143	18	83	244
27	Puducherry	29	0	4	33

28	Punjab	14243	1644	471	16358
29	Rajasthan	33679	1339	23374	58392
30	Sikkim	115	0	2	117
31	Tamil Nadu	5417	305	1428	7150
32	Telangana	3878	91	2031	6000
33	Tripura	17	2	10	29
34	Uttar Pradesh	75718	8933	16016	100667
35	Uttarakhand	7452	26293	589	34334
36	West Bengal	1593	26	94	1713
	Total	342226	84261	123587	550074

India has witnessed several cases of glanders outbreak since 2006. Re-emergence of Glanders in India was observed in 2006 after a gap of a decade. Subsequent, intensified surveillance resulted in detection of new cases almost every year from Uttar Pradesh and sporadic cases from Himachal Pradesh, Uttarakhand, Chhattisgarh, Andhra Pradesh, and Punjab. Enhanced surveillance efforts and enactment of the National Action Plan in 2019 revealed presence of equine glanders in various states such as Gujarat, Delhi, Jammu, Rajasthan, Maharashtra, Madhya Pradesh, Karnataka, and Tamil Nadu. From the resurgence in 2006 to 2023, a total of 1,844 glanders-positive cases were confirmed in 115 districts across 15 states in India, of which nearly 70% of cases were reported in Uttar Pradesh. This shows high prevalence of glanders across Uttar Pradesh, regarded as a highly endemic region.

Taking account of past five year's surveillance data, it was observed that only 11 states Uttar Pradesh, Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand, Rajasthan, Gujarat, Maharashtra, Chhattisgarh, Madhya Pradesh regularly participated in the glanders surveillance. On the other hand, negligible, irregular or no surveillance was conducted in North-East and South India. Therefore, pro-active participation of all State Animal Husbandry Department in the surveillance programme is necessary.

Taking into cognizance of the above fact of an increasing trend of Glanders outbreaks, it was felt necessary to revise the action plan for efficient surveillance across all the States, test-track-destroy approach shall be adopted with the ultimate objective of control and eradication of Glanders in India.

3. Activities

Testing and culling was/has been the policy throughout the world including India for control and eradication of Glanders, and many countries have got rid of this disease following this policy. In this regard, the Glanders Surveillance Plan shall be applicable to all States / UTs of the country having an equine population. During the first five-year span (2019-2023) of the action plan priority was given to States which have reported Glanders, viz., Andhra Pradesh, Chhattisgarh, Haryana, Himachal Pradesh, Jammu & Kashmir, Maharashtra, Punjab, Uttar Pradesh, Uttarakhand, Gujarat, Rajasthan, Madhya Pradesh, Tamil Nadu, Karnataka, and Delhi. Around 90% cases were reported in seven states in Northern India.

Sporadic cases of equine glanders have been reported from Southern Indian states specifically from Tamil Nadu, Karnataka and Andhra Pradesh. This indicates the presence of glanders in the Southern India. Regular surveillance of equids in these states is required to monitor the glanders in Southern states. Certain states, specifically North-Eastern, Bihar, Odisha and West Bengal were not regularly participated glanders in surveillance program. Therefore, the present status of Glanders in these states are not known. Hence, it is also imperative that the revised national action plan should cover surveillance of equine population across India. This will enable us to ascertain Glanders-free zone/state in the country.

At present, 18 network diagnostic facilities (Regional disease diagnostic laboratories (n= 4), Central Disease Diagnostic Laboratories(n=1) and State disease diagnostic laboratories (n=13) located in 16 states are involved in the glanders surveillance. These state includes Uttar Pradesh, Haryana, Himachal Pradesh, Jammu, Kashmir, West Bengal, Bihar, Maharashtra, Punjab, Uttarakhand, Gujarat, Rajasthan, Madhya Pradesh, Chhattisgarh, Tamil Nadu and Karnataka. However, in some of the states, designated laboratories are yet to be identified. States having a large population of equines like Uttar Pradesh, etc., need to identify more than one designated laboratory for glanders surveillance.

In general, serum samples shall be collected and sent along with information sheet for diagnosis of Glanders to such designated laboratory(s). In specific clinical cases of Glanders, serum samples, clinical specimens like nasal swabs/pus swabs/lesion swabs/pus samples shall be sent to ICAR-

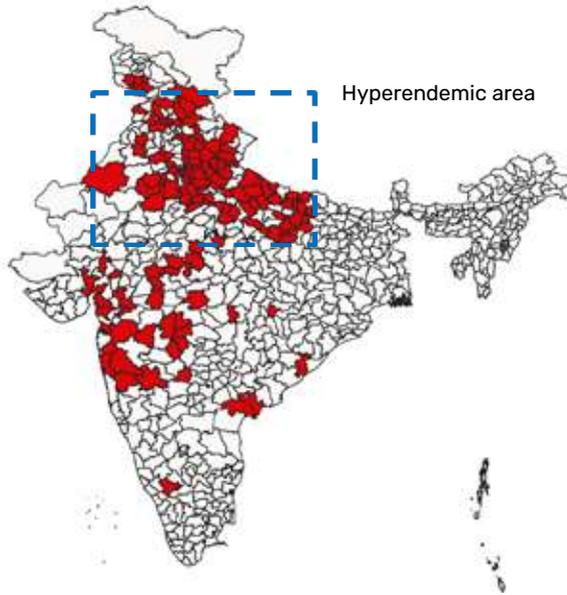


Fig. Glanders reported Districts in different States between 2006 and 2023

NRCE, Hisar along with the necessary information for serological, molecular and bacteriological analysis.

Screening may be done by serological testing (Hcp1 ELISA) by the designated laboratories. ICAR-NRCE, Hisar shall serve as the National Referral Laboratory for Glanders and declaration of any incidence/ outbreak in the country shall be done by ICAR-NRCE, Hisar only.

From zoonotic point of view, collection of blood serum samples from in-contact humans (equine owners/ handlers/ veterinarians) under One Health Approach shall be coordinated by the veterinarian of that area in consultation with the health authorities.

It is the responsibility of State Animal Husbandry Department to devise methodology and assign duties to participating officer for monitoring surveillance activities.

4. Sample size:

The sample size should be a representation of the State/UT concerned. The stratified random sampling method is to be adopted.

For states who have earlier reported Glanders, viz., Andhra Pradesh, Chhattisgarh, Haryana, Himachal Pradesh, Jammu & Kashmir, Maharashtra, Punjab, Uttar Pradesh, Uttarakhand, Gujarat, Rajasthan, Madhya Pradesh, Tamil Nadu, Karnataka and Delhi, sample size should be 30% of the population in each district should be sero-surveyed and 10 % population should be under physical surveillance. Samples should be sent for screening/diagnosis to the designated laboratories.

For other states, it should be 10% of the population for sero-surveillance and 10% under physical surveillance in each district. Samples should be sent for screening/diagnosis to the designated laboratories.

It is important to follow continuous surveillance strategy. Therefore, concerned district unit have to identify equine inhabited block and sequentially and rotationally collect samples in each month.

In the event of an outbreak, all in-contact animals should be sampled in a premises/area having epidemiological link and suspected samples to be sent to ICAR- NRCE, Hisar after initial screening.

For post-outbreak sampling: As per clause 4.4 and 4.5 of the action plan:

100% equids of the notified area shall be screened within 3 weeks of the incidence of the first case and repeated twice within next 2 months with samples taken 21-30 days apart.

25% equids shall be screened beyond the notified area up to 10 Km **(2-10 km)** thrice over a period of six months.

5. Diagnostic approach

As per WOAH guidelines, there are different diagnostic methods have been recommended for detection of glanders depending on the purpose as indicated in the table given below.

Purpose					
Method	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policy	Confirmation of clinical cases	Prevalence of infection-surveillance
Agent Identification					
PCR	-	-	-	+	-
Culture	-	-	-	+	-
Detection of immune response					
Complement fixation	++	++	+++	+	+++
ELISA	+	+	++	+	++
Mallein skin test	+	+	+	+	+
Western blotting	+	+	++	+	++

+++ Recommended method, ++ suitable method, + may be used in clinical cases, - not appropriate for this purpose

In India, in continuation with the previous action plan, two-tier sero-diagnostic approach will be followed for rapid and efficient execution of surveillance activities. Initial screening will be done by Hcp1-ELISA at State Designated Laboratory(s) for which necessary training of the staff concerned has been/shall be given by ICAR-NRCE, Hisar. Confirmation of all referred samples shall be undertaken by ICAR-NRCE, Hisar as described in clause 2.2 and 2.3.

2. Responsibilities

- 2.1 Sample record**–It is the responsibility of designated laboratory to keep sample record in hard copy as received from field and compile digital data. Serum samples should be tested for quality. Haemolysed serum and samples without animal details should not be included for testing.
- 2.2 Glanders ELISA**– Each designated laboratory shall cater diagnostic services to corresponding districts attached to them. Accordingly, each laboratory needs to develop facilities for carrying out Glanders ELISA. State Animal Husbandry Department needs to procure ELISA kit for distribution to designated laboratory according to demand after its commercial availability. The respective CVO/DMU/SMU should inform the designated laboratory in advance regarding the tentative submission of samples to the laboratory, enabling the laboratory to make necessary preparations and ensure timely testing and reporting
- 2.3 ELISA result**– If some samples show positive/ suspected (border line) by ELISA, it may be re-tested again. If same result persists, equines may be quarantined and repeat samples should be collected and should be sent to ICAR-NRCE for further confirmation. Repeat sample should be collected as mentioned in the clause 2.6.
- 2.4 Sharing of data:** District-wise sample information sheet, ELISA data, etc., shall be shared between designated laboratory and ICAR-NRCE through a quick and transparent communication system every quarter ending as per proforma developed by ICAR-NRCE. The data shall be analyzed by ICAR-NRCE and a quarterly progress report shall be shared with the Department of Animal Husbandry & Dairying, New Delhi. An annual report should also be submitted/ published in this regard by ICAR-NRCE.
- 2.5 Reviewing of Designated Laboratory(ies)** – All staff concerned of the State Designated Laboratories shall undergo capacity building including hands-on training at ICAR-NRCE as per module to be developed by it. ICAR-NRCE shall also develop Inter Laboratory Comparison (ILC) set in order to test the proficiency of the designated laboratory. Each laboratory shall send a minimum of 10 samples per year for ILC to ICAR-NRCE for evaluation.

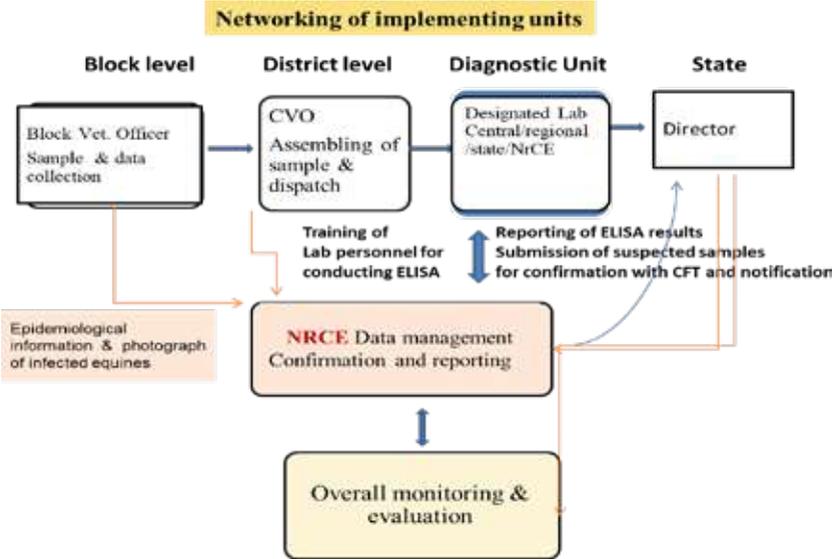
3. Confirmation and reporting & follow up action

Final confirmation and reporting of Glanders cases will be done by

ICAR- NRCE only. Post-outbreak follow-up measures will be performed as per existing act and Glanders advisory notified from time to time by DAHD, GOI. In the event of any outbreak, it should be reported immediately and the State should initiate action to control and contain the disease as per the provisions of the Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009.

All positive reactors showing clinical picture, positive by ELISA/CFT need to be eliminated. Equine keeper of Glanders affected equines has to be compensated according to rule of Government of India. Each and every outbreak should be investigated to know the epidemiology of the disease with forward and backward linkages. The in-contact animals should be under physical inspection and sero-surveillance for a period of two months. From the 2 km radius of the nuclei of infection 100% equine population and from next 2-10 km 25% equines should be put under surveillance to monitor disease transmission to susceptible animals. Disinfection of infection premises and implementation of bio-security measures will be followed. Adequate public awareness campaign will be organized in outbreak areas. Equine fairs shall be prohibited till de- notification of area by repeated surveillance within 6 months is done.

4. Data Flow mechanism



5. Screening of human samples for *B. mallei* infection

Surveillance of in-contact human samples: From zoonotic and one health point of view, in-contact human samples from equine owners, animal handlers, veterinarians will be tested. ICAR-NRCE (being reference laboratory of Human Glanders) will carry out human Glanders testing. Human Glanders surveillance through Integrated Disease

Surveillance Programme (IDSP), coordinated by National Centre for Disease Control (NCDC), New Delhi is already in practice from 2018. For collection of human samples, the veterinarian concerned shall coordinate in consultation with the health authorities.

6. Surveillance network

Following are the specific roles and responsibilities of the various monitoring units

- 6.1 Block monitoring unit-** Block Veterinary officer (VO) will ensure physical surveillance of equines, identification/tagging of animal and collection of samples or post-outbreak surveillance as per directions from the district fall under the category. VO should ensure dispatch of samples to district unit within 3 days of sample collection. In case of suspected Glanders cases, VO should implement necessary control & containment measures immediately and report the case to district unit. Health card for each equid will be issued to the equine owner whose animals are tested.
- 6.2 District monitoring Unit (DMU)-** Deputy Director/CVOs/DVOs will be the leader of district monitoring unit. DMU will be responsible for smooth execution of surveillance activity within the district during entire duration of the project. DMU will devise (village wise/block wise) surveillance plan. He/She will provide all necessary required infrastructure facilities for sample collection, timely dispatch to diagnostic lab, organizing awareness campaign, elimination of positive equines and implementation of control measures according to legislative provision. Samples received from different block unit should be sent to designated diagnostic lab/RDDLs within 7 days of collection. DMU will compile all record of serum samples including area of surveillance, number of equines sampled, positive cases and action taken report and next month target and submit to SMU at monthly basis.
- 6.3 Divisional Unit/Designated laboratory/RDDLs/CDDL-** This unit

will carry out Glanders diagnosis by ELISA. Joint Director/Deputy Director/In-charge designated laboratory will be the leader of this unit. The unit will receive samples from different districts identified by SMU. Samples should be tested within 7 days of receipt, and results should be communicated to DMU and block unit. For ELISA suspected cases, VO should be informed for quarantine of suspected animals and collection of repeat sample collection for confirmation. Samples from suspected clinical cases or repeat samples should be submitted to ICAR-NRCE, Hisar for confirmation by ELISA/CFT/PCR. District wise sample information sheet, ELISA data, etc. shall be shared between designated laboratory and ICAR-NRCE through a quick and transparent communication system.

6.4 State Monitoring Unit (SMU) - SMU will facilitate fund disbursement to divisional diagnostic lab, DMU for procurement of necessary consumables, recruitment of trained manpower and ELISA kit for sample collection and diagnosis. SMU will procure ELISA kit in advance and should have sufficient cold storage facility. SMU will devise work plan (district wise) and ensure proper execution of plan. In nutshell, SMU will look after overall progress of the project, reviewing of activity at defined interval.

6.5 Central Monitoring Unit (CMU) - CMU is to be headed by the Animal Husbandry Commissioner, Government of India and Director, ICAR-NRCE to be one of its members. The CMU shall oversee the overall monitoring and evaluation of the surveillance activities. A national-level technical advisory committee should undertake annual review of the plan's implementation across states. Additionally, external evaluation by independent agencies every 3 years can help identify performance gaps.

7. Role of ICAR-NRCE in development of Glanders diagnostic & state collaboration

Considering the intrinsic problems of complement fixation test (CFT) for Glanders diagnosis & research gap for better diagnostic test with higher sensitivity & specificity suitable for mass surveillance program, ICAR-NRCE has developed recombinant protein(s) based ELISAs for detection of *B. mallei* specific antibodies in equines. All the ELISAs were validated in different laboratories in India and internationally at WOAH Referral Laboratory on Glanders, Germany. The Hcp1-ELISA showing highest specificity & sensitivity have been rigorously evaluated at ICAR-NRCE. To tackle Glanders outbreak,

ICAR-NRCE has distributed Hcp1-ELISA reagents to state diagnostic laboratories. In the recent past, NRCE has trained laboratory personnel from different State Lab/RDDLs (Uttar Pradesh, Gujarat, Haryana, Rajasthan, Himachal Pradesh, Madhya Pradesh, Karnataka, Chhattisgarh, Punjab, Maharashtra, Bihar, Jammu, Kashmir) on glanders diagnosis by ELISA developed at NRCE. This was found useful for setting up of network laboratory for rapid and efficient execution of surveillance activities.

ICAR-NRCE is coordinating the glanders surveillance programme in collaboration with State Animal Husbandry Departments. Two tier diagnostic system (ELISA and CFT) is being followed for the surveillance purposes. In addition, molecular methods (PCR, qPCR) and culture isolation is applied in biological samples collected from a glanders outbreaks. At present, 18 designated laboratories are doing glanders surveillance using commercially available glanders ELISA kit manufactured by Genomix Diagnostic Pvt. Ltd. As per guidelines, net-work laboratories shall conduct initial screening by ELISA and positive samples should be retested for final confirmation by complement fixation test (CFT) and molecular methods at ICAR-NRCE.

8. Modalities for procurement & supply of ELISA kit

Recombinant protein-based ELISA developed at ICAR-NRCE will be used for mass screening of equines for Glanders diagnosis at designated diagnostic laboratory. ELISA technology has been transferred to Genomix Diagnostic Pvt. Ltd for manufacturing of ready to use ELISA kit. Quality control of kit will be monitored by random verification of kits from each batch at ICAR-NRCE. SMU will determine requirement and accordingly procure Glanders ELISA kit from company. SMU will distribute ELISA kit to divisional diagnostic unit as per requirement. Issuing of ELISA kit and actual test will be recorded at the State/ Divisional units.

Suggestive Photographs



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