



National Centre for Animal Health
Department of Livestock
Ministry of Agriculture & Forests
Royal Government of Bhutan



National Rabies Prevention and Control Plan



SECOND
EDITION
2017



National Rabies Prevention and Control Plan

Second Edition 2017

National Centre for Animal Health

Department of Livestock

Ministry of Agriculture & Forests

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Photo courtesy in this document

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FOREWORD

January 27, 2017

Rabies is one of the most important zoonotic disease affecting both animals and humans and cause an approximate of 59,000 human deaths annually in the world. Human rabies can be prevented through prompt administration of post-exposure prophylaxis (PEP) to victims of rabid animal bites, and infection can be eliminated at source through sustained mass vaccination of dog population achieving more than 70% of the dog population. However, these measures are either inadequate or lacking in most of the rabies endemic low income countries.

Rabies is a notifiable disease as per the Livestock Rules and Regulations of Bhutan 2008. Rabies commonly occurs in the southern belt of Bhutan along the borders with India; however, isolated cases have been documented in the interior northern parts of the country, as a result of incursion from south and across the border. Therefore, it is extremely important to control the disease at the source and prevent establishment of endemicity in the country. As part of the global effort to eliminate the disease by 2030, Bhutan is also actively implementing various strategies to control and eliminate dog mediated rabies in the country through stepwise approach. This document clearly outlines strategies and activities to achieve stage 4 by 2020.

I am happy to note that the National Centre for Animal Health and Animal Health Division, Department of Livestock have taken the lead in revising and updating the National Rabies Prevention and Control Plan. I would like to extend my appreciation to all individuals who have contributed towards producing this important national plan document and in particular Dr. Tenzin, Head, Disease Prevention and Control Unit (Rabies focal point), NCAH, Dr. Karma Rinzin, CVO, AHD and Dr. Kinzang Dukpa, Program Director, NCAH for their excellent coordination in production of this control plan.

I hope this revised plan document (Second edition 2017) will be useful as ready reference to all those involved in the prevention and control of rabies in the country. I am confident that this plan document will directly contribute in bringing down the incidence of rabies in the country thereby contributing towards healthy and happy life.


(Dr. Tashi Samdup)
DIRECTOR GENERAL

Director General
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Abbreviations

AHD	Animal Health Division
BAFRA	Bhutan Agriculture and Food Regulatory Authority
BPU	Biological Production Unit
CABC	Community-based Animal Birth Control
CNS	Central nervous system
CNVR	Catch-neuter-vaccinate-release
CVH	Centre Veterinary Hospital
DALYs	Disability-adjusted life years
DDM	Department of Disaster Management
DLS	Dzongkhag Livestock Sector
DoFPS	Department of Forests and Park Services
DOIT	Disease Outbreak Investigation Team
DVH	Dzongkhag Veterinary Hospital
ELISA	Enzyme Linked Immuno-Sorbent Assay
FAO	Food and Agriculture Organization of the United Nations
FAT	Fluorescent antibody test
FVNT	Fluorescent virus neutralization test
GT	Gewog Tshogdue
IEC	Information, Education and Communication
LEC	Livestock Extension Centre
MoAF	Ministry of Agriculture and Forests
MoF	Ministry of Finance
NCAH	National Centre for Animal Health
NGO	Non-governmental organization
NRPCP	National Rabies Prevention and Program
OIE	Office International des Epizootic (World Organization for Animal Health)
PEP	Post-exposure prophylaxis
QMCT	Quarantine and Movement Control Team
RABV	Rabies virus
RBP	Royal Bhutan Police
RFFIT	Rapid fluorescent focus inhibition test
RIDT	Rapid Immunodiagnostic test
RLDC	Regional Livestock Development Centre
RNE-EC	Renewable Natural Resources-Extension Center
RRT	Rapid Response Team
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAARC	South Asian Association for Regional Cooperation

SOP	Standard Operating Procedure
SVL	Satellite Veterinary Laboratory
TADInfo	Transboundary Animal Disease Information
WHO	World Health Organization

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01 Background

Rabies is a fatal viral infection that can infect all mammals, but domestic dogs cause over 99% of all human deaths from rabies. Globally approximately 59,000 human rabies deaths and over 15 million rabies exposures occur every year, mainly in low-income countries of Africa and Asia, equivalent to over 100 people dying every day. Rabies also cause over 3.7 million (95% CIs: 1.6-10.4 million) disability-adjusted life years (DALYs) and 8.6 billion USD (95% CIs: 2.9-21.5 billion) economic losses annually. Human rabies can be prevented through prompt administration of post-exposure prophylaxis (PEP) to victims of rabid animal bites, and infection can be eliminated at source through sustained mass vaccination of dog population achieving more than 70% of the dog population. However, these measures are either inadequate or lacking in most of the rabies endemic low income countries.

1.1. Situational analysis

Rabies is a notifiable disease in Bhutan as per the Livestock Rules and Regulation of Bhutan 2008, and that, as soon as suspected or detected, must be reported to the veterinary authority to contain the disease. Rabies commonly occurs in the southern belt of Bhutan along the borders with India; however, isolated cases have been documented in the interior northern parts of the country, as a result of incursion from south and across the border. Sporadic human deaths due to rabies are reported mainly in rabies endemic southern parts of Bhutan. For instance, 17 human deaths (mostly children) were reported between 2006 and 2016. However, the dog bites in human occur throughout the country with an estimates of over 5,000 cases per year. The people who are exposed to dog bites and presumed rabid animals are provided post-exposure prophylaxis (PEP) free-of-charge in the hospitals.

In 1980's, the elimination of stray dogs was generally thought to be the best method of rabies control in Bhutan. The erstwhile Department of Animal Husbandry initiated rabies control program funded by World Health Organization (WHO). In this control program, WHO supplied dart guns with syringes and medicines to eliminate stray dogs. This equipment was distributed to all Dzongkhags and a few staff from each Dzongkhag were trained in handling the equipment. In the beginning, there was some impact since the stray dog population was reduced. However, in due course, due to strong religious and public sentiments, the Department had to discontinue

the program. Nonetheless efforts were still continued to reduce the dog population, whereby the Department supplied poison to the municipal authority so that they could carry out poisoning of stray dogs using strychnine tablets without the notice of the general public. Later, poisoning of stray dogs in the municipality was also discontinued. Then the other control measures of mass vaccination of dogs coupled with sterilization campaigns on a smaller scale was followed. Impounding of stray dogs in various location in the country was also implemented in 2008, but was discontinued in late 2009.

Campaigns were organized every year by Dzongkhags to sterilize stray dogs by giving cash incentives to those who catch and bring stray dogs. These animals were also vaccinated during such campaigns. But the coverage and frequency of these various control activities were very variable between the Dzongkhags.

There were various constraints for the control of dog population and rabies in the country as follows:

Lack of a Comprehensive National Rabies Control Policy

Rabies is a typical zoonotic infection which does not fit into the domain of a single agency that can be entrusted with the task of controlling it. A nationally coordinated and supervised comprehensive programme is necessary for achieving success. This programme can yield tangible results only if it is backed by commitment at the highest level in the country through a national policy on control of rabies. The national policy is the statement of intention of the country revealing its commitment to control rabies and is accompanied by allocation of appropriate resources to achieve the desired objectives within stipulated period.

Weak Coordination in the implementation of Rabies Control Programme

There was no comprehensive written policy document to coordinate sustained rabies control activities program. Whatever the activities undertaken are carried out by the individual Dzongkhags/agencies without any defined indicators to measure success as well as to evaluate the output achieved with the input of human and financial resources.

Weak Institutional linkages

Inadequate co-operation between the ministry and other organizations/agencies has been one of the main constraints to achieve the success of the control programme. As of now, the Department of Livestock, has been taking lead role without much support from other relevant Ministries and agencies.

Non-implementation of a technically sound strategy

Globally it has been demonstrated that immunization of dogs will break the transmission chain of rabies virus if >70% of the dogs in the community are covered with effective vaccines on a sustained basis. We have been following an *ad-hoc* approach vaccinating a limited number of dogs with the available resources and are unable to sustain the level of immunization. Though this provides individual protection to the animal, it has no bearing upon the epidemiology of the infection. Further, sterilization of small number of dogs does not yield much benefit to the programme.

Long porous border

Bhutan shares long border with India in the south and rabies is a cross-border problem. Transboundary movement of stray dogs and disease spread is a concern and need regional approach towards prevention and control of rabies. Strategies to look at how the dogs at the border areas could be protected needs to be looked into.

Inadequate budget

For any disease control programme sufficient and sustained funding source is important and without it, success of any programme is difficult. Sufficient budget is required for procurement of vaccine, for campaign expenditure, infrastructure development, post intervention surveillance and monitoring program. Therefore, lack of continuity of the control programme as a result of budgetary constraints results in a lowered impact.

Weak Public Health Education

Community participation is an essential element of any disease control programme. There is low public awareness and participation in rabies control campaign and this adversely affect the success of the campaigns.

Weak surveillance system

Surveillance is the key for understanding the prevalence of the disease and to assess the control program. Therefore, a good surveillance system is necessary to assess the effectiveness and efficiency of the control program.

Stray dog population pressure

The large number of stray dog is one of the major problem for rabies control programme in the country. This is mainly due to the fact that the dog habitat and population density are totally dependent upon the availability of resources like food, garbage, water, shelter and access to mates. These resources are abundantly available to dogs in the urban areas, schools & religious establishments. Further, control of rabies in the endemic zones depends heavily on management of the dog population. Canine rabies brings into the picture of the social impact of man-dog relation and interaction. The human population providing abundant habitat and food out of social and religious sentiments severely hampers the dog population management.

Nevertheless, a more sustained dog population management and rabies control programme through “catch-neuter-vaccinate-release (CNVR)” had been started since 2009 through collaboration between the Department of Livestock and Humane Society International program. Between February 2009 and June 2015, 60,993 dogs (stray: 34,462, owned: 29,885) and 3,354 cats have been processed and vaccinated against rabies throughout the country with an estimated coverage of 64 % in the urban areas and 45% in the rural areas. In addition, an annual mass dog vaccination campaign have been implemented in the southern belt of Bhutan to create immune buffer.

1.2. Objectives

The goal of national rabies control strategy is to control and eliminate dog-mediated human rabies in the country by 2020 through stepwise approach. The pathway defines a series of stages, from zero to five, which range from a level of having no evidence-based knowledge of the disease to declaration of free from rabies. The lowest level, Stage 0, is assigned to a country in which rabies is known to be present, but there is a lack of structured information. One of the earliest and most important elements of the stepwise approach is disease monitoring and/or surveillance, which provides data for planning, executing and evaluating the national control programme.

In each distinct stages, a number of activities have to be undertaken by the animal and public health sectors and should include legislation, data collection and analysis, diagnosis, prevention and control and information-education and communication.

For each stage of the pathway, it is also very important to define goals and criteria for moving to the subsequent stage. It would be important that country establish inter-ministerial task force or rabies technical working group to address and assess the control of rabies in the different relevant sectors. This task force shall assess and take key decision like declaring certain areas as rabies free in a step wise approach.

It is considered that Bhutan is currently at Stage 3 and progression to Stage 5 by 2020 requires Bhutan to implement various control strategies as outlined in Figure 1. Bhutan shall follow the step wise approach towards elimination of rabies.

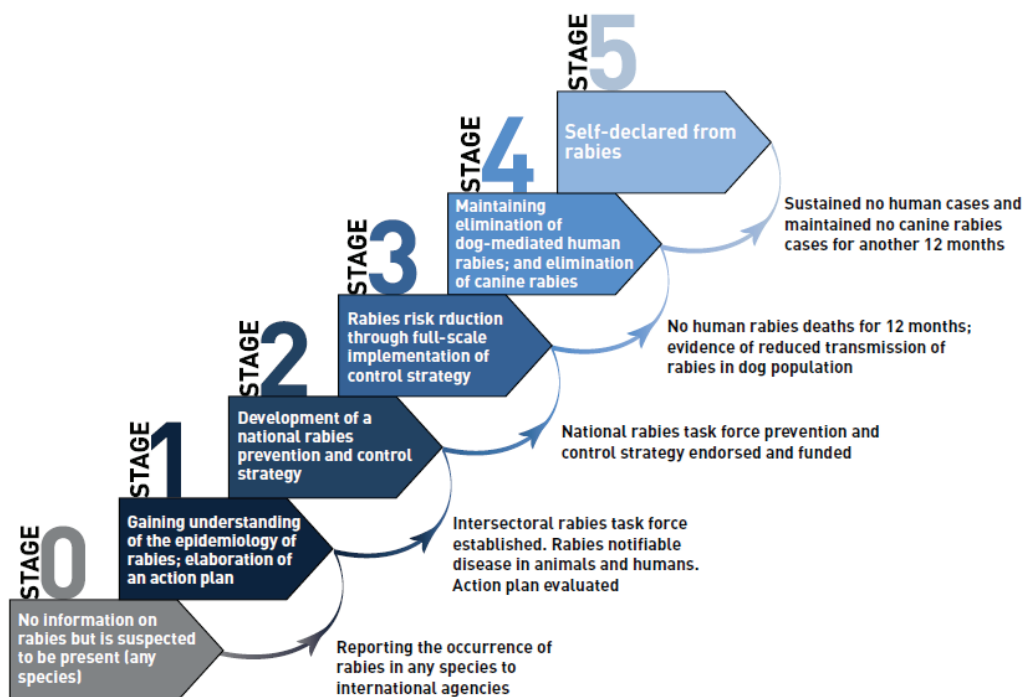


Figure 1: A stepwise approach to rabies control (Source: FAO)

1.3. Epidemiology of Rabies in Bhutan

1.3.1. Spatial distribution

Rabies was prevalent in most parts of Bhutan until the early 1990s but has been controlled by restrictive elimination of dogs. Currently the disease is endemic in the southern districts of Bhutan along the border with India and most commonly reported in the border towns/villages of Sarpang, Samtse, Samdrup Jongkhar and Chukha Dzongkhags (Figure 2). Sporadic incursions into interior rabies-free Bhutan have taken place, indicating possible re-emergence of rabies in the country. For instance, there was an incursion of rabies in east Bhutan (Trashigang, Tashiyangtse and Mongar) during 2005-2006 and in Orong villages of Samdrup Jongkhar during December 2015 as a result of movement of infected rabid dog from the border towns. The most recent incursion (July - December 2016) occurred in Merak-Sakteng, Rangjung town, Buna, Trashigang town and Radhi Gewog in Trashigang Dzongkhag.

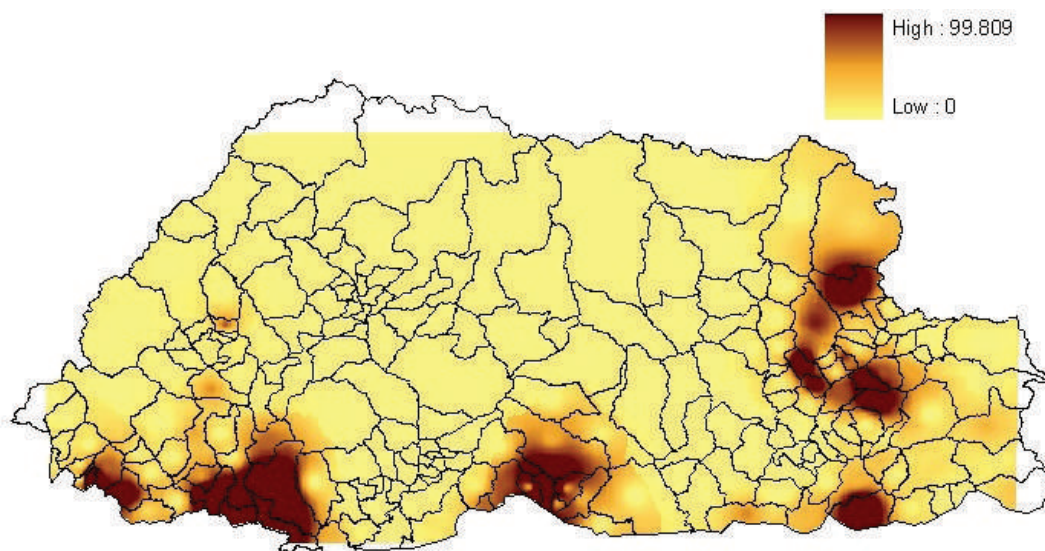


Figure 2: Risk map for rabies in Bhutan (source: Dr. Tenzin)

1.3.2. Species affected

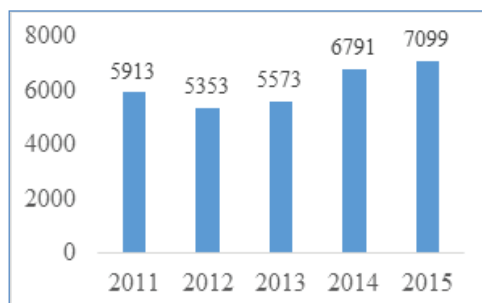
Between 1996 and 2016, 1070 rabies cases were reported in dogs (442, 41.3%), cattle (551, 51.5%), and other animals (cats, goats, pigs, sheep: 77, 7.2%) (Table 1). However, some cases in dogs (stray dog) could have been unreported due to passive nature of reporting system.

Table 1: Reported rabies cases in animals in Bhutan (1996-2016)

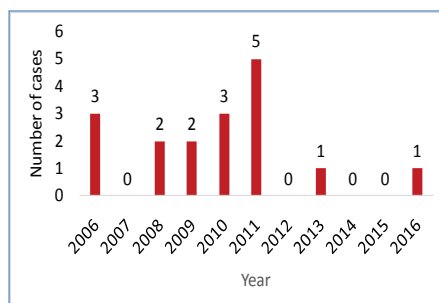
Year	Cattle	Dog	Cat	Goat	Horse	Pig	Sheep	Total
1996	8	10						18
1997	16	2						18
1998	28	32						60
1999	26	13		1	2	2		44
2000	15	4			1	1		21
2001	19	1		1		1		22
2002	26	20			1			47
2003	9	8				1		18
2004	12	4			2	2		20
2005	21	26			2			49
2006	138	103	3		5	2		251
2007	28	12	1	2		1		44
2008	72	65	7	2	4	2		152
2009	30	17	3			1		51
2010	9	8						17
2011	10	22	3	4	2	1	2	44
2012	13	12	4	2		1		32
2013	13	16			1			30
2014	16	19			1			36
2015	23	10		4		1		38
2016	19	38	1					58
Total	551	442	22	18	19	16	2	1070

Note: blank cell indicate zero cases

In human, although dog bite cases is common, rabies cases is sporadic. For instance, 17 human deaths (mostly children) were reported between 2006 and 2016, accounting for about 1.2 deaths per 100,000 population (Figure 3).



Reported dog bite cases in human (2011-2015)



Reported human rabies cases (2006-2016)

Figure 3: Reported dog bite and rabies cases in human in Bhutan

1.3.3. Temporal distribution

Rabies outbreaks in animals have occurred throughout the year with an average of 17 outbreaks reported every year (Figures 4 and 5).

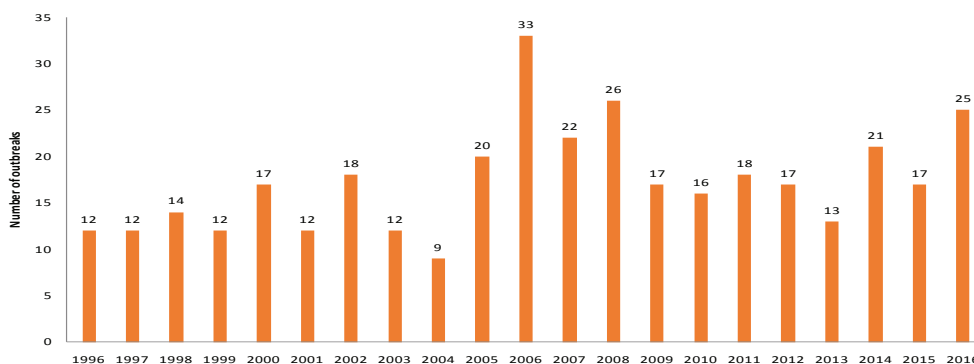


Figure 4: Annual reported rabies outbreak in animals in Bhutan (1996-2016)

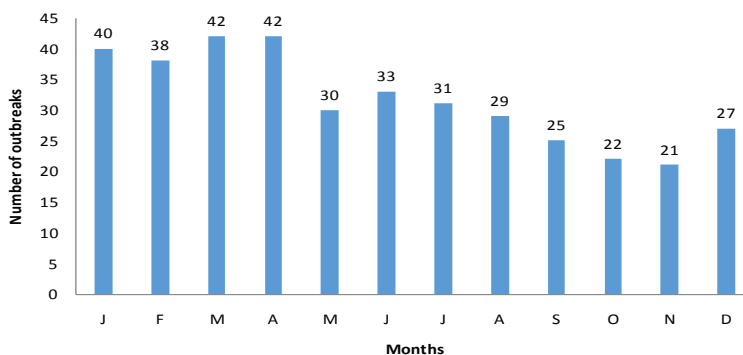


Figure 5: Monthly reported rabies outbreak in animals in Bhutan (1996-2016)

1.3.4. Virus strain

The molecular and phylogenetic analysis of the Bhutanese rabies virus isolates indicated that they were highly similar and were closely related to Indian strains and South Asian Arctic-like-1 viruses. More sampling from different rabies outbreak areas and time periods is needed to understand the RABV transmission dynamics in Bhutan.

02 The Disease

2.1. Causative agent

Rabies is caused by rabies virus, the prototype species of the genus *Lyssavirus*, family *Rhabdoviridae* and order *Mononegavirales*. According to the International Committee on Taxonomy of Viruses, 12 species are classified under the *Lyssavirus* genus: Rabies virus (RABV); Lagos bat virus (LBV); Mokola virus (MOKV); Duvenhage virus (DUVV); European bat Lyssavirus 1 (EBLV-1); European bat Lyssavirus 2 (EBLV-2); Australian bat Lyssavirus (ABLV); Aravan virus (ARAV); Khujand virus (KHUV), West Caucasian bat virus (WCBV) and Shimoni bat virus (SHIBV). Other lyssavirus (Bokeloh bat lyssavirus (BBLV) and Ikoma lyssavirus (IKOV)) have been detected. More lyssaviruses in bats species are expected to be detected and identified in future as the increasing scientific research efforts in the bat population continues. However, rabies virus (RABV) is the most widespread and recovered from terrestrial mammals globally and from *Chiropteran* bats in the Americas. Other rabies-related lyssaviruses are more restricted in their host range and geographical distribution.

2.2. Excretion of virus

The excretion of rabies virus and the levels of virus excreted is the most important factors for transmission. Rabies virus can be excreted in saliva of infected animals for many days after the onset of clinical signs of disease. Rabies virus has been found in dog saliva up to seven days before signs of rabies were observed. Rabies virus can be excreted from the saliva of cats for one to three days and cattle for one to two days prior to onset of signs.

2.3. Virus survival outside host

Rabies virus is quite fragile and is inactivated rapidly on exposure to sunlight and can be killed by lipid solvents (e.g. soap solution) and susceptible to all commonly used disinfectant in the health centers. Simple soap and water can destroy the virus. Soap is a detergent which can break up the lipid envelope surrounding the virus, thus, the virus can be destroyed by a combination of both the detergent and anti-viral actions of soap.

However, the virus can remain stable for a few days at 0 to 4°C in the internal organs; in saliva in temperate climate for about 24 hours and survives indefinitely when freeze dried or kept at -70°C. Glycerol preserves the virus and therefore, brain and other tissues for laboratory examination are preserved in 50% glycerol in phosphate buffered saline solution.

2.4. Host range and susceptibility

All warm-blooded animals are vulnerable to infection by rabies virus, however the degree of species susceptibility varies considerably. In developing countries, the domestic dog is the major reservoir of rabies.

Rabies exists in two epidemiological forms

Urban Rabies: The domestic dog population maintains the urban cycle. This cycle is responsible for 99% of human infections through dog bites. This cycle is common in developing countries.

Sylvatic or Wild life Rabies: The sylvatic cycle is mainly maintained by wild canid species (e.g. foxes, jackals, wolves, coyotes, shunks, mongooses, etc.,) and bats. These wild animals transmit the infection to domestic animals and human through bites.

2.5. Transmission

2.5.1. Direct contact: animal bites and licks

Rabies is mainly transmitted by the bite of a rabid animal that contain rabies virus in the saliva. The virus can also be transmitted via direct contact of fresh wound or intact mucosal surface (eyes, nose) with infectious saliva or by licks of infected animals, and transdermal scratches contaminated with infectious material. The virus cannot penetrate the intact skin.

2.5.2. Ingestion and oral transmission

Transmission of rabies in humans through handling and skinning of infected carcasses and subsequent consumption of raw meat has been reported. Similarly, transmission of rabies through ingestion of raw dog meat has also been reported. The skinning and handling of carcasses with bare hands and touching eyes or lips with hands while they are contaminated by traces of the dog's fluids have been suggested as the main cause of contracting rabies. Pasteurization and cooking

will inactive rabies virus; therefore, drinking pasteurized/boiled milk or eating thoroughly cooked animal products do not constitute rabies exposures. However, it is recommended not to consume the meat and milk originated from rabid animals.

2.5.3. Aerosol transmission

Transmission of rabies by inhalation of virus-containing aerosol is rare but has been reported and can be a potential hazard for laboratory workers. Inhalation of an aerosolized rabies virus during homogenization of fixed virus in the laboratory had resulted in two human rabies cases. Similarly, aerosolised virus from bat urine has been suspected as an exposure pathway for wildlife investigators.

2.5.4. Transmission via organ transplantation

Rabies virus is present in many tissues in the terminal stages of disease. Caution should be exercised before transplanting organs from people who have died with neurological symptoms and signs, as several cases of rabies due to organ and tissue transplantation have been documented. Corneal transplantation, which is common in developing countries, should be performed with caution. Although rabies virus has been isolated from a variety of tissues and body fluids, including cerebrospinal fluid, saliva, tears and urine sediment, no well documented evidence of transmission of virus from rabies victim's saliva and secretions to other humans including the close attendant, relatives, friends and medical staff exists. However, the person handling rabid patients (hospital nurses and family members of patients) should take necessary precautionary measures.

2.5.5. Transplacental transmission

Rabies in pregnancy is very rare. There is only one recorded case of transplacental transmission of human rabies reported in Turkey. A nine-month pregnant woman was bitten by dog 34 days before she gave birth to a baby boy by induction. The baby died after 40 hours and 30 minutes, and the laboratory examinations confirmed rabies in both the mother and the baby. In contrast, infants (n=5) have survived delivery from mothers infected with rabies, when the child was given a series of post-exposure rabies vaccination.

2.6. Pathogenesis

The lyssavirus is a highly neurotropic virus that causes an acute encephalomyelitis of the central nervous system (CNS). After entry of the virus, commonly through infiltration of virus-contaminated saliva from a rabid animal into a bite wound, the virus replicates in the muscle cells and in the neuromuscular spindles at the site of the bite. The virus then enters the peripheral nerves, and is transported by retrograde axoplasmic flow via peripheral nerves to the CNS. This occurs via sensory and motor nerves at the initial site of infection. The speed of virus retrograde transport has been estimated between 50 to 100 mm per day but depends on the amount of virus inoculated at the site of bite. The exposed individual will not show any symptoms during this time. Once the virus reaches the brain, it further replicates (due to the large numbers of neuronal cell bodies in the brain) and disseminates within the CNS. The patient or animals will show first signs of rabies after the virus has multiplied in the brain. Finally, the virus travels centrifugally from the CNS through peripheral nerves to various tissues, most notably the salivary glands, and the transmission cycle is repeated. Salivary gland infection and shedding of virus in saliva is essential for the transmission of virus to its natural susceptible hosts, again usually through a bite wound or contamination of mucous membranes by virus-contaminated saliva, and thus maintaining the epidemiological cycle.

2.7. Clinical signs

2.7.1. Rabies in Dog

The incubation period of rabies in dogs is 3–8 weeks on average, but may vary from 10 days to as long as 6 months, but is rarely more than 4 months. In general, rabid animals of all species commonly exhibit typical signs of central nervous system disturbances with behavioural changes.

A rabid dog may show either furious or paralytic (dumb) form of rabies.

Furious form of rabies

The major clinical signs in furious form of rabies in dogs are change in behaviour and become very excitable, aggression, abnormal barking, biting unusual things like sticks and stones, roaming, laryngeal paralysis, and excessive salivation, tremors, ataxia, muscular incoordination and convulsions, coma and death. The characteristic symptoms of hydrophobia are absent in animals.

Paralytic or dumb form of rabies

The paralytic form (dumb form) of rabies is characterized by the inability to swallow, hanging of jaw due to paralysis of the jaw muscles and the dog is unable to close the mouth leading to a typical sign of foaming saliva around the mouth. There will be ascending paralysis which begins at the hind extremities and eventually complete paralysis is followed by death. The entire course of the disease up to death takes 1 to 7 days.

2.7.2. Rabies in cattle

The average incubation period of rabies in cattle is 15 (depend on the site of bite) days and the average morbidity period is 4 days. The major clinical signs in cattle includes excessive salivation, behavioural changes, muzzle tremors, vocalization (bellowing), low-pitched voice due to paralysis of vocal cord (may mistake for heat sign), aggression, hyperesthesia and/or hyperexcitability, and pharyngeal paresis/paralysis, coma and death.

2.7.3. Rabies in sheep and goats

The clinical signs in sheep includes muzzle and/or head tremors, aggressiveness, hyperexcitability, and/or hyperaesthesia, trismus, salivation, dropping ears, vocalization, and recumbency and death.

2.7.4. Rabies in horse

In horse, the average incubation period is 12 days (depend on the site of bite) and the average morbidity period is 6 days with majority of the horses developing furious rabies. Muzzle tremors, pharyngeal spasm or pharyngeal paresis, ataxia or paresis, lethargy or somnolence are the common signs manifested by rabid horse.

2.8. Diagnosis

Although primary diagnosis may depend on clinical signs, rapid and accurate diagnosis by laboratory tests is important for deciding on post-exposure prophylaxis in humans and initiating rabies control in animals. The main principles of rabies diagnosis are based on antigen and antibody detection.

2.8.1. Clinical diagnosis

The rabies can be diagnosed based on clinical signs, history of exposure and epidemiological information (as discussed above).

2.8.2. Laboratory diagnosis

2.8.2.1. Fluorescent Antibody Test (FAT)

The direct fluorescent antibody technique is a rapid, sensitive, specific method for diagnosing rabies in animals and humans and is the gold standard for rabies diagnosis. However, the sensitivity of the test depends on the degree of autolysis (autolyzed samples can reduce the sensitivity and specificity of the test), sampling of the brain, the competencies of the technicians, the quality of the reagents (conjugates) used, including the fluorescence microscope (Refer FAT SOP). In addition, rabies can also be diagnosed using Rabies Immunoperoxidase antigen detection test, which does not require fluorescence microscope (Refer RAID SOP).

2.8.2.2. Rapid Immunodiagnostic test (RIDT)

Rabies virus antigen can also be detected from brain samples using RIDT which works based on the principles of immunochromatography. The immunochromatographic lateral flow strip test is a one-step test that facilitates low-cost, rapid diagnosis of rabies virus without the need for laboratory equipment, and can be conducted in the field (refer SOP).

2.8.2.3. Molecular techniques

Various molecular diagnostic tests, e.g. detection of viral RNA by reverse transcription polymerase chain reaction (RT-PCR), PCR-ELISA, real-time PCR, hemi-nested PCR, and nested PCR are used as rapid and sensitive tests for rabies diagnosis. The RT-PCR with subsequent nucleotide sequencing permits the diagnosis of rabies, typing and molecular epidemiological studies and also for ante-mortem diagnosis in humans.

2.8.2.4. Cell culture and Mouse inoculation test

These tests are based on the principles of detecting the infectivity of a rabies virus tissue suspension in cell cultures or in laboratory animals (mouse) after inoculation. These tests should be used if the result of antigen detection tests gives an uncertain result (to confirm) and for further amplification or characterization of an isolate, but is not commonly used because of the availability of other alternative methods.

2.8.2.5. Serological tests

Serological tests are used to measure the level of virus neutralizing antibody in vaccinated individuals and to detect host response to rabies infection by measuring antibodies in cerebrospinal fluid/serum in suspected rabid cases. Rapid fluorescent focus inhibition test (RFFIT) and fluorescent virus neutralization test (FVNT) are currently recommended by the WHO for detecting the virus neutralization. However, these tests require a specialized laboratory and facilities to handle tissue culture and the virulent rabies virus, and also are too complex for large scale screening of field sera. Several enzyme-linked immunosorbent assays (ELISA) based methods using either the whole virus or purified G glycoprotein as the detection antigen are available to effectively detect and quantify rabies antibody in the sera of vaccinated animals or humans, and are applicable for testing large numbers of field sera.

2.8.2.6. Ante-mortem diagnoses of rabies

Although the various intra-vitam laboratory methods can be used to confirm a clinical case of rabies while the patient (human) is still alive, use of intra-vitam techniques for the diagnosis of rabies in animals is, however, strongly discouraged. The sensitivity of a technique for diagnosing rabies varies widely according to the stage of the disease, immunological status, intermittent viral excretion and the training of the technical staff. While a positive validated result is indicative of rabies, a negative result does not necessarily rule out the infection.



Brain tissue sampling from rabies suspected case

03 Prevention and Control Strategies

3.1. Institutional arrangements

The animal health services in the country are delivered to farming communities through the network of various Livestock extension centres (Veterinary hospitals, livestock extension and RNR Centres). Technical and laboratory support are provided through National Centre for Animal Health (NCAH), Regional Livestock Development Centres (RLDCs), City Veterinary Hospital & Satellite Laboratory (CVH & SL), Satellite Veterinary Laboratories (SVLs), National Animal Hospital (NAH) and Dzongkhag Veterinary Hospitals (DVHs).

3.1.1. Department of Livestock

The Animal Health Division (AHD) at the Department of Livestock (DoL) shall oversee policy formulation related to National Rabies Prevention and Control Program (NRPCP) in the country. The specific roles include the following:

- Mobilize resources including fund for Rabies prevention and control program in the country
- Liaise with different stakeholders/agencies/international organizations (e.g. FAO, OIE, WHO, SAARC) for facilitating better implementation and ensuring success of the control program
- Collaborate with BAFRA to enable better enforcement of the Livestock Acts and By-laws of the country
- Collaborate with relevant national agencies (DoFPS, DoPH (MOH), DDM, NGOs) for ensuring and mobilization of support required for Rabies control
- Coordinate border harmonization meetings with the neighboring countries
- Explore the projects/donor agencies for the skill development of manpower and undertaking the disease prevention and control activities in the country

3.1.2. National Level

The NCAH shall function as the national focal agency for the overall planning, coordination and implementation of the National Rabies Prevention and Control Program (NRPCP) in the country. The responsibilities for the national focal agency are to:

- Coordinate the overall implementation of the NRPCP in the country
- Mobilize resources at the national level in terms of supply of vaccine and equipment
- Support the activation of rapid response team (RRT) in the event of disease outbreak
- Liaise with different stakeholders/agencies for facilitating better implementation and ensuring success of the control program
- Coordinate conduct of epidemiological research on Rabies in collaboration with national, international diagnostic and research institutions
- Production of education (IEC) materials and make available for wider circulation for advocacy campaign
- Ensure maintenance of database on Rabies control program (e.g. vaccination coverage), analysis and dissemination of information/progress report to the Department/Ministry/other stakeholders including the Department of Public Health (MOH) regarding the progress of the control program
- Conduct Rabies coordination workshops at national level to review and realign the control program
- Coordinate the conduct of field simulation exercise among RRT
- Monitor and evaluate the control programs implemented by the field units
- Ensure vaccination coverage as per the risk zone identification
- Assess the status of the risk zones by regular surveillance and monitoring
- Declaration of risk zones for Rabies
- National referral laboratory for the diagnosis of Rabies including the standardization of protocol for diagnosis for ensuring uniformity across diagnostic laboratories in the country.
- Regular information update on Rabies to the Department of Livestock and MOH (Department of Public Health) using the existing database
- Regularly update the National Rabies Prevention and Control guidelines
- Conduct researches on rabies

3.1.3. Regional Level

The Regional Livestock Development Centres (RLDCs) shall function as regional focal agency for Rabies control program. The main roles of the regional focal agency are to:

- Coordinate the overall implementation of the NRPCP at the regional level
- Coordinate the activation of rapid response team (RRT) in the event of disease outbreak
- Provide support and coordinate logistics arrangement at the regional level
- Liaise with the BAFRA at the regional level for facilitating better enforcement of the Livestock Acts and By-laws
- Monitoring and evaluation of the control programs in their respective regions
- Ensure prompt reporting of outbreak and updating the disease status in the existing database.
- Ensure maintenance of database on Rabies control program (e.g. vaccination coverage), and submit progress report to the NCAH
- Monitor and evaluate the control programs implemented by the field units
- Information update on Rabies control program and outbreaks to NCAH, DoL and the Regional/District/Local Health authorities in the respective areas.
- Test the functioning of RRTs in the region during the outbreak

3.1.4. SVL and CVH Level

The SVL and CVH shall function as the focal agency for Rabies control program in the southern border/frontier areas. The main roles of the SVL and CVH are to:

- Coordinate the overall implementation of the NRPCP through vigilance and surveillance along the border areas
- Coordinate the activation of rapid response team (RRT) in the event of disease outbreak
- Provide support and coordinate logistics arrangement in the event
- Liaise with the BAFRA at the border areas for facilitating better enforcement of the Livestock Acts and By-laws
- Monitoring and evaluation of the control programs in their respective areas

- Ensure prompt reporting of outbreak and updating the disease status in the existing database.
- Ensure maintenance of database on Rabies control program (e.g. vaccination coverage), and submit progress report to the NCAH
- Monitor and evaluate the control programs implemented by the field units
- Information update on Rabies control program and outbreaks to NCAH and the District Health authorities in the respective areas
- Conduct disease surveillance through testing of brain tissue samples (dogs/wildlife/other animals) using rapid antigen detection test
- Conduct awareness/advocacy campaigns to the public and relevant stakeholders
- Provide sensitization on the pet registration and responsible pet ownership
- Maintain dog registration and vaccination database

3.1.5. Dzongkhag Level

At the Dzongkhag level, the Dzongkhag Veterinary Hospital (DVH) shall function as the focal agency for implementation of the Rabies control program. The Dzongkhag Livestock Officer shall adequately liaise with the Dzongkhag Administration for the declaration of outbreak and lifting of the ban of the disease as outlined in the Livestock rules and regulations. The Dzongkhag focal agency should carry out the following tasks:

- Implement the NRPCP in the field
- Arrange logistics at the Dzongkhag level and assist the Gewog staff with their logistics
- Liaise with the BAFRA at the Dzongkhag level for facilitating better enforcement of the Livestock Acts and By-laws
- Support the activation of rapid response team (RRT) in the event of outbreak in the dzongkhags
- Mobilize manpower in the Dzongkhag for routine and ring vaccination program including the mass vaccination campaigns
- Ensure prompt reporting of outbreak and updating the disease status in the existing database.
- Conduct awareness/advocacy campaigns to the public and relevant stakeholders
- Provide sensitization on the pet registration and responsible pet ownership
- Maintain dog registration and vaccination database
- Ensure maintenance of database on Rabies control program (e.g. vaccination coverage)

- Submit monthly reports to the RLDC regarding status of the disease in the Dzongkhags
- Conduct disease surveillance in the Dzongkhags including Gewogs through testing of brain tissue samples (dogs/wildlife/other animals) using rapid antigen detection test
- Receive the inputs from the NCAH/RLDC and maintain inventory in the Dzongkhags
- Confine the Rabies suspected or confirmed animals till further directives from RLDC
- Coordinate and or assist the RLDC/NCAH in the disease outbreak investigation and sample collection

3.1.6. Gewog Level

The Livestock Extension Centre/RNR Extension Centres/Veterinary hospitals at Gewogss shall be the focal agency for that Gewog. The in-charge of the Gewog Livestock office shall adequately involve the Gewog administration in the implementation of the NRPCP. They would play a very crucial role in the implementation of the Rabies control program in their respective Gewogs. The main roles of the Gewog focal agency are as follows.

- Implement the Rabies control program in the field as per the NRPCP.
- Ensure prompt reporting of outbreak through FLASH report and updating the disease status on regular basis
- Implement provisional emergency control measures in the locality in the event of outbreak
- Ensure maintenance of proper recording of vaccinated dogs and cats and other required records
- Liaise with the BAFRA at the Gewog level for facilitating better enforcement of the Livestock Acts and By-laws
- Liaise with the Gewog administration (GT), BHU and other local leaders and farmers for facilitating proper implementation of the program in the field
- Play lead role at the time of vaccination campaign and disease outbreak investigation in their respective Gewogs
- Conduct regular disease awareness campaign for the farmers and other clients
- Assist the DVH (Dzongkhag)/RLDC/NCAH in the disease outbreak investigation, sample collection and containment of disease
- Report the findings of the laboratory to BHU of MOH and other agencies.

- Confine the Rabies suspected or confirmed animals till further directives from DVH
- Inform the exposed human against confirmed or suspected cases of Rabies for reporting to the nearest health facilities
- Monitor the disease situation at the Gewog level
- Conduct rabies surveillance and report monthly to the DVH through existing animal health reporting system even if there was no reported cases of rabies in the Gewog (zero-reporting system)
- Maintain dog registration and vaccination database

3.1.7. Bhutan Agriculture and Food Regulatory Authority

Bhutan Agriculture and Food Regulatory Authority (BAFRA) as a Regulatory Authority under MOAF is mandated to enforce and implement Livestock Act of Bhutan 2001 and Livestock Rules and Regulations 2008:

- Enforcement of movement ban of livestock and livestock products in and out of the Rabies outbreak areas
- Quarantining of infected animals in the affected areas
- Inspection and certification of livestock products originating from outbreak areas
- Carry out bio-security and food safety measures during outbreak.
- Vigilance on the illegal movement of dogs and cats
- Participate in the RRTs for the control of rabies during the outbreak
- Awareness on Livestock Rules and Regulations

3.1.8. Other relevant agencies/organizations

The prevention and control of Rabies require joint effort at various levels including the regional and international organizations.

Department of Public Health (MOH)

Rabies is a zoonotic disease and it requires a close collaboration with Department of Public Health in terms of disease reporting and support for the prevention and control of the disease. The major collaborations and support required with MOH are as follows:

- Management of dog bite database in the country
- Sharing of data/information on dog bites and suspected human rabies cases

- Develop IEC materials on Rabies and public awareness
- Provide rabies prophylaxis to the livestock officials and other frontier workers in the rabies endemic areas
- Participate in RRT activities in the event of rabies outbreak in animal
- Develop and update the human rabies guidelines
- Facilitate fund support for rabies prevention and control activities
- Liaise with NCAH related to sample collection from suspected human rabies cases and conduct of laboratory test to confirm the case

Department of Forests and Park Services

The domestic livestock graze freely in the forest where there is a possibility of mixing domestic animals with wild animals (wild dogs, wild cats, foxes, jackals). There may also be disease transmission (rabies) at the domestic-wild life interface since most of the villages in the country are surrounded by forests. Therefore, collaboration between livestock and forestry sectors (wildlife) is important for disease surveillance, sharing of disease outbreak information and prevention and control program. There is also need for the regular reporting of wild life diseases to NCAH as it is mandatory for the country to report to OIE on wildlife diseases.

Dzongkhag Administration

The Dzongkhag, Dungkhag and Gewog administration support is important for coordinating rabies prevention and control activities including management in the event of outbreak in their areas. Local government support is crucial for strict implementation of NRPCP at the village and community level.

Royal Bhutan Police

The Royal Bhutan Police (RBP) support is necessary for enforcement of Rabies control measures and regulations especially in the event of outbreak. The RBP support is also necessary in the event of legal matter that arises in association of rabies and nuisance due to dogs. The involvement of RBP at the border towns is crucial in monitoring of the illegal import of dogs and cats which are one of the causes of disease introduction in the interior places in the country in the recent years.

Ministry of Finance

The Ministry of Finance (MOF) should provide adequate fund for implementation of NRPCP in the country. The adequate budget provision for rabies prevention and dog population control should be made available at different levels (Gewog, Dzongkhag (DVH), SVL/ CVH, RLDC, NCAH and DOL). MOF should provide contingency fund for disease outbreak containment in addition to the routine prevention and control activities.

City Corporation (Thromde)

A Thromde support in the urban areas is important towards sustainable dog population management and rabies control program in the country through proper waste management thereby reducing the habitat and food resources for dogs. A MoU shall be signed between relevant Thromde and DoL for mass dog vaccination and animal birth control program.

Non governmental organizations

The NGOs and Animal Welfare organization in the country would also play an active role during mass vaccination campaign, creating public awareness on rabies, and by providing fund support for rabies control program

International Organizations

It is important to build linkages with the international organizations such as the Food and Agriculture Organization (FAO), World Organization for Animal Health (OIE), World Health Organisation (WHO) and other organizations for seeking fund and technical support, human resource development, and referring of samples for laboratory diagnostic services.

The SAARC Regional Support Unit and WHO SEARO should play an important role in helping with developing coordinated program for Rabies control in the SAARC countries and also sharing facilities like laboratory services, disease information centre, and facilitate in quality vaccine procurement. In addition, international organizations should facilitate cross border rabies control projects focussing on the southern international borders. The cross border harmonisation meeting, mass vaccination campaign against Rabies and also animal birth control program at the border towns shall be pursued with priority with the involvement of the relevant states/districts at the local level.

3.2. Prevention strategy

3.2.1. Risk zone identification and prevention strategies

Risk zone identification and categorization for rabies is important for defining the strategies to be used in each zone and for defining disease status in accordance with the stepwise approach toward control of rabies in the country.

The country has been divided into two risk zones (high and low risk zone) based on the epidemiology of the disease (number of outbreaks), sharing of border with neighbouring countries, and road connectivity (Figure 6). This will guide strategies for surveillance and vaccination programme. However, the risk zones would be reviewed periodically based on the disease status to increase the efficiency of the prevention and control program.

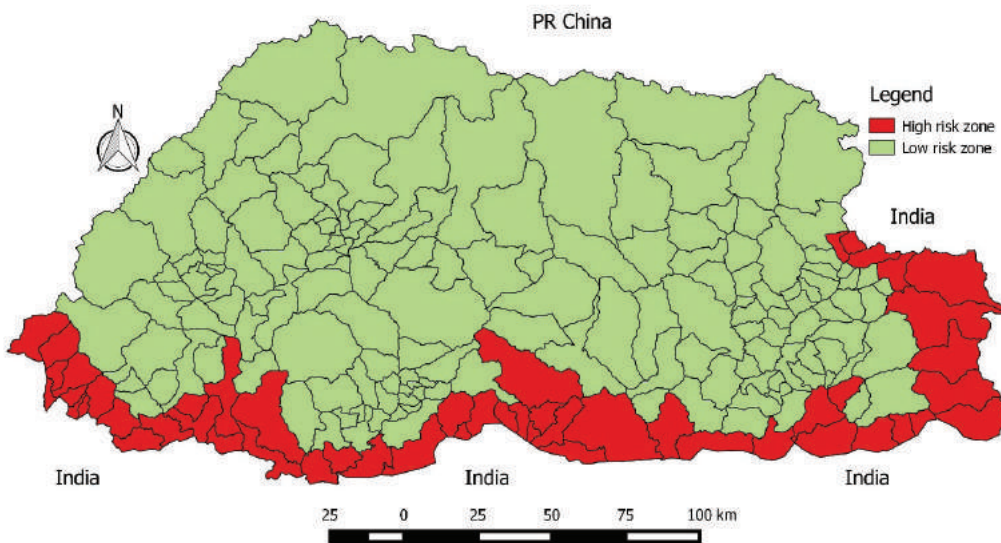


Figure 6: Rabies Risk zone in Bhutan

High risk zone

A total of 51 Gewog sharing borders with Indian states and also the next nearest Gewogs, fall under this zone (Figure 6, Table 2). Some of these areas experience frequent outbreaks of rabies owing to sharing open border with India. Annual vaccination of dogs (both stray and owned) in the high risk zone would create an immune buffer (*cordon sanitaria*) and prevent incursion of virus/disease from across the border thereby reducing the risk of spread to interior rabies-free Bhutan. In this zone a minimum of 70% of the total dog population (including both owned and stray dogs/cats) should be vaccinated during each annual pulse campaign. This zone shall remain under stringent vaccination schedule as stipulated above until such time when the disease risk is reduced to zero. In addition, both stray and owned dogs shall be vaccinated during the time of CNVR and CABC programme.

Low risk zone

All other Gewogs that is not under high risk zone fall in the low risk zone (Figure 6). The low risk zone has been defined as the villages/Gewogs that fall under remote and mountainous terrain and where the incidences of rabies has not been reported at all or there has been few incidences of incursion of rabies from the high risk zone/border areas in the past. Owing to low incidences of rabies in this zone, one of the objectives is to achieve recognition of freedom from rabies in accordance with the stepwise approach of rabies control program (Figure 1). In this zone, stray dog should be vaccinated every three years with more than 70% coverage in each pulse vaccination campaign. The frequency of the vaccination campaign requirement will be assessed based on the findings of the sero-monitoring studies, rabies surveillance, and dog population turnover rate. However, owned dogs can be registered and vaccinated annually at their respective veterinary centres. In addition, both stray and owned dogs shall be vaccinated during the time of CNVR and CABC programme (refer surveillance system).

Table 2: Rabies risk zone in Bhutan

Dzongkhags	Gewogs
High Risk zone	
Samtse	Tading, Pugli, Samtse, Chengmari, Ugentse, Yoeseltse, Chargharey, NamgayChoeling, Sipsu, Biru, Tendu, Bara
Chukha	Bongo, Darla, Sampheling, Phuntsholing, Logchina, Geling
Sarpang	Taraythang, Umling, Serzhong, Chhuzagang, Gelephu, Bhur, Dekiling, Shompangkha, Hilley, Singye; Jigmecholing
Samdrup Jongkhar	Langchenphu, Samrang, Pemathang, Phuntshothang, Dewathang; Orong, Gomdar, Shinkhar Lauri, Serthig
Dagana	Nichula, Deorali, Lhamoizingkha
Pemagatshel	Norbugang, Chokhorling
Zhemgang	Ngangla, Phangkhar
Trashiyangtse	Khamdang, Teotsho, Yalang (shares open border with Tawang, Arunachal Pradesh and there has been incidence of rabies incursion in the past)
Trashigang	Merak, Sakteng, Phongme (shares open border with Tawang, Arunachal Pradesh and there has been incidence of rabies incursion in the past)
Low Risk zone	All other Gewogs that do not fall under high risk zone

Note: Some of the villages (remote villages) under HR zone may not be necessary to follow the vaccination schedule of high risk zone. Vaccination program should be considered based on risk assessment and technical judgement.

3.2.2. Vaccination program

3.2.2.1. Vaccine procurement and supply

The NCAH should procure vaccine and supply as per the annual indents submitted by the Dzongkhags. The total anti-rabies vaccine doses requirement in each Dzongkhag should be calculated based on the susceptible animal population [dogs (both stray and owned) and cats], risk zone and 70% coverage target during each campaign. A vaccine bank at the regional level should be established at SVLs (Gelephu, Deothang, Nganglam, Phuntsholing and also at RLDC Khangma) for emergency supplies during outbreaks. A vaccine that provides a minimum immunity of 3 years should be used.

3.2.2.2. Vaccine strain and quality

Vaccine containing tissue culture Rabies virus, CVS strain in appropriate cell line and inactivated with suitable adjuvant (Aziridine compound Aluminium hydroxide gel). The minimum requirement for antigen potency should be greater than one international unit per ml (>1 IU per ml) as per the standards specified by WHO. The potency is calculated by comparison of ED₅₀ (median effective dose) of the sample and that of a simultaneously tested reference vaccine with an established potency (IU). The quality of vaccine should be monitored through periodic sero-monitoring study post vaccination.

3.2.2.3. Cold chain maintenance

To preserve the immunological properties of rabies vaccine, the manufacturers' recommendations for storage should be respected. Particularly, prolonged breaks in the cold chain, exposure to sunlight and temperature fluctuations should be avoided. As vaccines are susceptible to extremes of temperature, including freezing, care should be taken to ensure that the cold chain is maintained within an acceptable temperature range. The quality of vaccine should be maintained throughout the cycle (storage, transportation and handling of vaccine at all levels). The maintenance of optimal temperature during the supply of vaccine by the supplier to NCAH should be monitored by Biological Production Unit (BPU) and storage at BPU should be monitored by Program Director, NCAH. The distribution of vaccines from the BPU to the field should be done in a refrigerated van to the RLDCs and the Dzongkhags. During the distribution of vaccine, it should be ensured that the optimal temperature is maintained in the refrigerated van by use of temperature data logger, which should be monitored by BPU. At the RLDCs and Dzongkhags, the concerned staff should record the temperature of the refrigerators daily and maintain the data at all levels using standard form (refer FORM 1), which should be checked and monitored by the RLDC. It is the responsibility of the concerned Gewog in-charges to ensure that vaccines are kept under proper cold storage during the entire phase of the vaccination program. Vaccine should be carried to the field in cool-boxes using ice-packs. Normally the ice packs may last only up to 24 hours and should be replaced with new ice packs.

3.2.2.4. Target species for vaccination

All dogs (stray and owned) should be vaccinated with the aim of covering at least 70% of the population. As cats are also important vectors of rabies to humans, cats should also be vaccinated.

3.2.2.5. Age for vaccination against Rabies

The primary vaccination for dogs and cats is 4 weeks (1 month) irrespective of the vaccination history of the dam with booster vaccination at 3 months and then provide annual vaccination (see risk zone vaccination schedule).

3.2.2.6. Mass vaccination campaign

Dog mediated rabies can be eliminated through mass vaccination of dogs covering more than 70% of the dog population.

Information on dog ecology

Initial determination of the dog population size in a given community is important in the preparatory and evaluation stages in order to determine campaign logistics (number of dogs to vaccinate) and to calculate the resources required and the appropriate methods for accessing dogs for vaccination.

Vaccination plan and approach

- All members of a vaccination team who handle dogs should receive pre-exposure prophylaxis one month before the campaign. Post-exposure prophylaxis should also be given for people exposed (bites) during the campaign.
- Vaccination campaigns must be strategically planned, well managed and adequately resourced and funded.
- Awareness at the national and community level is necessary to ensure commitment of the policy makers and community participation.
- Detailed vaccination schedule including date and place to be visited by the teams should be prepared in advance and informed to all the concerned Gewog livestock in-charges or to the public so that they can bring their pets and community dogs to the designated areas.
- Several temporary vaccination points should be set up at the central area which is convenient for the community
- The vaccination team comprising of 3-4 person (livestock) per team should be formed and assign the vaccination points. A local official or village Tsokpa should be engaged along with the team to implement the program smoothly and also for maximum coverage

- As vaccines are susceptible to extremes of temperature, including freezing, care should be taken to ensure that the cold chain is maintained within an acceptable temperature range. Vaccine should be carried to the field in cool-boxes using ice-packs. Normally the ice packs may last only up to 24 hours and should be replaced with new ice packs.
- In case of free-roaming/stray dog vaccination, dog handlers could be used to catch and restrain dogs humanely for vaccination.
- All vaccinated dogs should be given temporary identification mark (colour paint) at the time of vaccination so as to assess the vaccination coverage
- The owned dog that has been vaccinated should be given vaccination certificate to the owner or update the dog registration card of the dog if the owner have the dog registration card
- The vaccination coverage should be assessed by making transect walk in the vaccinated areas and conduct proportional counts (count the number of dogs with colour mark) and also questionnaire survey of the household
- Vaccination can also be conducted along with CNVR or CABC program
- Intensive vaccination campaigns lasting from 1 day to 1 month is effective in rabies control. Campaigns must, however, reach at least 70% of the dog population, and coverage should not be compromised in pursuit of speed

Timing of mass vaccination campaign

Rabies vaccination campaigns should be conducted annually in southern belt of Bhutan which is endemic for rabies (high risk zone) and every 3 years in interior Bhutan (low risk zone). Campaigns may be organized during school holidays to improve turnout, as children often bring their dogs for vaccination. The campaign should be done preferably during winter seasons. However, owned dogs shall be vaccinated based on their schedule of vaccination. Rabies vaccination in owned dogs can also be conducted along with other vaccination schedule in livestock (e.g. along with FMD campaign since FMD vaccination in animals are done through door-to-door visit of the households).

3.2.2.7. Vaccination during the time of outbreak

In the face of an outbreak, vaccination in the protection zone (dogs and cats) should be carried out to prevent further spread of the disease based on the recommendation of RRT (refer SOP).

3.2.2.8. Reporting the progress of vaccination coverage

Identification of vaccinated dogs is necessary in order to evaluate the vaccination coverage rate and to differentiate unvaccinated dogs for follow-up vaccination. Registration and permanent identification of all vaccinated dogs should be done and issued a card to the owner.

During the mass vaccination campaign, a survey should be undertaken within one week of the campaign to assess the numbers of marked and unmarked dogs. A revaccination campaign should be organized if the coverage is found to be below 70% of the estimated dog population.

The details of animals vaccinated in the field should be reported using the Monthly Animal Health Report Form and then enter the data in the existing Veterinary Information System database.

3.2.3. Dog population management

3.2.3.1. Registration and dog ownership

Chapter IX of the Livestock Rules and Regulations of Bhutan 2008 require registration of owned dogs with the nearest veterinary centres and also covers aspect of responsible dog ownership. Owning a pet is a privilege and should result in a mutually beneficial relationship. However, the benefits of pet ownership come with obligations which includes:

- Committing to the relationship for the life of the pet(s)
- Carefully selecting pet(s) suited to one's home and lifestyle
- Recognizing that ownership of pet(s) requires an investment of time and money
- Keeping only the type and number of pets for which an appropriate and safe environment can be provided, including appropriate food, water, shelter, health care and companionship
- Ensuring that the pets are registered with the local authority/Animal Health Centres
- Ensuring pets are properly identified (i.e., tags, microchips, or tattoos) with a unique registration number
- Controlling pet(s)' reproduction through managed breeding, containment, or spay/neuter thereby helping to address animal control and overpopulation problems

- Providing preventive (e.g., vaccinations, parasite control) and therapeutic health care for the life of pet(s) in consultation with, and as recommended by the animal health official
- Socialization and appropriate training for pet(s), which facilitates their well-being and the well-being of other animals and people
- Preventing pet(s) from negatively impacting other people, animals and the environment, including proper waste disposal, noise control, and not allowing pet(s) to stray or become feral
- Providing exercise and mental stimulation appropriate to the pet(s)' age, breed, and health status
- Making alternative arrangements if caring for the pet is no longer possible

In order to manage the pet dog population and prevent rabies spread through pet dog, dog registration system and dog ownership system shall be implemented in accordance with Livestock Act of Bhutan 2000 and Livestock Rules and Regulations of Bhutan 2008 (refer dog population management guideline).

3.2.3.2. Animal Birth Control and Dog population management

The free-roaming/ stray dog population in the country shall be managed through Animal Birth Control program –Catch-Neuter-Vaccinate-Release (CNVR) and Community Animal Birth Control (CABC) program and also through habitat control. Reduction of the dog carrying capacity of the environment can be achieved by improved solid waste management (community garbage collection and sanitary waste disposal), removal of shelter, clean-up of specific habitats, and education of the public (refer dog population management guideline).

3.3. Control strategies

3.3.1. Outbreak investigation and reporting

Since rabies is a notifiable disease, the pet owners/livestock owners/public should immediately report even a mere suspicion of this disease to Gewog Livestock extension official(s) or to the Gewog administration. The Gewog Livestock Office should immediately investigate all suspected cases of rabies and should be declared as “suspected Rabies case” if the affected animal shows clinical signs as per disease case definition. Following this, the concerned Gewog livestock staff should immediately report to the Dzongkhag Veterinary Hospital, Regional Livestock Development Centre or National Centre for Animal Health through telephopne/SMS/e-mail and by using Flash Report Form 6B. The DVH/RLDC

should send Disease Outbreak investigation team to investigate the suspected case immediately (refer SOP for disease outbreak investigation). This team should undertake a comprehensive epidemiological assessment in the field to confirm the disease as well as collect appropriate samples. The clinical diagnosis should be further confirmed using rapid antigen testing in the field as per SOP for rapid test, wherever possible, and then collect appropriate samples for final laboratory confirmation at NCAH as per SOP for sample collection. The disease outbreak investigation team should also update the detail of the outbreak through e-mail/telephone and update the data using online disease information database followed by weekly update of the disease outbreak situation.

Case definition for Rabies

Suspect case:

Dogs and cats (Furious form of rabies): Animal exhibiting strange behaviour progressing to aggression, disorientation, impaired mobility, unusual vocalizations, salivation, biting inanimate objects or people, coma and death.

Dogs and cats (Dumb form of rabies): Animal exhibiting strange behaviour progressing to disorientation, impaired mobility, unusual vocalizations, salivation, hanging of jaw coma and death.

Cattle and other animals: Frequent bellowing with low-pitched voice, salivation with foamy froth, aggressiveness, nervous signs, reddened eyes, butting other objects and people, and death.

Confirmed case:

The above clinical signs complemented by positive detection of rabies virus in brain tissues by rapid rabies antigen test in the field and subsequently confirmed by fluorescent antibody test (FAT) or RT-PCR at NCAH.

3.3.2. Declaration of Provisional Infected Zone

When Rabies is suspected, Gewog Livestock Staff should immediately quarantine the suspect infected place (premises/town/village/farm) and the surrounding area (based on risk assessment) and declare it as a **Provisional Infected Zone**. The geographical limits of the Provisional Infected Zone should be determined after due consideration of the epidemiologic risk, settlements and natural geographical settings. The Provisional Infected Zone should be declared by the Gewog Livestock Office with the consent from the Gewog Administration.

The following control measures should be implemented in the Provisional Infected Zone to prevent spread of the suspected disease (refer table 3 and 4 for management of exposed animal).

- Immediate segregation of affected animals including separate management (feeding, watering and shelter). This would include proper restraint of the suspected animals away from the rest of the animals
- Management of the affected animals and their products (in case of rabies in cattle)
- Disinfection/ Decontamination of the contaminated premises as per SOP.
- Provisional ban on the movement of animals and their products from the infected premises as per advice of the DOIT
- Awareness and education of the livestock owners/public in the affected village on zoo sanitary measures

3.3.3. Declaration of the Infected Zone

Once the case definition of Rabies is met, the area where the disease has occurred within a radius as decided by disease outbreak investigation team should be immediately declared as **Infected Zone**. The geographical limits of the infected zone should be determined after due consideration of the epidemiologic risk, settlements and natural geographical settings. The disease outbreak investigation team should also declare vaccination zone within certain radius of infected zone where immediate vaccination in the surrounding villages needs to be carried out to prevent further spread of outbreak. Based on the recommendation of the disease outbreak investigation team, the Dzongkhag Administration shall issue the disease outbreak declaration order with information to the Gewog Administration, DOL, BAFRA, NCAH, RLDC and DVH.

All the provisional control measures should be continued with reinforcement of the efforts in the required areas once the disease outbreak is officially declared. If the disease suspected is not Rabies, all the provisional control measures that are being implemented shall be immediately discontinued and the alternate measures specific to that disease should be undertaken.

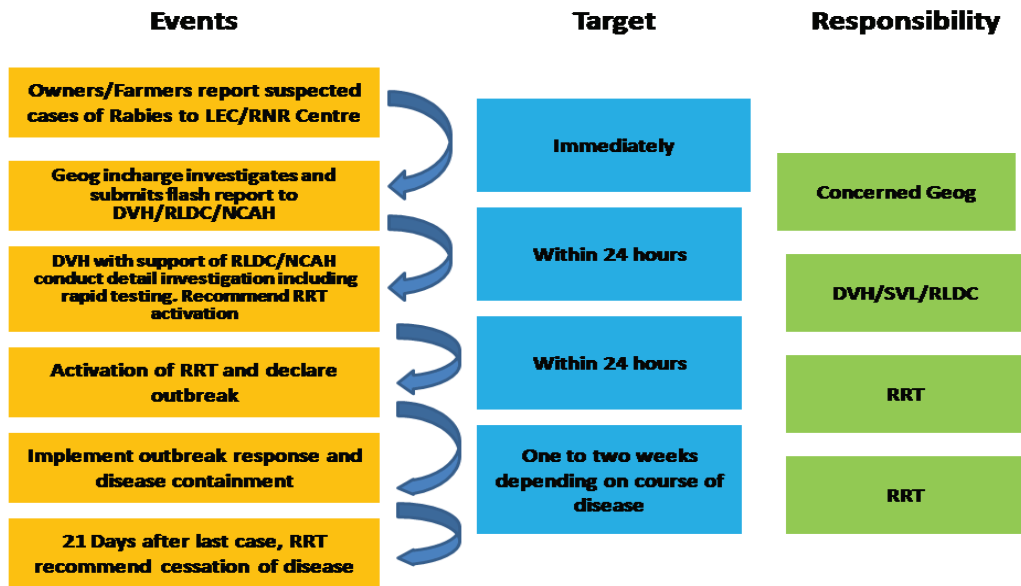


Figure 7: Time line for containment of rabies disease

3.3.4. Activation of the Rapid Response Team

Once the disease is confirmed by the disease outbreak investigation team (DOIST), Rapid Response Team (RRT) should be activated immediately to rapidly contain the disease without allowing it to spread to other places. RRT should mainly constitute four team based on the nature of outbreak to effectively implement disease control measures– DOIST, Mass Dog Vaccination Team (MDVT), Heath Team (HT) and Quarantine and Movement Control Team (QMCT).

The DOIST should be responsible for disease outbreak investigation (including sampling), disinfection/decontamination and disposal of carcass, treatment of the affected animals, disease surveillance, public education while the MDVT is to carry out vaccination of susceptible dogs and cats in the vaccination zone (need-based). The QMCT (BAFRA officials) should be responsible for quarantine and movement control of susceptible livestock and livestock products from the infected zone as recommended by DOIT. The Health team should be responsible for creating public awareness and also asses the human risk from rabies. The hospitals and BHU shall provide post post-exposure prophylaxis to the dog bite victims and other exposed individuals as well as provide pre and post bite prophylaxis to the members of DOIST and MDVT. All team should work under the spirit and concept of One Health for rapid containment of outbreak.

3.3.4.1 Management of rabies suspected/confirmed and exposed animals

This section provide generic as well as specific guidelines related to the management of rabies suspected/confirmed and also exposed animals. The animal owner/handler and also the veterinarian/RRTs should follow the following procedures and also the specific SOPs:

- Verify the case and approach the case(s) diligently and always keep in mind that rabies is a fatal disease and can be transmitted through bite wound and also through direct contact of the infected saliva with mucous membranes or a break in the skin
- Appropriate Personal Protective Equipment (PPE) should be worn while handling the case and also the animal owners should also be provided with PPEs while handling animals (refer SOP)
- All livestock officials handling rabies cases should receive pre or post exposure prophylaxis
- Any potentially rabid animal which has exposed another animal and is not available for laboratory testing should be presumed to be rabid
- Domestic animals that bite other domestic animals are not usually considered as rabies suspects unless showing signs compatible with the disease and confirmed through laboratory test
- All the suspected/infected/exposed animals should be restrained and isolated as per SOPs and as outlined below in Table 3 and Table 4
- The infected/exposed animal should be housed in a building, pen or by some other suitable escape-proof method or enclosure. The animal should not be removed from confinement unless on a leash and under the immediate control of a responsible adult. The animal should not be moved from the premises unless permission is obtained from the authorized Veterinarian/District Livestock Officer/ Regulatory Officer from BAFRA
- In the high risk zones (southern belt of Bhutan) any dog or cat (whether vaccinated or unvaccinated) that may be apparently healthy and that bites a person/livestock must be approached accordingly (as outlined below in Table 3).

Table 3: Management of dogs and cats

Sl. No	Scenarios of exposure	Action to be taken/Recommendations
1	Vaccinated pet dog and cats exposed to a confirmed or suspected rabid animal (bitten by rabid or suspected rabid dog)	<ol style="list-style-type: none"> 1. Wash the bite wound with soap and water and perform antiseptic dressing 2. Provide PEP on day 0, 3, 7, 14, 28 3. Confine animal for 45 days observation and then release if the animal is healthy 4. Monitor the animal and if the animal shows signs that are compatible with rabies, the animal should be humanely euthanized and tested for rabies, or if the animal dies, it should be tested for rabies
2	Unvaccinated pet dog and cats exposed to a confirmed or suspected rabid dog (bitten by rabid or suspected rabid dog)	<ol style="list-style-type: none"> 1. Wash the bite wound with soap and water and perform antiseptic dressing 2. Provide PEP on day 0, 3, 7, 14, 28 3. Confine animal for 2 months observation and then release if the animal is healthy 4. Monitor the animal and if the animal shows signs that are compatible with rabies, the animal should be humanely euthanized and tested for rabies, or if the animal dies, it should be tested for rabies
3	Pet dog and cat showing clinical signs of rabies or suspected to be rabid	<ol style="list-style-type: none"> 1. Confine the animal immediately 2. The animal may be euthanized upon the consent of the owner, brain samples collected/tested and dispose the carcass as per SOPs 3. If the owner is unwilling to euthanize, the animal should be placed in strict isolation and the brain tissue be collected at the time of death and dispose the carcass as per SOP 4. No treatment should be given to the animal
4	Stray dog/cat suspected to be rabid (showing clinical signs of rabies)	<ol style="list-style-type: none"> 1. The animal may be tranquilized and euthanized immediately, brain samples collected/tested and dispose the carcass as per SOPs 2. Follow guideline on rapid containment of rabies outbreak and SOPs

Sl. No	Scenarios of exposure	Action to be taken/Recommendations
5	Managing stray/pet dogs in rabies outbreak areas	<ol style="list-style-type: none"> 1. Vaccinate dogs against rabies 2. Engage only few people during the vaccination program. They should be given post exposure prophylaxis even if not bitten by the dogs 3. Assess the vaccination coverage by spraying colour paint on the vaccinated dogs and by conducting dog count survey after the program (see SOP) 4. All pet dog should also be vaccinated and advised the owner to confine their pets within the home premises 5. Selective elimination of stray dogs may be considered on a case by case basis based on the gravity of the situation 6. Monitor the disease situation and conduct awareness campaign
6	Apparently healthy pet dog or cat that bite a person or other animals	<ol style="list-style-type: none"> 1. The person should be immediately referred to the hospital for advice/PEP 2. In southern Bhutan or in interior places wherein pets have been recently moved from rabies endemic areas, the animal may be suspected for rabies and should be placed for strict isolation and observed for 10 days 3. If the animal is normal after 10 days of observation period, one dose of rabies vaccine should be administered before release 4. If the animal shows sign of rabies during observation period, the animal may be euthanized upon the consent of the owner, brain samples collected/tested and dispose the carcass as per SOPs 5. If the owner is unwilling to euthanize, the animal should be placed in strict isolation and the brain tissue be collected at the time of death and dispose the carcass as per SOP

Table 4: Management of domestic livestock (cattle, horse, sheep, goat, pig, etc.,)

Sl. No	Scenarios of exposure	Action to be taken
1	Animal bitten by rabies suspected or confirmed rabid dog	<ol style="list-style-type: none"> 1. The bite wound should be washed with plenty of soap and water thoroughly and then apply antiseptic agents (Tr. Iodine/ povidone iodine) - <i>see the precautionary measures for the handlers</i> 2. Do not suture the bite wound 3. Provide PEP on day 0, 3, 7, 14, 28 (only if the date of bite is known) and should NOT give PEP if the date of bite is unknown since the animal may be under incubation period of rabies and may not be effective) 4. Place animal under strict isolation for 45 days observation and then release if the animal is healthy (but monitor the animal) 5. If the animal shows sign of rabies during observation period, the animal may be euthanized upon the consent of the owner, brain samples collected/tested and dispose the carcass as per SOPs 6. If the owner is unwilling to euthanize, the animal should be placed in strict isolation and the brain tissue be collected at the time of death and dispose the carcass as per SOP 7. No medication should be given once the animal show sign compatible with rabies
2	Animal bitten by rabid animal and showing clinical signs of rabies	<ol style="list-style-type: none"> 1. If the animal shows sign of rabies, the animal may be euthanized upon the consent of the owner, brain samples collected/tested and dispose the carcass as per SOPs 2. If the owner is unwilling to euthanize, the animal should be placed in strict isolation (secure fencing) and the brain tissue be collected at the time of death and dispose the carcass as per SOP 3. Notice indicating “<i>RABIES INFECTED ANIMAL, DANGER, DO NOT GO NEAR THE ANIMAL</i>” should be displayed on the shed for avoiding the people going near the animal or the owner should be advised to keep people away from the animal 4. All the exposed people especially the one involved for the daily management of sick animal should be sent to hospital for medical advice/PEP

Sl. No	Scenarios of exposure	Action to be taken
3	If the suckling calf shows sign of rabies (after bitten by rabid dog) but the dam is apparently healthy and normal (dam is milking)	<ol style="list-style-type: none"> 1. The rabid calf should be isolated and follow the above procedure (Scenario 2) 2. The calf should not be allowed to suckle the milk (udder) of the dam 3. Isolate the dam and look for any bite wound on the teat/udder. If the calf have been salivating and suckled the milk/udder, there is some chance that the calf may have injured the udder/teat and transmitted the rabies virus to the dam (so far not documented yet). In this circumstances, wash the bite wound on the teat/udder and provide PEP on day 0, 3, 7, 14, 28. OR provide PEP even if the bite mark is not observable. 4. Isolate the dam for 45 days and then release if the animal is healthy 5. Do not milk the animal during 45 days observation period (if it is unavoidable, the animal may be milked and discard the milk (do not consume raw milk). 6. The milker/handler should receive PEP 7. Monitor the animal and if an animal shows signs that are compatible with rabies, the animal should be humanely euthanized and tested for rabies, or if the animal dies, it should be tested for rabies.
4	If the dam is confirmed rabid and calf had been suckling milk from infected dam	<ol style="list-style-type: none"> 1. The calf should be restricted from suckling and kept under observation 2. Provide PEP to the calf on day 0, 3, 7, 14, 28 3. Isolate the calf for 45 days and then release if the animal is healthy 4. Monitor the calf and if the animal shows signs that are compatible with rabies, the animal should be humanely euthanized and tested for rabies, or if the animal dies, it should be tested for rabies. 5. For the dam, follow the procedure to manage the rabid animal as above (Scenario 2)

Sl. No	Scenarios of exposure	Action to be taken
5	Management of other animals within the affected herd (<i>How to manage other animals when there is a confirmed case of rabies within the herd</i>)	<ol style="list-style-type: none"> 1. Herbivore-to-herbivore transmission is uncommon 2. The owner may have concern that the other members of the herd or flock may have been similarly exposed to rabies. In situations like this, monitoring the remaining animals in the herd or flock for 45 days beyond the day the index case became ill should be sufficient to rule out exposure to other animals. 3. However, there should not be any sale or movement of the animals during this time. 4. In case, if any of the remaining herd shows sign of rabies, it could have been due to rabid dog bite exposure and not due to animal-to-animal exposure (and follow the above procedure) (Scenario 2)
6	Animals bitten by an apparently healthy and normal dogs	<ol style="list-style-type: none"> 1. The bite wound should be washed with plenty of soap and water thoroughly and then apply antiseptic agents (Tr. Iodine/ povidone iodine)- <i>see the precautionary measures for the handlers</i> 2. Do not suture the bite wound 3. No medication and PEP be given to the animal 4. Not necessary to isolate or confine the animal, but if animal shows signs compatible with rabies, follow the above procedure (Scenario 2)

Note: The post exposure treatment in animals (e.g. pet dog and cattle/other livestock) should be assessed through seromonitoring studies. If the animals is selected for PEP administration, blood/serum samples should be collected on day 0 (prior to the administration of vaccine), day 14, 45, 180 and 365 post vaccination and submit the sample to NCAH for antibody titre estimation. In addition, animal details should also be collected at the time of sampling.

3.3.4.2 Ban on the movement of livestock and livestock products

In order to prevent further spread of the disease, the DOIT may recommend ban on the movement of live animals and their products (meat, milk, meat, butter, cheese, hides etc) out of the infected zone/herd as per the risk assessment. Where possible, animal product originated from clean herd on a case by case basis should be certified by BAFRA as fit for human consumption.

3.3.4.3 Surveillance and regular follow-up

The RRT should also carry out the clinical surveillance in the infected zone on the occurrence of any new cases as well as to monitor any mortality of the suspected animals. An update on the disease outbreak situation should be done as follow-up report on weekly basis through the existing database until the complete cessation of the disease.

3.3.4.4. Logistic support

In order to implement the successful containment of Rabies outbreak by RRT adequate logistic support should be mobilized by respective focal agencies depending on the seriousness of the disease outbreak situation (refer 3.1 Institutional arrangement).

04 Surveillance system

Surveillance is a collection, collation and analysis of data that enables the prompt dissemination of the information to take timely and appropriate action. Surveillance is needed to understand the health status of the animals in the country, so that problems can be identified and actions taken. However, different countries have different surveillance needs and surveillance capabilities. For an appropriate surveillance activity to be performed, we should have a clear objective and understanding of the need. There are large numbers of reasons why veterinary authorities undertake surveillance activities and can be summarized into four general purposes:

- Demonstrating freedom from disease
- Early detection of disease
- Measuring the level of disease
- Finding cases of disease

Surveillance is a key element of the national rabies control plan and will become even more important as Bhutan works to move along the stepwise approach towards elimination of dog mediated rabies by 2020 and globally by 2030.

4.1. Clinical surveillance

4.1.1. Syndromic surveillance

This surveillance should be conducted through monthly reporting system on the absence or presence of rabies in the country. Each livestock centres and district veterinary hospitals should collect information on rabies status in animals from their respective areas (village/chiwog). The reporting should be done from Gewog to Dzongkhag office. The Dzongkhag Livestock Sector (DVH) should then enter the data into existing VIS database. The NCAH shall maintain national data, perform analysis and provide feedbacks to all the stake holders in the country. The analysed report shall support validating WHO/OIE/FAO Stepwise approach towards elimination of rabies in Bhutan and determine stages of rabies eradication. In the border areas, the concerned DVH, SVL, CVH and Livestock Extension centres should also conduct regular surveillance for early detection of cases especially monitor the movement of rabies suspected dogs along the highways into interior Bhutan.

4.1.2. Surveillance during outbreak

Once the rabies outbreak is confirmed by the disease outbreak investigation team, continuous surveillance should be carried out by RRT. The update on the disease status shall be submitted on weekly basis to RLDC and NCAH using Form 6B (appendix) and/or online database system and through email/telephone communication.

4.2. Laboratory surveillance

4.2.1. Sero surveillance

A structure based survey shall be conducted regularly to determine sero-conversion post mass dog vaccination and community animal birth control (CABC) and CNVR programme using ELISA test (see SOP) or other antibody test protocol. Further, seroconversion studies shall also be conducted in animals that has been treated with anti-rabies vaccination following rabid dog bites (see foot note of Table 4). The study findings will support policy decision on rabies control program.

4.2.2. Virological surveillance

During the outbreak period, brain tissue samples should be collected and tested using rapid antigen detection test (see SOP). Samples tested negative shall be subjected to fluorescence antibody test (FAT) (see SOP) as confirmatory diagnosis. Samples tested positive to FAT shall be archived for molecular analysis and research purpose.

The brain tissue samples from carcasses (especially dogs and cats) shall be collected from any part of the country and subjected to rapid antigen detection test and FAT to find a case or demonstrate freedom from rabies. The laboratory technicians based in the DVH, CVH/SVLs and RLDC should coordinate sample collection, testing or referral to NCAH. The brain tissue from animals died due to vehicle accident (especially dog and cats or wild animals) should also be collected and tested against rabies virus. This would enhance the surveillance system and provide guidance for policy decision on provision of anti-rabies vaccine (PEP) to dog bite victim in interior rabies-free Bhutan.

4.3. Wildlife surveillance

Surveillance shall be conducted in collaboration with Wildlife Conservation Division under Department of Forests and Park Services. Blood samples shall be collected from immobilized felid and canid species operated under rescue and rehabilitation programme. Serum samples shall be subjected to antibody test using ELISA. Additionally, carcasses of wild animals not limiting to canid and felid species shall be collected to obtain brain tissue sample that will be tested for the presence of rabies virus using antigen detection tests. The findings will support to detect a case or demonstrate freedom of rabies in wildlife species.





Sample (brain tissue) collection from rabies suspected cases



05 Other support plan

5.1. Awareness education

Public awareness education are needed to ensure that the general public and the livestock industry are made aware of rabies control document and its potential benefit and the activities of the programmes being implemented.

Following are the awareness programmes to be implemented during prevention phase:

- Awareness on rabies and its economic impact
- Role of civil society organizations in animal welfare and disease control
- Implications of dog bites and post exposure prophylaxis (PEP) for exposed animals and humans
- Importance of vaccination
- Importance of dog registration and ownership, CABC and CNVR of free-roaming dogs
- Dog habitat control and dog population management
- School children education on dog bites and rabies prevention

Following are the awareness programmes to be implemented during outbreak phase:

- Awareness on regulation of movement of livestock, livestock products and consumption of infected product
- Awareness on importance of timely reporting of disease outbreak
- Awareness on safe disposal of animals that died of rabies
- Clinical diagnosis of disease in different animal species
- Biosafety, sampling and transport of samples for laboratory diagnosis
- Awareness on the protective measures to be undertaken to avoid contact with the rabid animals
- Awareness on avoiding dog bites and importance of bite wound washing, handling suspect rabid or exposed animals and use of rabies PEP

5.2. Border harmonization

Coordination meetings between Bhutanese livestock officials and the officials from the state veterinary departments of the adjoining Indian states (Assam, West Bengal, and Arunachal Pradesh) are needed to develop common platform in controlling disease along the border areas.

The Ministry of Agriculture and Forests should interact with the relevant veterinary authority, Government of India for bringing about understanding between the two countries (political sanction) and actively participate in disease control programme. The Department of Livestock shall coordinate border harmonization meeting with support from relevant agencies. Vaccination programme on either sides of the border shall be synchronized to achieve maximum possible vaccination coverage.

5.3. Research and development

Research on specific areas such as cost involved in disease control programme, economic losses and sero-monitoring will benefit in providing directives for formulating effective policies. Molecular characterisation of circulating virus will provide information on how different is the virus detected in Bhutan that from other places. The role of wildlife in the epidemiology of rabies in Bhutan needs to be clarified. Research at advanced level shall be collaborated with institutions outside the country.

Data from clinical surveillance and from areas that had not reported rabies for a considerable period of time should be analysed to categorise the area provisionally free of rabies. This will aid in validating the status of progress towards eradication of rabies in South Asia and the world.

5.4. Capacity building

Capacity building on rabies prevention and control programme is essential to implement the national rabies prevention and control plan.

5.5. Rabies coordination workshop

It is necessary to conduct annual national rabies coordination workshop where all the stakeholders come together to review and revise the program.

06 Programme financing

Rabies control programme will require development of annual business/operational plan which includes clear specification of physical requirements and the estimated costs of implementation. The required budget should be proposed to the Ministry of Finance each year by AHD, NCAH, RLDCs and Dzongkhags based on the roles and responsibilities required to be executed by respective agencies. It will also need clear specification of the output, key performance indicator, responsibility and timeline. Additionally, the programme financing component may also be borne by Ministry of Health, respective municipalities, WHO-FAO-OIE, and non-governmental agencies such as Humane Society International and other Animal Welfare Organizations.



Capturing of stray dog using net and paint marking after vaccination



Rapid antigen detection test indicating positive to rabies virus



Rabid dog on the move



Sampling brain tissue using swab

07 Monitoring and evaluation

At the national level NCAH should monitor and evaluate the progress of the rabies prevention and control programme using the data submitted by Dzongkhags. RLDCs should monitor and evaluate the progress in their respective regions at the regional level. The disease control plan shall be amended periodically based on the M & E findings.

The NCAH shall present the progress and situation of rabies in the country to the Rabies Task Force. An inter-sectorial task force should also evaluate the progress of the control program and assess in which Stages of the Stepwise approach of the rabies control program have Bhutan achieved. Based on these, a revised strategy may have to be implemented to achieve Bhutan goal of rabies elimination by 2020. The methods and criteria for evaluating the control program will be developed and decided by the Task force members.

Annexure 1: Rapid Response Team (RRT) during Rabies Disease Outbreak

Background/ Rationale

Rabies is a highly contagious and fatal disease that can spread rapidly through movement of infected dogs. Therefore, a Rapid Response Team (RRT) to respond against outbreak should be formed with the following objectives:

- To conduct thorough investigation of disease outbreaks to identify the source(s) of outbreak.
- To rapidly contain the outbreak without allowing it to spread to other places, which includes certain actions to be taken by the team even before the disease/agent is confirmed
- To conduct risk communication on the disease and its control measures to educate the general public and field staff.
- To coordinate with the different stakeholders in responding to the disease outbreak within a shortest possible time to reduce the cost of outbreak

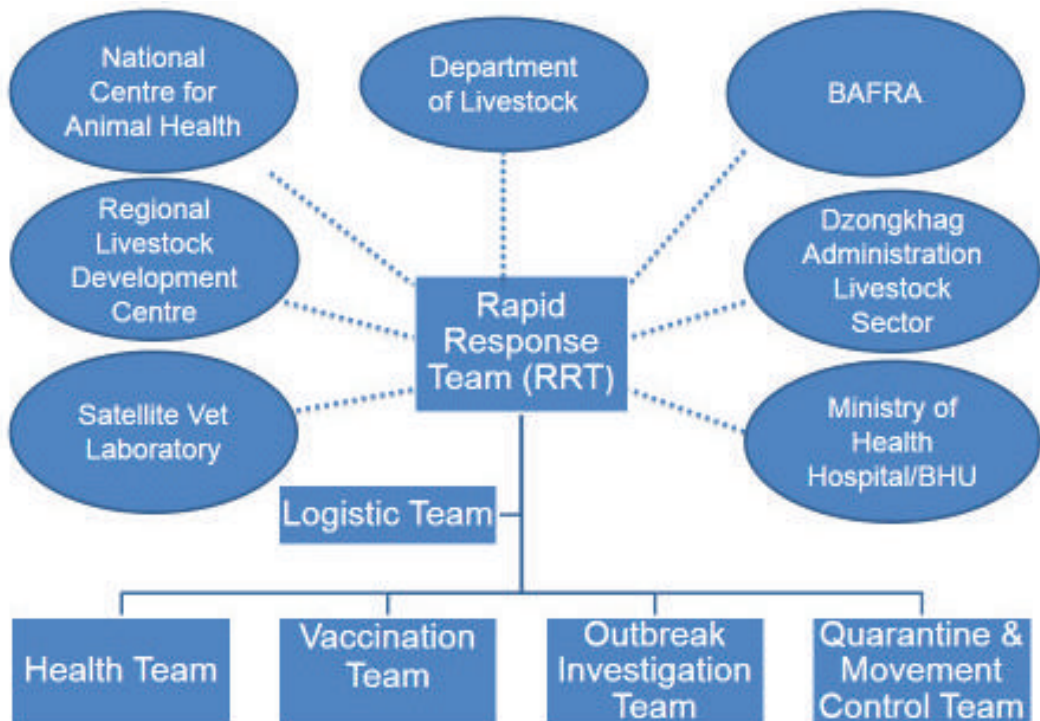


Figure 8: Rapid Response Team for Rabies outbreak containment

Disease Outbreak Investigation and Surveillance Team (DOIST)

The DOIST team should comprise of the following:

- Veterinary Officer from DVH/ CVH-Team Leader
- Veterinary Officer from RLDC/SVL
- Laboratory Technician from DVH/RLDC
- Livestock In-charge of the concerned Gewog
- Local Government representative (Magmi/Tsokpa)
- Public Health official
- BAFRA

The team will be reinforced with Veterinary Epidemiologist or Disease Expert from NCAH and also Rabies Expert from Human Health sector based on the epidemiology and severity of outbreak. Once these additional team is in the field, Veterinary Epidemiologist/Disease Expert from NCAH shall coordinate and lead the investigation team.

Roles of Disease Outbreak Investigation and Surveillance Team

- As soon as suspected case(s) is reported, conduct a thorough investigation and find out the source of outbreak.
- Collect samples from the carcasses, conduct rapid test and refer samples to NCAH.
- They should be responsible for the identification and establishment of infected premises and declaration of Infected Zone and vaccination zones
- Recommend Dzongkhag Administration to issue outbreak declaration order.
- Isolation and management /treatment of affected animals (refer Tables 3 and 4).
- Disinfection of infected premises using appropriate disinfectants.
- Conduct surveillance in infected and vaccination zones.
- Conduct case-tracing and eliminate suspected/confirmed rabid cases (refer SOP)
- Constantly monitor the outbreak situation in the affected and at-risk areas.
- Daily recording of the disease outbreak status and update the outbreak status to Dzongkhag/RLDC/NCAH/DoL/BAFRA/MoH
- Create public awareness education

Mass Dog Vaccination Team (MDVT)

Team members

- Dzongkhag Livestock Officer or Asst. DLO -Team Leader
- Pooled staffs from RLDC
- Pooled staff from DVH
- Pooled staff from Geog Livestock Centre
- Local Government representative (Magmi/Tsokpa)

Roles of mass dog vaccination team

The number of vaccination team shall be determined by the nature of outbreak, geographical setting and risk of disease spread to other areas.

- Carry out emergency an emergency vaccination in the designated areas (vaccination zone) based on the risk assessment and recommendation of DOIST (refer SOP).
- Maintain proper record of the vaccination.
- Assess the vaccination coverage through field and household surveys for the stray and owned dogs respectively
- Create public awareness at the time of vaccination
- Conduct surveillance and inform to the disease investigation team if any suspected cases is observed.

Quarantine and Movement Control Team

The Quarantine and movement control team (QMCT) will be composed of following members:

- Livestock Regulatory and Quarantine Officer, BAFRA
- Police personals (optional and only if the situation demand involvement of police personal)

Roles of QMCT

- The Quarantine and Movement Control Team shall be responsible for enforcement of movement ban of livestock and livestock products out of the infected zone. Detailed procedure on enforcement of quarantine and movement control measures shall be done as per the Livestock Rules and Regulations of Bhutan 2008 by BAFRA.
- The team shall also report any suspected cases to the DOIST
- Create public awareness on importance of movement ban

Health Team

Team members

- Dzongkhag Health Officer/Asst. Dzongkhag Health Officer-Team leader
- Medical officer
- Health assistant of the respective BHU

Roles of Health Team

- Create public awareness along with Livestock officials (DOIST) through One Health approach
- The team shall find out the human exposure cases and assess the risk
- Refer bite cases to BHU/Hospitals for PEP and HRIGs.
- Provide Pre or post exposure treatment to RRT

Note: The team composition shall vary depending on the magnitude of the outbreak which will be decided by overall coordinator of the RRT

Modalities/ Modus Operandi

- Following the report of disease outbreak in the field, RLDC should decide on the activation of the RRT based on the disease situation.
- RRT should be activated in the field within 24 hours based on the advice of the Disease outbreak investigation team.
- RRT should seek the support of NCAH, Department of Livestock and MoHas and when required.
- Once the ring vaccination is completed the RRT Team Leader should decrease the number of members involved in Rabies control activities as the main activities of the remaining team will be on the surveillance in the vaccination zone and other nearby areas.

Logistics

Manpower:

- Mobilize additional staffs from nearby centres, RLDCs, NCAH, Department and other Dzongkhags.

Vehicle

- Mobilized from Dzongkhags, RLDCs, NCAH, projects and other central programs if required

Diagnostics

- RLDC/ NCAH should facilitate rapid diagnostic kits and other sampling equipment.

Communication equipment

- Recharge vouchers should be provided to the team members

Vaccines

- NCAH shall arrange the required quantity of vaccines during the emergency in consultation with RRTs

Fund

- The fund required for the purchase of vaccines and consumables should be made available by NCAH.
- The fund for payment of DSA to team members should be met from the respective unit/Dzongkhag/RLDC/ NCAH.
- Expenses for the working lunch/refreshment during the diseases containment program should be arranged by RLDC and Dzongkhag. If there are no fund provision or insufficient fund, NCAH and Department should provide required fund support to RRT.

Annexure 2: Standard Operating Procedures

2.1. Standard Operating Procedure for Disease outbreak investigation

A disease outbreak is defined as the occurrence of one or more cases of Rabies in a herd or village in a town in time and space. An outbreak can be considered as separate outbreak if the case(s) occur in a herd, village or town separated from other herd, village or town by physical barriers and or occurs after one month apart in the same village.

An outbreak investigation is a systematic procedure to help identify causes and sources of epidemic with a view to control an existing epidemic and prevention of possible future ones.

Purpose

- To identify the causes and sources of disease outbreak
- To identify measures to prevent further spread of disease
- To control and contain the existing disease outbreak

Scope

- This SOP outline the general principles and steps for investigation of Rabies disease in the field

Users or targets

- Veterinary Officers and para-veterinarians
- Rapid Response Team

Team composition

- Veterinary Epidemiologist/Dzongkhag Veterinary Officer-Team Leader
- Veterinary Officer from RLDC/SVL
- Laboratory Technician from DVH/RLDC
- Livestock In-charge of the concerned Gewog
- Local Government representative (Magmi/Tsokpa)
- Public Health official
- BAFRA

Materials and equipment

Disposable gloves	-	20 pairs
Gumboot	-	5 pairs
Apron	-	5 nos
Shoe covers	-	10 pairs
Scissors	-	2 nos
Forceps	-	2 nos
50% PBS glycerine saline	-	5 nos
Cotton	-	1 roll
Marker pen	-	2 nos
Cool box	-	2 nos
Bio-hazard bags	-	10 nos
Disinfectant (Virkon-S/bleaching powder)	-	1 kg
Antiseptic hand wash	-	1 package
Ice pack	-	As required
GPS	-	1 no
Disease outbreak investigation form	-	5 nos
Laboratory sample submission form	-	5 nos
Written Instructions/SOP Print-outs	-	3 nos
Notebook and pens	-	2 nos

Steps for Investigation

Pre-investigation preparation

- Formation of investigation team and planning the response among team members
- Discuss each person's roles and responsibilities
- Arrangement of materials and logistics (refer materials and equipment requirement)
- Epidemiological materials: Investigation form, note book, laptop, GPS
- Laboratory: swabs, needles, cool box, sampling kits and rapid rabies antigen detection kit
- Educational: SOPs, guidelines
- Decontamination
- Mobility - vehicle
- Extension gears

Gather preliminary information: Following information needs to be collected by the team prior to their departure

- Farmers' name and phone number
- Name of village, Gewog, Dzongkhag
- Species of animal affected
- Date and time of report of outbreak from farmer to LEC/ DVH
- Date and time of report from LEC/ DVH to RLDC/NCAH
- Date and time of visit by veterinarian or field staff
- Name of contact field staff, address and phone number
- Provide information about the team visit to outbreak area
- Date and time of visit

Field investigation

Background information to collect

- General information regarding introduction of any new dogs and cats
- General information regarding buying and selling of any livestock and livestock product
- General information about the affected village/ farm (no. of households;
- General information about any recent local festival or gathering in the village/ locality
- Collect XY coordinates (using GPS), altitude, road network, Government offices, frequency of movement of people in and out of the outbreak area

Baseline morbidity, mortality and clinical signs

- Determine baseline mortality for period (week or month) before the outbreak and in previous year, both generally, and more specifically for the same seasonal time period as the present outbreak in the previous year;
- General information of the present disease outbreak such as number of households affected, population at risk, livestock population in the surrounding villages etc.
- Record of the daily morbidity and mortality figures
- Record of the detail clinical signs.

Wild animals

- Determine the presence of any wild dogs and cats or jackals and foxes in the area
- Determine whether there are any suspected Rabies cases in the vicinity
- Determine whether the animals have been bitten by wild animals or not

Vaccination history

- Record vaccination programs and verify whether the dogs/cats in the affected herd/ villages are vaccinated against Rabies and other diseases
- Determine the vaccination coverage of dogs in the area

Laboratory investigation

Laboratory investigation in the field (refer specific SOP for sampling, packaging and transportation to the laboratory and rapid field test)

- Put on proper PPE (apron, gloves, gumboots, goggles and shoe cover)
- Carry out physical examination of the sick animals and check whether the animal has any bite wound/lesions on the body or body parts
- Collect brain tissues from the dead animals in 50% Glycerine saline and transport to the laboratory (refer SOP)
- Carry out rapid diagnostic test for Rabies in the field (refer SOP)

Laboratory Diagnosis (refer specific SOPs for laboratory diagnosis)

Following laboratory tests will be done for further confirmation

- Rapid antigen detection test
- FAT
- RT-PCR

Characterize the outbreak

- Establish or verify the outbreak
- Provisional diagnosis made on clinical signs, epidemiological pattern.
- Provisional disease control measures should be in place before the confirmatory diagnosis is made

Establish the case definition for Rabies

Suspect case:

Dogs and other canines:

Furious form of rabies

Animal demonstrating strange behaviour and become very excitable progressing to aggression, disorientation, impaired mobility, unusual vocalizations, excessive salivation, biting inanimate objects and people, muscular incoordination and convulsions, coma and death.

Paralytic or dumb form of rabies

Animal demonstrating strange behaviour characterized by the inability to swallow, hanging of jaw due to paralysis of the jaw muscles and the dog is unable to close the mouth leading to a typical sign of foaming saliva around the mouth followed by ascending paralysis which begins at the hind extremities and eventually complete paralysis, coma and death.

Cattle

Animal demonstrating excessive salivation with foamy froth, behavioural changes, muzzle tremors, vocalization (frequent bellowing) with low-pitched voice due to paralysis of vocal cord (may mistake for heat sign), hyperesthesia and/or hyperexcitability and aggression (butt other object/people if released), and pharyngeal paresis/paralysis, coma and death.

Sheep and goat

Animal demonstrating muzzle and/or head tremors, aggressiveness, hyperexcitability, and/or hyperaesthesia, trismus, excessive salivation, dropping ears, vocalization, and recumbency and death.

Horse

Animal demonstrating muzzle tremors, aggression, pharyngeal spasm or pharyngeal paresis, ataxia or paresis, lethargy or somnolence, coma and death.

Confirmed case

The above clinical signs plus positive detection of rabies virus in brain tissues by rapid rabies antigen test in the field or by fluorescent antibody test (FAT).

Differential diagnosis

- Toxoplasmosis
- CNS infection
- Feline infectious peritonitis
- Canine distemper
- Neoplasia
- Trauma
- Hepatic encephalopathy
- Thiamine deficiency (cats)
- Oral and pharyngeal foreign bodies
- Poisoning with, for example, lead, organochloride compounds, benzoic acid strychnine
- Spongiform encephalopathy (cats)
- Several weakness seen in moribund animals from various generalized infections

Describe outbreak in terms of time, animal and place

Time (draw epidemic curve by plotting cases against the time from available data-preferably time series)

- When was the index case?
- What is the exact period of outbreak?
- Given the diagnosis what is probable period of exposure?
- Is the outbreak most likely to be point source or propagated or both?

Animal (attack rates, risks etc.)

Estimate the attack rate

Place (plot the location of outbreak on a map with physical characteristics such as road, water bodies, mountains, infrastructures etc.)

- What are the geographical distributions of the cases?
- What is the pattern of the cases among different livestock species in different management system?
- Whether case farm is close to the international borders, national highways, migratory routes or other spatial risk factors?

Develop hypothesis based on the pattern of disease (animal, time and place).

- Source of disease outbreak-forward and backward contact tracing
- Mode of transmission.
- Whether the outbreak is a common source or propagating
- If a common source, whether it is point or multiple exposure
- What are the risk factors associated with problem?

Control and Prevention (Refer specific SOPs for RRT and management of Rabies affected or exposed animals (SOP)

- Provisional control measures should be in place before the outbreak is officially declared.

Declaration of Provisional Infected Zone

When Rabies is suspected, Gewog Livestock Staff should inform DVH, DLO, RLDC, NCAH, DOL and BAFRA and immediately quarantine the suspect infected place (farm or a village) and the surrounding area based on risk assessment as a Provisional infected zone. The Geographical limits of the provisional infected zone should be determined after due consideration of the epidemiologic risk and natural geographical settings. The Provisional Infected Zone should be declared by the Gewog Livestock Office and Gewog Administration.

All places with susceptible livestock species within the Provisional Infected Zone shall be considered at-risk/suspect and should be visited to establish their infection status.

Quarantine and movement control of susceptible livestock species and their products should be imposed (refer SOP for quarantine and movement control).

Strict surveillance and movement control should be maintained on all other areas within the infected Zone.

Declaration of Infected zone

If the case definition of Rabies is met, the area as decided by risk assessment team/ Disease Outbreak Investigation Team should be immediately declared as protected zone. The geographical limits of the protected zone should be determined after due consideration of the epidemiologic risk and natural geographical settings.

Once the outbreak is confirmed by the Disease Outbreak Investigation Team (DOIT) and officially declared by the Dzongkhag Administration, RRTs should be immediately activated. Quarantine and movement control on susceptible livestock species and their products be imposed (refer SOP for quarantine and movement control).

Declaration of Vaccination Zone

In order to create buffer zone around the infected zone vaccination zone should be declared by the DOIT. The geographical limits of the vaccination zone should be determined after due consideration of the epidemiologic risk and natural geographical settings. RRT should carry out ring vaccination and other containment activities on all the properties/villages within vaccination zone based on the direction of disease outbreak investigation team (refer specific SOPs). Public awareness and strict surveillance should be undertaken within the vaccination zone.

Reporting

- Document the findings (background; investigation procedures, epidemiological and laboratory findings; economic impact etc)
- Provide recommendations to all the relevant stakeholders (farmers/producers; Managers; DoL, BAFRA and other agencies)
- Submit the final report detailing the source of outbreak and recommendation

Surveillance and monitoring (will be done by the disease investigation team)

- Is the frequency of the disease remaining constant; increasing or decreasing?
- Is the control program effective?
- Does the disease have any impact on productivity or profitability

2.2. Standard Operating Procedure for mass dog rabies vaccination campaign

Purpose

To organize mass dog vaccination campaign so as to cover more than 70% of the dog population within short duration.

Scope

This SOP outlines the general methods and steps to carry out mass dog vaccination against rabies

Responsibility

Dzongkhags/VH/DVH

User

Veterinarians, Paravets and Extension agents of RLDCs, DVH/ NCAH/VH

Manpower

Veterinarians
Para-veterinarian
Dog catchers /Animal Welfare Officers

Materials/equipment required

- Dog Registration card
- Anti-rabies vaccine
- Needle and syringe (18 gauze, 10 ml)
- Hand gloves
- Cool box with ice pack
- Marker pen (permanent)
- Cotton/tissue paper
- Sample label
- Ethanol 70%

Procedure

- Vaccination team who handle dogs should receive pre-exposure prophylaxis before the campaign.
- The vaccination campaign should be planned properly to increase awareness at the national and community level to ensure commitment of policymakers (financial support) and community for their participation in dog rabies control operations
- The dog population and dog-keeping practices should be estimated prior to the campaign in order to calculate the resources required and the appropriate methods for accessing dogs for vaccination
- Detailed vaccination schedule of the place to be visited by the teams should be prepared in advance and distributed to all the concerned Geog in-charges who in turn informed the public so that they can bring in their pets and community dogs to the designated areas. The designated areas should be identified at the central area and convenient for the dog owners to bring their dogs for vaccination
- The vaccination teams will be divided into groups and will be briefed on the schedule, location and route they would be taking.
- Local official will accompany them so that no areas were left un-covered.
- Required number doses of anti-rabies vaccine will be provided by NCAH based on the requisition by the concerned Dzongkhags.
- Accessible rural communities: along with continual vaccination at fixed vaccination posts in well-recognized sites within the community (e.g., government veterinary clinics).
- In dispersed communities: Central-point vaccinations, consisting of mobile teams that set up temporary vaccination posts in central village locations. Dog handlers could be used to catch and restrain dogs humanely (The trained dog-catchers with nets).
- In very remote communities combined approaches using central point and house-to-house vaccination conducted by either mobile teams of permanent staff or trained community animal health worker.
- Registration and permanent identification of all vaccinated dogs should be done with issuance of a card for pet animals and with owners.
- The use of color spray of all vaccinated dogs as temporary marking should be done. A survey should be undertaken within 3 days of the campaign to assess the numbers of marked and unmarked dogs.
- Supply of adequate vaccine
- Maintenance of strict cold chain within an acceptable temperature range

2.3. Standard Operating Procedure for the management of rabies suspected/infected and exposed animals

Purpose

The purpose of this SOP is to effectively manage the Rabies suspected/infected and exposed animals for the prevention and control of the disease within a short duration

Scope

The management of Rabies infected and exposed animals is described

Users

Veterinarians and Para-veterinarians

Manpower

Animal Health Supervisor
Veterinarian

Materials/Equipment

- Potassium permanganate powder
- Virkon powder/ Savlon
- Cotton
- Scissors
- Gumboots
- Disposable hand gloves
- Apron
- Goggles
- Disposal and post-mortem gloves
- Biohazard bags
- Tr. Iodine

Procedure

- Persons handling exposed animals, carcasses, or tissues should be vaccinated prior to handling (pre-exposure rabies vaccination)
- Staff entering the affected premises shall put personal protective equipment like the gumboot/ shoe cover, apron, goggles, hand gloves and take biohazard bags
- Select a clean area for the display of the treatment/management items and accordingly ask the owner to tether the sick and or exposed animals in the isolated and confined area (the animal handler/owner should also wear PPE and receive PEP)
- Person feeding and watering the infected or exposed animals should use minimum required PPE (Personal Protective Equipment) while handling the case

Management of rabid animals (animal showing clinical signs of rabies)

The animal shall be euthanatized immediately but if the owner is unwilling, strict confinement procedures should be followed as follows:

- A temporary confinement shed with minimum facilities with fencing (woods/ bamboo/barbed wires) should be constructed for confinement of the rabid infected animal
- The animal should be properly tethered till the animal dies
- The animal should be provided with water and feed as per the standard feeding schedule
- Notice indicating “**RABIES INFECTED ANIMAL, DANGER, DO NOT GO NEAR THE ANIMAL**” should be displayed on the shed for avoiding the people going near the animal
- Any items and utensils used for feeding, watering and treating the animals should be properly washed with soap and water or disinfected using the standard disinfectants before reuse
- Once the animal dies, the brain tissues should be collected and carcase disposed through deep burial along with infected materials as per the SOP.
- All the exposed people especially the one involved for the daily management of sick animals should be provided with Pre or Post Exposure Prophylaxis (PEP) as per the guidelines (they should be advised to visit the hospital for PEP).
- The premises where the sick animals were kept should be disinfected using Cetrimide & Chlorhexidine (savlon) or potassium permanganate solution or the infected materials be burnt or buried

For details, refer the scenario-based management of animals 3.3. Control strategies and sub-section 3.3.4.1 Management of rabies suspected/confirmed and exposed animal (Tables 3 and 4)

2.4. Standard Operating Procedure for disposal of Rabies carcasses by deep burial

Purpose

The purpose of the SOP is to have standard procedure for safe disposal of Rabies infected carcasses and infected materials.

Scope

This SOP describes procedures for site selection and burial of Rabies carcasses in a safe manner to avoid transmission of virus to other animals and humans

Users

Veterinary Officer/Para veterinarians

Manpower

- Veterinarians
- Para-veterinarians
- Village Tshogpa
- Animal owners

Materials/Equipment required

- Hand gloves
- Face masks
- Gum boot
- Goggles
- Post-mortem gloves
- Disinfectant -Lime/Virkon-S/ bleaching powder
- Digging tools: spades, crowbars, peak-axe, ropes and others.

Procedure

- Select an appropriate site for carcass burial. The site should be away from the water source, residential areas, livestock facilities and pastures. Preferably it should be away from any footpaths or roads leading to the site.
- Prepare a pit with sufficient width to accommodate the carcass with a minimum dimension of 2 meter deep considering the size of the carcasses.
- Wear appropriate PPE (apron, face masks, goggles, gumboots, apron, overall) and hand gloves (double gloves) before handling the carcasses.
- Drop the carcasses into the pit and dispose the hand gloves, face mask, apron into the pit
- Cover the carcasses with soil, 400 mm is suggested, and add an unbroken layer of lime (calcium carbonate). Do not spray lime directly on to the carcasses as it will slow decomposition process.
- Close the pit with sufficient soil and make a heap over the site.
- Then put a layer of lime over the soil
- Disposal site should be secured by putting stones, thorns, and logs or fenced temporarily with locally available fencing materials.
- All tools, utensils, equipment used for burial should be thoroughly cleaned and disinfected with disinfectant solution using the above disinfectant.
- The animal handlers should thoroughly clean and disinfect themselves before leaving the burial site.

2.5. Standard Operating Procedure for disinfection and decontamination of contaminated premises and materials

Purpose

To have standard procedure for effective disinfection and decontamination of contaminated premises and materials

Scope

The document describes procedures for disinfection and decontamination of contaminated materials and premises.

Users

- Veterinarians/Para-veterinarians

Manpower

- Veterinarians
- Para-veterinarians
- Animal owners/ Helpers

Materials/ Equipment required

- Hand gloves
- Apron (disposable)
- Gum boots
- Buckets
- Mugs/jugs
- Water

Disinfectants (any one of the following can be used as disinfectant)

- Bleaching powder (calcium hypochlorite) as 1%
- Lime powder (Calcium hydroxide) 1%
- Virkon-S (1-2%)
- Gluteraldehyde (Korhsolin) 2%
- Formaldehyde

Antiseptics (any one of the following can be used as antiseptic)

- Cetrimide and Chlorhexidine as 1-3% solution (Savlon)
- Dettol liquid/soap

Procedure

- Prepare 2% Virkon solution or 1% solution of bleaching powder or any other disinfectants specific to Rabies in a bucket.
- Collect the bedding materials and bury them with carcasses if it is in small quantities; or collect and burn it in a pit if in larger quantities.
- Contaminated premises should be disinfected thoroughly with 2% Virkon-S. Allow contact time of 2-3 hrs.
- Disposable items, including used PPEs must be buried in a pit.
- While leaving the infected premises the personals and vehicles should be thoroughly disinfected.
- All tools, utensils, equipment used for burial should be thoroughly cleaned and disinfected with disinfectant solution using 2% Virkon-S.
- Once the disinfestations of the premises is over, use the appropriate antiseptics like 1-3% cetrimide and Chlorhexidine solution or Dettol or the soap and water for proper sterilization of the persons involve during the disinfection of the premises.

2.6. Standard Operating Procedure for quarantine and movement control during rabies outbreak

Purpose

To have standard procedure for effective quarantine and movement control during rabies outbreak

Scope

The document describes procedures for quarantine and movement control to contain the disease

Users

Regulatory Officers (BAFRA officials)
Veterinarians/ Para-veterinarians

Manpower

Regulatory Officers (BAFRA officials)
Royal Bhutan Police (if required)
Veterinarians/ Para-veterinarians (if required)

Materials/Equipment required

Barrier tape
Sign board
Basic PPEs (mask, gloves, eye protections)

Procedure

- Quarantine and Movement Control Team shall implement regulations of movement of animals and animal products out of rabies infected areas;
- Ban and movement restriction of all high risk animals from the outbreak areas;
- Ban on slaughter and sale of affected animals of food origin;
- Suspected animal should be confined and observed for clinical signs;
- Should seek local government support for enforcement;
- Regularly update implementation of regulations to concerned higher authorities;

2.7. Standard Operating Procedure for euthanasia of dogs and other animals (rabid/rabies suspected cases)

Purpose

To have standard procedure for effective euthanasia of rabid/rabies suspected animals to prevent spread of the disease

Scope

The document describes procedures for performing euthanasia of animals

Users

Veterinarians/ Para-veterinarians

Manpower

Veterinarians/ Para-veterinarians

Materials/Equipment required

Thiopentone sodium injection
Syringe and needle
Blade/scissor
Basic PPEs (mask, gloves, eye protections)

Procedure

- In case of owned animals, get the written consent from the owners
- Put on the PPE set
- Restrain the animal properly;
- Locate the jugular vein (in case of large animal/small ruminant) and inject the inject Thiopentone sodium until sufficient
- In case of dog, the solution can be injected via vein or directly into the heart
- Monitor the heart beat and declare the health status
- Follow SOP for disposal of the carcasses
- Care should be taken to avoid accidental injection of the solution into your vein or other person assisting the procedure

2.8. Standard Operating Procedure for collecting brain tissue sample for diagnosis of rabies

Purpose

To have standard procedure for collecting appropriate samples from host for laboratory diagnosis

Scope

This standard Operating Procedure (SOP) describes the steps and precautions required while collecting samples that includes appropriate time of collection and type of samples to be collected for each type of indicated test.

User

Veterinarians/ Laboratory Officer/ Laboratory Technicians /Para veterinarians

Manpower

Veterinarians
Para-veterinarians
Laboratory technician
Animal attendants
Animal owners

Materials/equipment required

1. Sample submission form
2. Phosphate buffered saline (PBS) tablet or pre-prepared saline
3. Tissue bottle
4. Gum shoes
5. Eye protection
6. Mask
7. Disinfectant*
8. Apron
9. Hand gloves (double gloving)
10. Cool box
11. Marker pen (permanent)
12. Cotton/tissue paper

13. Sample label
14. Self-sealing plastic bags/snap lock bag
15. Scissor
16. Blades
17. Trepine set
18. Hand saw (hack saw)
19. Packing tape
20. Ethanol 70%
21. 50% PBS glycerine (PBSG) (7.4 pH)
22. Waste disposal bag/bio-hazard bag
23. Sharp container

* Calcium carbonate, Virkon-S, aldehyde compound, bleaching powder and iodophore compound

Brain tissue sample collection procedure

1. Put on coverall, gumboots, apron, face mask, eye protection (google) and hand gloves (double gloving);
2. Spray the carcass with disinfectant solution especially all around the head and also surrounding the carcass;
3. Flap out the skin from the head covering the skull using blades and scalpel;
4. When the skull is exposed, mark the area of skull with imaginary line and use hacksaw blades to cut and separate the mark area of skull;
5. Once the brain is exposed, scoped out the brain tissue, dissect and collect cerebellum (cattle)-Figure 10 and hippocampus (dog and cat) as shown in the figure below
6. Transfer brain sample to tissue bottle with 50% PBSG and cap properly and label the botte with permanent marker pen (this is for referring the sample to laboratory) and cover the bottle with adhesive tape to prevent leakage;
7. Complete sample submission form with all the details
8. Label bottle reference number corresponding to sample submission form
9. Pack properly in the plastic bags and keep in cool box
10. Rapid antigen detection test can be done at the site (refer SOP)

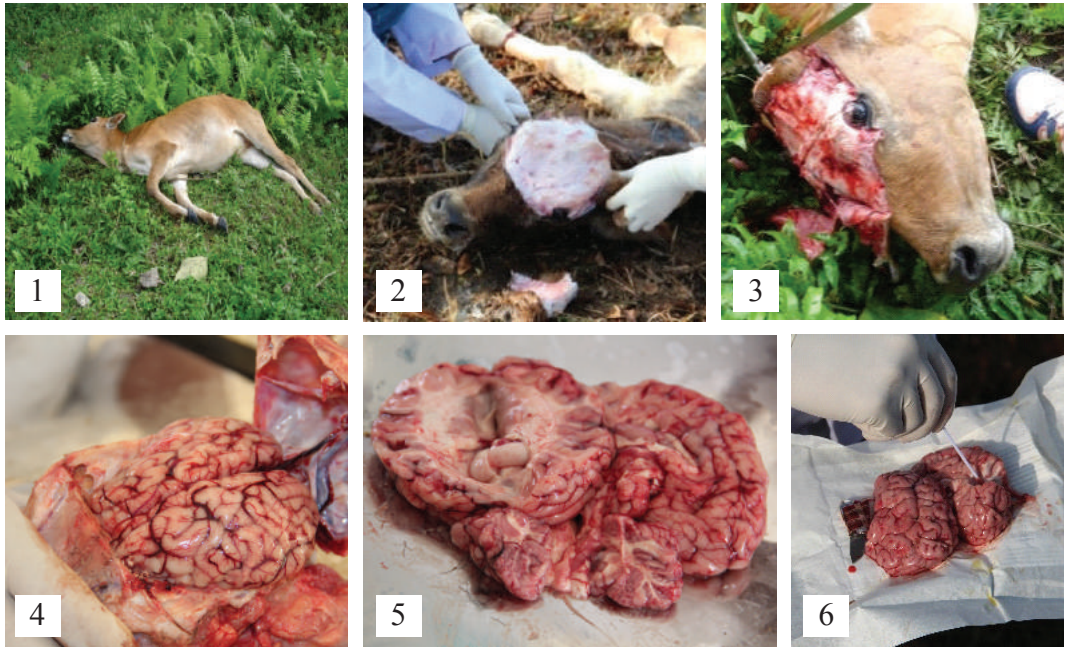


Figure 9: Procedure to collect brain tissue sample from cattle (photo source: Dr. Tenzin)



Figure 10: Procedure to collect brain tissue sample from dog (photo source: Dr. Tenzin)

Waste disposal

1. Used PPEs should be collected at one site and burnt;
2. Wherever possible such waste should be sprayed with disinfectant, double bagged in a waste disposable bag, sealed and carried back to office or laboratory and disposed after being autoclaved (if the sampling site is near to laboratory);
3. Sharps such as blades should be collected into a sharp container, sprayed with disinfectant and destroyed appropriately;

Disinfection and decontamination

1. Instrument, apron and gum shoes should be sprayed with disinfectant;
2. Apron and gum shoes should be changed while leaving sampling site;
3. Sampling site should be surface sprayed with disinfectant;
Refer SOP for carcass burial and decontamination

Hand washing and sanitization

Although rabies virus is fragile and do not survive long in the environment it is important to follow personnel disinfection procedure to ensure that the virus do not access individuals handling brain tissues. After doffing all the PPEs the individual handling carcass or brain tissue should sanitize their exposed skin particularly the hands. Hands may be first washed with water and carbolic soap. Product such as Dettol may be used for thorough hand washing. Thereafter, hand sanitizing agents containing alcohol may be used.

2.9. Standard Operating Procedure for collecting blood sample for detection of antibodies in vaccinated animals

Purpose

To have standard procedure for collecting appropriate samples from host for determining immune profile

Scope

This standard Operating Procedure (SOP) describes the steps and precautions required while collecting samples that includes appropriate time of collection and type of samples to be collected for each type of indicated test.

User

Veterinarians/ Laboratory Officer/ Laboratory Technicians /Para veterinarians

Manpower

Veterinarians;
Para-veterinarians;
Laboratory technician;
Animal attendants;
Animal owners;

Materials/equipment required

1. Sample submission form
2. Vacuutainer blood tubes (red cap)
3. Phosphate buffered saline (PBS) tablet or pre-prepared saline
4. Glycerine
5. Needle and syringe (18 and 22 gauge, 2.5 ml)
6. Needle holder
7. Serum tube
8. Gum shoes
9. Disinfectant*
10. Apron
11. Hand gloves (double gloving)
12. Cool box

13. Marker pen (permanent)
14. Cotton/tissue paper
15. Sample label
16. Self-sealing plastic bags/snap lock bag
17. Scissor
18. Blades
19. Packing tape
20. Ethanol 70%
21. PBS 50% glycerine (PBSG) (7.4 pH)
22. Waste disposal bag/bio-hazard bag
23. Sharp container

* Calcium carbonate, Virkon-S, aldehyde compound, bleaching powder and iodophore compound

Blood collection procedure

1. Put on apron and hand glove and restrain animal properly
2. Clean cephalic vein area with 70% ethanol
3. Collect the 5 ml blood in the disposable plastic syringe or vacuutainer
4. Allow the blood to clot within the syringe or in the tube. The syringe should be placed at 45 degree angle for serum separation at room temperature or at 37°C for 20-30 minutes.
5. Separate the serum in eppendorf tubes/cryo vials for sending to the laboratory
6. Complete sample submission form with all the details
7. Label each tube with reference number corresponding to sample submission form
8. Pack properly in the plastic bags and keep in cool box

Waste disposal

1. Used glove, disposable apron, mask and other waste should be collected and sprayed with disinfectant, put into a waste disposable bag, sealed and carried back to office of laboratory and disposed after being autoclaved;
2. Other materials such as blades, needle, syringe should be collected and sprayed with disinfectant and destroyed appropriately;

2.10. Standard Operating Procedure for rapid field test (BioNote) for detection of virus in brain tissue samples

Purpose

To have standard test protocol to diagnose rabies virus in the field using rapid antigen detection test.

Scope

This SOP outlines the general principles and steps to detect rabies virus antigen in brain tissue samples homogenates

Test Principle

The Antigen Rapid Rabies Antibody Test is a chromatographic immunoassay for the qualitative detection of Rabies virus antigen in canine, bovine, raccoon dog's secretions of saliva, and brain homogenates.

The Antigen Rapid Rabies Ag Test Kit has a letter of "T" and "C" as test line and control line on the surface of the device. Both the test line and control line in result window are not visible before applying any samples. The control line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. A purple test line will be visible in the result window if there is enough Rabies virus in the specimen.

The specially selected Rabies virus antibodies are used in test band as both capture and detector materials. These enable the Antigen Rapid Rabies Ag Test Kit to identify Rabies virus antigen in canine, bovine, and raccoon dog's secretion of saliva, and brain homogenates with a high degree of accuracy.

Materials

- Ten(10) Antigen Rapid Rabies Ag Test Kits
- Ten(10) Specimen tubes containing assay diluents buffer
- Ten(10) Sample collection swabs
- Ten(10) Disposable droppers
- One(1) Instruction for use
- The specimens (mainly brain tissues) should be tested immediately as soon as the samples are collected from canine and bovine

Safety Consideration (refer sample collection SOP)

- Wear mask and gloves during sampling
- Disposing the tests materials accordingly
- Dispose the tests materials safely
- While doing post mortem, maximum precautions are to be taken (this is a technical discipline to be followed every time)

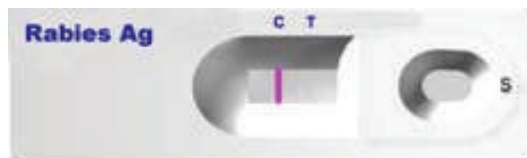
Laboratory Procedures

- Collect the samples - brain homogenates using the swab
- Insert the swab into the specimen tube containing 1ml of assay diluent
- Mix the swab samples with assay diluent to extract well
- Remove the test device from the foil pouch, and place it on a flat and dry surface
- Using the disposable dropper provided, take the samples from extracted and mixed specimens in the tube
- Add four (4) drops into the sample hole using the disposable dropper
- As the test begins to work, you will see red/purple color move across the result window in the centre of the test device. If the migration has not appeared after 1 minute, add one more drop of the mixed assay diluent to the sample well

Result Interpretation

- Interpret test results at 5 ~ 10 minutes. Do not decide after 10 minutes
- A color band will appear in the left section of the result window to show that the test is working properly. This band is the control band. The right section of the result window indicates the test results. If another color band appears in the right section of the result window. This band is the test band.

Negative result: The presence of only one band within the result window indicates a negative result.



Positive result: The presence of two color bands ("T" and "C") within the result window, no matter which band appears first indicates a positive result.



Precautions/Limitation of Procedure

- Do not open or remove test kit from their individually sealed pouches until immediately before their use
- Do not use the test kit if the pouch is damaged or the seal is broken
- Do not reuse test kit
- All reagents must be at room temperature before running the assay
- Do not use reagents beyond the stated expiration date marked on the label
- The kit can be stored at room temperature (2 to 30°C) or refrigerated
- DO NOT FREEZE. Do not store the test kit in direct sunlight
- Although the Antigen Rapid Rabies Ag Test kit is very accurate in detecting Rabies virus antigen, a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the veterinarian after all clinical and laboratory findings have been evaluated

Reporting

After the completion of laboratory test the result based on the interim format of NCAH may be sent to the concerned In-Charges. Results are reported either as positive or negative.

Quality Control

- The components in this kit are quality control tested for standard batch unit.
- If the purple color band is not visible within the result window after performing the test, the result is considered invalid. It is recommended that the specimen be re-tested.

2.11. Standard Operating Procedure for fluorescence antibody test (FAT) in the brain tissue sample of suspected rabies infected animal

Purpose

To have standard test protocol to diagnose rabies virus using fluorescence antibody test (FAT).

Scope

FAT method is used for detecting rabies inclusion bodies (viral antigen) in cells of acetone-fixed brain smears

Test Principle

The smears are incubated with FITC-labelled anti-lyssavirus polyclonal antibody. Un-bound antibody is then removed by washing and smears are examined by fluorescence microscopy. In rabies positive specimens the antibodies bind to the antigen and produce apple-green fluorescing inclusion bodies or viral aggregates when viewed under a fluorescent microscope.

Safety Consideration

- All laboratory personnel who handle and work with suspected rabies virus infected tissue specimens must be well trained, competent and comply with national bio containment and biosafety regulations to protect staff from contact with pathogens.
- All personnel involved in rabies testing should receive pre-exposure immunisation.
- Only personnel who demonstrate an antibody titre of 0.5 international units (IU)per ml or higher should be allowed to handle the suspected rabies infected specimens.
- Personnel should be routinely monitored every 6 months for adequate rabies neutralising antibodies. Booster vaccinations must be given when the titre falls below 0.5 IU/ml. In the absence of serological monitoring, the vaccination regimen should consist of a booster vaccination at 1 year and thereafter every 1-3 years, depending on the vaccine.
- Appropriate protective clothing must be worn at all times.
- The specimens must only be processed in a Class II Biological Safety Cabinet.

- Aerosols – high speed centrifugation and any procedure that generates aerosols should be carried out in tightly closed containers and possibly under a negative draught hood.
- All contaminated instruments and utensils must be sterilised by autoclaving immediately after the test procedure is finished and before they are washed.
- Disposable items must be placed into sterilising bags and must be sterilised by autoclaving before disposal.
- Disinfection of biological safety cabinets and used instruments should be done with disinfectants such as 1% Virkon and/or other virucidals efficient to kill lyssaviruses.

Materials

Equipment and Facilities

- Fluorescent microscope, Zeiss or equivalent, with mercury vapour lamp, 50 or 100 watt (450- 490 nm excitation filters, and 510 nm stop filter).
- Refrigerator (+2 to +8°C).
- Freezers, chest or upright, with temperature of not less than -20°C (e.g. -70°C).
- Incubator, temperature capability of 37°C±2°C).
- Double door autoclave, any brand.
- Class II biological safety cabinets, any approved brand.
- Single channel Micropipettor for drawing 100µl volumes.
- Single channel Micropipettor for drawing 0.5-10µl volumes.
- Vortex mixer.
- pH meter.
- Dark room.
- Forceps.
- Scissors.
- Standard laboratory timers.

Other supplies

- Closed plastic container for use as a humidified chamber
- Microscope slides, clear, frosted one end, any brand, non-fluorescent
- Cover slips 13 mm thick, any brand
- Petri dishes, disposable or other suitable container to place brain material
- Non fluffing high quality absorbent paper towel
- Coplin staining
- HB pencil for labelling slides

- Lens cleaning tissue
- Squeeze/wash bottle with PBS
- A suitable containers with virucidal disinfectant capable of killing rabies virus
- PPE including Nitrile/Latex powder free disposable Gloves
- Disposable tips (1-10 μ l, 20-200 μ l)
- Autoclavable bags

Reagents

- 0.01M Phosphate buffered saline (PBS), pH 7.2-7.4
- Heat sterilized distilled or deionized water, or water of an equivalent purity.
- High-grade (99.9 to 100%) acetone (CH₃)₂CO (MW 58.8), Assay by (GC) is minimum of 99.5%, ASC grade, or similar.
- High grade glycerol
- Anti-lyssavirus FITC polyclonal conjugate (can be obtained from the ARC-Onderstepoort Veterinary Institute (www.arc.agric.za), SANOFI Pasteur (www.sanofipasteur.com), Chemicon (www.chemicon.com) with a predetermined working dilution.
- Lens cleaning fluid.
- Mounting media/mountant.
- Positive and negative control brain specimens.
- Fresh or 50% glycerol saline preserved test brain specimen.
- Evans blue. Counterstain added to the working dilution of the conjugate is optional. Evans Blue counterstain (0.5%) can be aliquoted and stored at +4°C for up to 6 months and indefinitely at -20°C. The amount of counterstain added to the conjugate is determined by titration when the working dilution of the conjugate is determined. Due to counterstain, the cells will be noticeably red, but should not be strongly red as to diminish the specific green fluorescence. An Evans Blue concentration of 0.00125% works very well in many laboratories. This concentration is prepared by adding 2.5 μ l of 0.5% stock dye solution per ml of conjugate diluent.

Laboratory Procedures

Preparation of reagents, test specimens, controls and other materials

- Prepare the diluents for the conjugate which is 0.01M PBS pH 7.2±0.2 with 0.5% Evans blue. This can be prepared and aliquoted into 1 ml volumes at -20°C.
- Determine the optimum working dilution of the anti-lyssavirus FITC polyclonal conjugate as per manufacturer's instructions to provide an excellent staining of viral antigen in brain smears. On the day of specimen testing, prepare only enough of the optimum working dilution conjugate for the number of samples to be tested by adding working dilution of the conjugate into the diluent above (PBS with 0.5% Evans Blue).
- On each day of performing a test, ensure that there is sufficiently chilled acetone preferably kept -20°C.
- Keep microscope slides in an acetone bath at room temperature for degreasing and take them out when needed for staining.
- Dry the slides using appropriate absorbent paper towel.
- Phosphate buffered saline (PBS) 0.01M pH 7.2-7.4 (Refer to Appendix 2 for instructions on how to prepare the buffer).
- Keep fresh filtered acetone in a Coplin or staining jar in a freezer (-20°C).
- Preserve fresh brain specimens that cannot be tested on the day of arrival in 50% glycerol- saline (refer to Appendix 3 for instructions to prepare glycerol-saline). Specimens in 50% glycerol-saline can be stored at room temperature for a maximum of 2 weeks, or frozen at -20°C or at a lower temperature overnight.
- Thaw frozen control samples on the day of testing by placing them in a bio-safety cabinet at room temperature for 30-60 minutes.
- Prepare mounting media (Refer to Appendix 4 for instructions on how to prepare the mounting media)

Sample preparation

- Remove clean slides from acetone and dry them with appropriate absorbent paper.
- Label the frosted end of the slide with the corresponding rabies specimen identity using a pencil.
- Using forceps take out the brain specimen from its original container and place on a petri dish or any other suitable shallow container while exposing the different parts of the brain required for the test.
- Using a pair of scissors, cut small samples (about 2 to 3 mm) of the fresh brain specimen from the brain stem (thalamus, pons, medulla oblongata), cerebellum and hippocampus and place each piece onto two ends of the microscope slide/ wooden tongue depressor to constitute a composite of samples in a pair. The composite samples should be placed within a close distance so that when an impression smear is prepared all the brain samples in the composite are exposed and the resulting smear is about 1cm in diameter. In case the brain specimen is preserved in 50% glycerol saline, each cut brain sample should be blotted on paper towel to remove excess glycerol and then treated as fresh ones.
- Using the forceps, return the original brain specimen into its original container and place it at -20°C or lower temperature for further storage.
- Make impression smears by lightly pressing the microscope slide for staining on the two sets of brain composite samples.
- Prepare the negative and positive controls in the same manner as test samples and include them in every staining.
- Air dry smears for five minutes at room temperature.
- Discard all the contaminated equipment/materials used to obtain the smear into a container with disinfectant.

Fixation

- Immerse the slides into fresh cold acetone and then keep them at -20°C for 20 minutes.
- Air-dry the fixed slides for approximately 5 minutes in the Class II Biological Safety cabinet until all traces of acetone and moisture have evaporated.

Staining procedure

- Prepare a sufficient amount of anti-lyssavirus FITC conjugate according to conjugate dilution titration table.
- Place the slides into a humidified chamber. The humidified chamber is prepared by placing absorbent paper in an enclosed container with a flat bottom, and then pouring some PBS on the paper to create moisture during the incubation step. The paper should not be too wet as this may interfere with conjugate.
- Apply freshly-prepared working dilution conjugate to cover the smear (approximately 150 µl per smear will suffice for a 1 cm diameter of smear. Too little conjugate will result in excessive drying while too much of the conjugate will increase the possibility of the conjugate running off the slides and these could both produce undesirable results).
- Place lid on humidified chamber.
- Incubate the slides in the humidified chamber at 37°C for 30 minutes.
- At the end of the 30-min incubation, remove excess conjugate and wash or rinse the slides in
- PBS three times and then blot dry them on paper. Please ensure that the slides are not completely dry

All procedures should be performed in a class II biological safety cabinet and gloves and a gown must be worn at all times.

- Preferably, submitted specimens need to be tested on the day of arrival and if this is not possible, should be tested at the earliest convenience. Use a fresh set of sterile, clean equipment and supplies for each brain specimen (forceps, scissors, and glass slide).
- Always prepare the control slides last.

Mounting

- Place one drop of mounting media on the slide and place a cover slip on top, ensuring that the mounting fluid is evenly distributed over the smear, while minimising air bubbles on the smear.
- Place slides in covered slide container and take to microscope room (Dark room) for reading.

Result Interpretation

- Two people must read all the stained slides and control smears. Positive controls must be positive and the negative control sample must be negative for the results to be acceptable.
- The stained slides should be examined using the 40X objective starting with positive and negative controls. In positive controls, the presence of rabies antigen is demonstrated by appearance of apple green oval to round shaped inclusion bodies of various sizes, some as dust particles studded against a black to dark greenish background, while such fluorescing inclusion bodies should not appear in negative control smears.
- Continue reading the test smears.
- Grade the smear quantitatively with a score of +1 to +4, depending on the abundance of viral antigens and give a quantitative grade of +1 to +4 depending on the intensity of fluorescence and record the results in the appropriate test results recording system as Positive, Negative or Doubtful

Precaution/Limitation of Procedure

All the generated waste should be sterilised in autoclave before disposal according to the waste disposal SOP of NCAH/RLDCs/SVLs/DVLs/NAH.

Reporting

As in result interpretation

Quality Control

- Each new lot of acetone must be checked to ensure that the acetone does not interfere with the staining of the brain smears.
- Select tissue from 4 to 6 previous specimens that have stained both weakly and strongly positive for rabies virus.
- Prepare two slides from each specimen.
- Fix one set of slides in the acetone in use and the other set in the new lot of acetone.
- Follow the procedures for fixing and staining.
- Read both sets of slides noting the amount of virus as well as intensity of staining.
- Both sets of slides must have identical results. If the new acetone does not meet this criterion, discard and purchase another lot.

- Similarly, the optimal working dilution of a new batch of conjugate must be determined by titration. Naturally, the working dilution may differ between laboratories depending on the microscope optimal system.
- Select known positive and negative brain material to prepare controls.
- Inoculate suckling mice with a 10% suspension of sample that previously tested positive with a score of +4. Pool brain samples from which succumb and test positive for rabies virus antigen. To prepare the negative control, inoculate suckling mice with PBS. Harvest brain tissues after 28 days, pool and place in vials.
- Prepare two-fold dilutions of the conjugate in PBS starting from 1:80 to 1:2560.
- Prepare two slides for each specimen.
- Fix the slides as described in the protocol.
- Follow the procedures for fixing and staining.
- Read both sets of slides noting the amount of virus and intensity.
- The least conjugate dilution that gives an excellent staining will be used as a working dilution of that batch.
- Prevent conjugate contamination by dispensing 1-ml quantities of the diluted conjugate into eppendorf tubes and store at -20°C or lower.
- External quality control will involve an annual participation by our laboratories in an FAT proficiency testing on a panel of rabies positive and negative samples.

2.12. Standard Operating Procedure for Rabies Immunoperoxidase Antigen Detection Test

Purpose

To have standard test protocol to diagnose rabies virus using Rabies Immunoperoxidase Antigen Detection Test (RAID).

Scope

RAID method is used for detecting rabies inclusion bodies (viral antigen) in cells of acetone-fixed brain smears using ordinary light microscope (do not require FAT microscope).

Test Principle

It is an indirect Immunoperoxidase detection of Rabies antigen in brain smears. It uses an in house produced anti-Rabies rabbit serum against an expressed Rabies virus nuclear protein in E.coli. The secondary antibody is an anti-rabbit peroxidase conjugate and uses AEC as the substrate/chromogen for detection. This enables stained Rabies antigen to be read with a light microscope. However, little validation has been done to date and this is not a NATA accredited test. Further work needs to be done.

Materials

- Equipment requirement
- Class II Biosafety Cabinet
- Fumehood
- Light microscope
- Humidified chamber (plastic)
- Petri dish, 90mm
- Scalpel with handle (disposable no: 22)
- Glass slides or positively charged slides-commercial
- Coverslips 50mm x 22mm
- Glass slides plain
- Slide rack-metal or glass not plastic
- Polypropylene and glass Coplin slide jar
- 10ml sterile polypropylene tubes
- Sharps and Biological bin

- Pipettes – currently verified for use
- Sterile plugged 200µl & 1ml tips
- Sterile dissecting instruments
- Autoclave

Freezers/Refrigerators

- 80°C Freezer (for reagent storage),
- 4°C Refrigerator (for reagent storage)
- 4°C to -20°C freezer (fixing)

Reagents requirement

Chemicals

- Saline (AAS) 3-aminopropyltriethoxysilane(Mw. 221.37) Sigma cat# A36-48
- 100% Acetone (Analar)
- 100% Ethanol(Tech Grade)
- Tris Buffer pH7.6 DAKO cat # K8007 (or in house recipe see appendix)
- 30% Hydrogen Peroxide H₂O₂
- AEC 3-Amino-9-ethyl-carbazole (sigma product) Sigma cat# A5754
- N,N-Dimethylformamide (DMF) Sigma cat# D4551-250
- Sodium Acetate or Sodium Acetate anhydrous
- Glacial Acetic Acid
- Distilled water pH = 6.9-7.2
- Virkon or equivalent disinfectant
- Skim milk powder
- Lilly Meyer's Haemotoxylin counter stain

Biologicals requirement

- Rabbit anti Rabies polyclonal serum #663(AAHL reagent)
- Anti-Rabbit HRPO conjugated antibody Jackson cat# 711-036-152
- Positive control Rabies infected brain
- Negative brain (not Rabies infected)

Precaution: ALL WORK SHOULD BE DONE IN A CLASS II BIOSAFETY CABINET WITHIN A DESIGNATED RABIES LABORATORY

Sample Storage

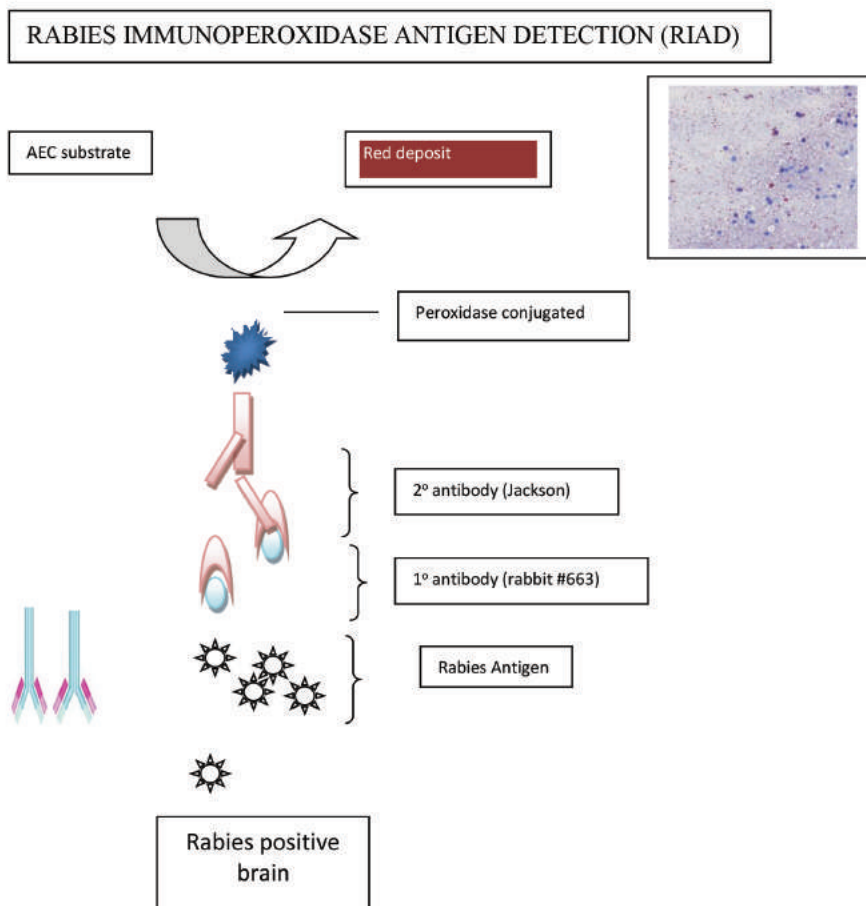
Fresh or frozen brains stored at -80°C can be used for this method. It is recommended that the samples stored in glycerol buffer should be removed or rinsed off before smears are made as per Fluorescent Antibody Test methods.

Control Brain Smears

Positive and negative control brains are stored at -80°C . Smears can be fixed ahead of time and stored dry at -20°C . To date they have been stored for 21 days but further testing needs to be done to validate the time they can be stored for.

TEST METHOD

Schematic Representation of Rabies RIAD tests



Preparation of Brain Smear – Slip Smear Technique

This method is preferred to the impression smear technique as it gives a thin film smear, which reduces non-specific binding of the antibodies during the detection steps. Working inside a Class II Biosafety Cabinet

- Place the brain in a 90mm Petri dish (larger brains can be worked with on a large clean plastic bag).
- Identify brain stem, cerebellum, hippocampus and cerebral cortex.
- Remove 0.5cm³ portions of each of the nominated regions to a clean Petri dish, cutting with a sterile handled scalpel blade.
- If it is not possible to identify these regions of the brain then remove tissue from four separate sections of the tissue.
- Cut the tissue with the scalpel blade finely and place a small amount on the surface of an AAS treated slide. Place a second slide underneath the test slide for support.
- Place the two slides along your index finger of your left hand for extra support.
- Produce the smear by using a 100% Ethanol cleaned slide held in your right hand, placing it on top of the tissue with your thumb over the top slide, pressing down and sliding the top slide away from the bottom 2 slides.
- Allow the brain smear to dry.
- Discard the top slide, the supporting bottom slide can be re-used to make the other smears.

Slide Fixation

- Place air dried smears into a fresh batch of cold -20°C 100% Acetone (make sure the container has a lid).
- Fix for 30-45min at -20°C.
- Remove from the Acetone and allow to dry in the Class II Biosafety cabinet

Rabies Antigen Detection-Immunoperoxidase

General notes before starting

- It is very important that the distilled water has a pH 6.9-7.1 (please check before commencing)
- All incubations are done at room temperature 21-25°C
- Rinse well after each step for 2 min.
- Have all the reagents at 21-25oC temperature for use.
- Keep the slides moist at all times-very important (if slides dry out non-specific staining will result).
- Have the reagents for the next step prepared before the wash.
- Prepare a squeeze bottle with Tris Buffer and one with distilled water for rinses.
- Prepare fresh Tris Buffer with 1% skim milk
- Dilute the Antibodies in Tris Buffer with 1% Skim Milk Powder.
- The test is done in a humidified containerto prevent the smears from drying - moisten some lint free tissue with distilled water and place in a plastic container with a lid.
- Include a positive and negative control with each test

Antigen detection

- Prepare the 3% Hydrogen Peroxide blocking solution

3% Hydrogen Peroxide	Volume
30% Hydrogen Peroxide stock	1ml
Distilled water	9ml

- Prepare the Antibody Diluent(1% Skim Milk in 1X Tris buffer pH7.6)

1% Skim Milk in Tris pH7.6	Quantity
1X Tris pH7.6	50 ml
Skim milk powder	0.5 g

- Rinse the smears through 3 serial baths of Tris-Buffer to moisten the smears

1X DAKO Tris pH7.6	Volume
20X Tris pH7.6 stock	50 ml
Distilled water pH=7.0	950 ml

- Place smears in the humidified container at Room Temperature
- Treat the each slide with 300µl of 3% Hydrogen Peroxide,10min at room temperature (close the lid- to keep moist)
- Dilute the Rabies polyclonal serum (Rabbit#662) 1:1000in antibody diluent

1o Antibody (Rabbit #663) 1:1000	Volume
Rabbit #663 antibody	2 µl
Antibody Diluent	2000 µl

- Rinse the slides through 3 serial baths of Tris-Buffer (the last bath should have fresh Tris buffer)
- Place back into the humidified container at room temperature add 300µl of the 1° antibody diluted 1:1000 per smear
- Incubate at room temperature 45 min in the humidified chamber
- Dilute the 2° Antibody (Jackson) 1:500 in antibody diluent

2° Antibody (Jackson) 1:500	Volume
2° Antibody	4 µl
Antibody Diluent	2000 µl

- Rinse the slides through 3 serial baths of Tris-Buffer as in step 7
- Add 300µl of the 2° antibody diluted at 1:500 per smear
- Incubate at room temperature 45 min
- Prepare AEC Substrate

AEC Substrate	Amount
AEC powder(pre-weighed)	2 mg
Di-Methyl-Formamide (DMF)	500 µl
Mix well	
0.05M Acetate buffer pH5.0	9500 µl
30% H ₂ O ₂ (just before use)	5 µl

- Wash slides 3 times with Tris Buffer
- Activate the AEC substrate with 5µl 30%Hydrogen Peroxide (H₂O₂)
- Add 300 µl of activated substrate for each brain smear
- Incubate at room temperature for 10 min-monitor colour development
- Wash with distilled water pH7.0 once (to stop the reaction)
- Counter Stain with Lilly Meyers Haemotoxylin 20 sec
- Rinse excess stain off with distilled water and wash 1X in distilled water
- Dip slides into Tris buffer
- Rinse and leave in distilled water until cover slipping
- Coverslip using aqueous mounting medium

RESULTS

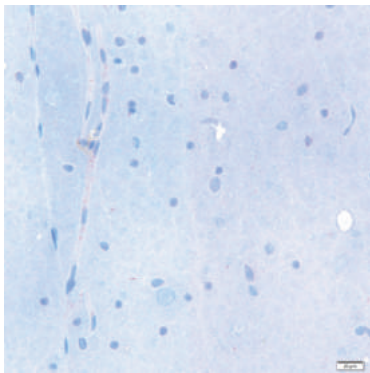
Reading

Slides are read under a light microscope- 40X lens

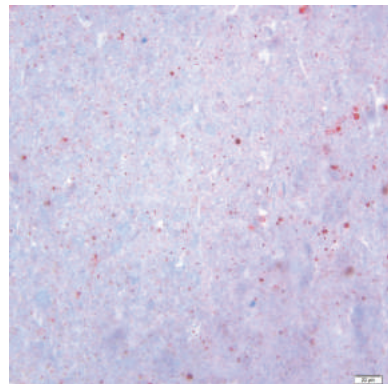
Recording and Interpretation

The presence of Rabies antigen is seen as brick red deposits in the smear against blue brain tissue. It is important to distinguish non-specific staining especially on the edges of the smear. For this reason positive and negative control smears must always be included with each test.

Smears are scored as positive or negative for antigen. A description of the quantity of antigen present can be included, for example high or low positive.



Negative Canine brain smear



Positive Canine brain smear (red deposit)

Annexure 3: Standard Forms

FORM: 1 Temperature recording form (to record temperature of vaccine storage in the refrigerator)

Name of Centre:
Month:

Gewog:

Dzongkhag:

		Date																																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31				
Temperature (vaccine storage refrigerator)		>10																																		
		10																																		
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Remarks:

FORM 3: Flash Report form for Disease outbreak reporting

Sl. No	Parameters	Data/Information
1	Village	
2	Location	Latitude: Longitude:
3	Gewog	
4	Dzongkhag	
5	Date of outbreak	
6	Date of report by owner to LEC/ RNREC/DVH	
7	Date of report by LEC/RNR/ DVH to RLDC/NCAH	
8	Disease suspected	
9	Species affected	
10	Number of cases	
11	Number died	
12	Number of household affected	
13	Number of susceptible animals in the village	
14	Probable source of outbreak	
15	Contact person in village (Name and phone number)	
16	Control measures taken	

FORM 4: Disease Outbreak Investigation Form

Name & position of the Investigator:	Date:
--------------------------------------	-------

Owner details		
Name of the owner:		Contact telephone number:
Village:	Gewog:	Dzongkhag:

Information about the disease outbreak
Date of onset of clinical signs:
Date of onset of mortality:
Date of report of outbreak from farmer to LEC/ RNEC/DVH:
Date of report from LEC/ DVH to RLDC/ NCAH:

Details of animal affected									
Date	Species	Breed	Age	Sex	No. affected		Number at Risk	Location of outbreak (Geo-coordinate)	
					Cases	Death		Longitude (N)	Latitude (E)

Clinical signs observed:
Post-mortem findings:

Samples collected							
Sample ID	Species	Specimen type	No. of specimens	Sample packing	Name of laboratory referred to	Date of shipment	Test requested for

Initial laboratory test findings (Rapid antigen test result)

Disease suspected/confirmed by clinical observation and laboratory test
--

Probable source of infection:

Number of humans bitten by the rabies suspected dogs/cats:

Whether the bitten person have visited hospital for PEP: Yes/ No

Rabies vaccination history	
Whether the affected animals (dog/cat) were vaccinated in the last vaccination? Yes No	
If yes, date of the last vaccination.....	
If No, date of the last vaccination.....	
Whether other dogs/cats in the affected village were vaccinated against rabies? Yes No	
If yes, date of the last vaccination.....and approximate vaccination coverage (%) or number of dogs vaccinated.....	
Any action taken so far	
Recommendations	
Name & Designation of Investigation Team	Signature

FORM 5: Reporting form to health facility for confirmed rabies in animals

(to be fill in by Veterinary authority and submit to Medical hospital at the time of refereeing people for PEP)

Reporting Animal Health Centre:

Date of report:

Geog:

Dzongkhag:

Date of case/outbreak	
Date of report to LEC/RNR-EC/DVH	
Geographical location of outbreak village name :	
Species of animal affected	
Total no. of cases	
Total no. of deaths	
Probable source of outbreak/infection	
Laboratory confirmation	
Control measures undertaken	
No. of people exposed* to the infected animal (provide list separately)	

*Exposure to rabid dog bites/livestock products from rabid animals



Animals died of rabies (cattle and dog)



Mass vaccination of free-roaming dogs and colour painting of the vaccinated dogs for identification purpose by RRT

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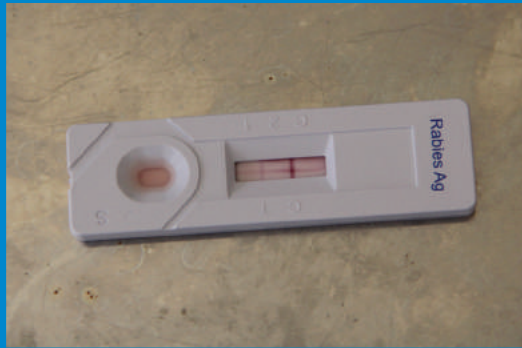
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