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POLICY PAPER ON DIAGNOSIS AND CONTROL OF RABIES IN ANIMALS IN INDIA BY 2030

Published By

National Academy of Veterinary Sciences (India)
(Registered with the Registrar of Societies vide Regn. certificate No.S-2/4471 of 1993 dated 7th July 1993
and
Centre for One Health
College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004 (Punjab)

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Policy Paper

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MISSION OF THE NATIONAL ACADEMY OF VETERINARY SCIENCES

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NATIONAL ACADEMY OF VETERINARY SCIENCES (INDIA)

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FOREWORD

I am immensely pleased to present this Policy Paper on 'Diagnosis and Control of Rabies in Animals in India by 2030' as an official publication of the National Academy of Veterinary Sciences (India). It has critically examined the detailed epidemiology, available diagnostics, relevant prevention and control measures and future recommendations on rabies in India. I am confident that this policy paper booklet will be useful in addressing the existing epidemiological scenario of rabies under the lens of One Health in India. I sincerely thank and compliment the contributors for compiling and editing the manuscript in an excellent manner. The collaboration from Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana has been vital in bringing out this important publication for guiding the authorities in veterinary and medical disciplines alike besides the policy makers for initiating the suitable interventions to curb the incidence of this dreaded disease.

Dr. D.V.R PRAKASH RAO
President, NAVS (India),
New Delhi

PREFACE

Rabies (Latin meaning '*rage* or *madness*') is one of the oldest zoonosis known to mankind. Rabies is close to 100% fatal, but at the same time almost 100% preventable. It is the deadliest disease on earth and fatal in the absence of preventive measures immediately after getting infected.

Rabies is present on all continents except the Antarctica. The disease is endemic in more than 150 countries and territories. Thousands of people die from rabies every year with 95% of human deaths occurring in Africa and Asia where this disease causes around 59,000 deaths every year. India alone accounts for 20,000 deaths; more than one-third of the world's total.

Countries predominantly affected by rabies often have poor diagnostic and reporting capacities, leading to a lack of accurate data and considerable uncertainty around the estimates of global burden. Most cases of human rabies occur in rural areas where effective treatments, such as human vaccines and immunoglobulins, are not readily available or accessible.

The present policy paper provides an outline on the epidemiology, host spectrum, clinical signs and available diagnostics for the rabies in India. A comprehensive outline of prevention and control strategies along with the recommendations for the policy makers and relevant stakeholders in order to achieve the goal of 'zero human rabies death by 2030'.

I congratulate the National Academy of Veterinary Sciences (India) on this initiative to bring out the policy paper on this important disease and its confidence in Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) for collaborating on the same. I appreciate the sagacious inputs of learned fellows of NAVS and faculty of Centre for One Health and Department of Veterinary Pathology at this University.

Dr. Inderjeet Singh
(Vice Chancellor, GADVASU)

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Preamble

Rabies, the archaic zoonotic disease causes a fatal meningo-encephalomyelitis condition in both humans and animals once the clinical symptoms start appearing. At the same instance, rabies is 100% preventable by prophylactic immunization, and the most cost-effective strategy for prevention of human rabies is by eliminating rabies in canines and wild animals through effective vaccination regimen. Rabies is widely distributed all over the world and is currently endemic in around 150 countries or territories with its major impact in the developing countries. India contributes around 35% of the global human mortality associated with canine rabies with an approximate death of over 20,000 per annum, primarily of children in rural or marginalized population(s). Although, vaccines and proper regimen of treatment exists to prevent rabies, but the lack of awareness, irregularity in availability of post exposure vaccines and/or delay in vaccination/ immunoglobulin, high cost of prophylactics, lack of structured national and/or regional surveillance and co-ordination (One health approach), and shortage of trained personnel etc. still contribute to fatality in both humans and animals.

The economic importance of rabies is well recognized and the worldwide data reported that canine rabies affect direct and indirect economic costs (Post-exposure prophylaxis [PEP], animal tests, dog vaccination, and livestock losses) and other various costs amounting to 8.6 billion US dollars loss per year. Livestock deaths alone amounted to 512 million US dollars per year, with major losses in African countries and in more populous countries of Asia. In addition, the effects also included the costs associated with the risk of human mortality and a global cost for canine rabies has been pegged as 120 billion US dollars. The United Nation's Sustainable Development Goals envisions aims to end the epidemics of neglected tropical diseases, including rabies, by the year 2030. By vaccinating 70% of dogs, countries can create "herd immunity", effectively slowing the spread of rabies until it wanes out entirely. India along with many other associations and expert organizations, committees etc. launched National Rabies Control Programme (NRCP) in the year 2013.

Keeping in view the public health significance of rabies, a policy paper was contemplated by National Academy of Veterinary Sciences (NAVS), India, on the basis of interaction with policymakers, public health scientists and leading veterinarian(s) having expertise on rabies through various National and International Webinars, Regional workshops organized by various Institutions and Organizations. To have a blue print to develop strategies for diagnosis, control and prevention of canine rabies, the observations and recommendations are advocated.

Introduction

Despite of centuries old, rabies remains a neglected viral zoonosis which is characterized by an acute progressive disease of the central nervous system being invariably fatal in both terrestrial and airborne mammals such as dogs, wolves, foxes, coyotes, jackals, cats, bobcats, lions, mongooses, raccoons, skunks, badgers, rodents, lagomorphs, bats, monkeys and humans (Oliveira *et al*., 2020; Fitzpatrick *et al*., 2014; Menezes, 2008). The rabies virus is a negative sense single stranded RNA virus belonging to the family *Rhabdoviridae*, order Mononegavirale, genus *Lyssavirus*. Currently, there are 16 members of *Lyssavirus* genus which are further divided into 02 phylogroups on the basis of serum cross-reactivity against the viral protein and thresholds in genetic sequencing differences. Classical rabies virus (RV-genotype1) and its field variants are classified under Phylogroup I which are responsible for causing rabies in human and animals (Banyard *et al*., 2014; Badrane *et al*., 2001; Bourhy *et al*., 1993). In India and other Asian countries, more than 90 percent of human infection(s) occur due to exposure to rabid dogs (97%), while cats (2%), monkeys and other wild animals (1%) are reported to transmit the infection in limited number of cases (WHO, 2013). Mongooses may also act as a source of human rabies with the involvement of some other intermediate animal species (Mani *et al*., 2016; Messenger *et* al., 2002). In India, the first case of rabies in a wolf (*Canis Lupus Pallipes*) was reported based on laboratory evidence in 2014 (Isloor *et al.,* 2014).

After the onset of clinical signs, the disease rapidly progresses and cause 100 percent mortality in infected individuals, the early observed symptoms include fever, tingling at the site of bite/ exposure, anorexia and change in behavior. The disease occurs in two forms - furious form and dumb form (paralytic rabies). Paralytic form is the most common clinical sign in cattle and sheep exhibiting incoordination, paresis and paralysis of the pelvic limbs, besides recumbency, paddling, coma and death (Pedroso *et al*., 2009; Rissi *et al.,* 2008). More than 75 percent of rabid dogs manifest furious form of rabies followed by incoordination and paralysis (42-48%). Rabid cats show more aggression than dogs (Fogelman *et al*, 1993). Muzzle tremors and pharyngeal paralysis, decreased or absence of sensitivity of anus, tail and varying degree of paresis and paralysis are observed in horses (Luciano *et al*., 2009; Hudson *et al*., 1996). Hyperexcitability, self-biting of forelimbs, attacking and biting inanimate objects, colic, drooling of saliva, sternal recumbency, paralysis of hindlimbs are seen in camels (Afzal *et al*., 1993), paralysis, difficulty in walking, opisthotonus, pedalling of posterior limbs and aggressiveness are observed in many wild animals (Delpietro *et al*., 2009).

Geographical distribution of rabies reflected that rabies is an endemic disease in about 150 countries/territories, with majority of cases reportedly occurring in developing countries. Densely populated South-East Asia and Africa contributes to the major chunk of rabies mediated deaths globally, while countries such as Australia, Ireland and many Pacific Island nations have always been free from dog-mediated rabies. Western Europe, Canada, the United States of America (USA), Japan, and some Latin American countries have recently eliminated rabies through exhaustive vaccination campaigns and are declared free from dog-mediated rabies (Carrara *et al.,* 2013). However, in Europe, Africa, Asia and Australia, rabies virus related lyssa virus causes the same fatal disease as rabies virus, circulate in bats, mongoose etc. and normal rabies virus targeted control efforts do not always provide immunity against these viruses (Fisher *et al*., 2018; Birhane *et al*., 2017; Castillio-Neyra *et al*., 2017; Banyard *et al*., 2014).

Global Burden

Globally, rabies imposes a huge impact on health (both human and animal population) and economy, particularly in the developing countries. A worldwide human death due to dog-mediated rabies has been estimated to be 59,000 per year, with an annual loss of over 3.7 million disability-adjusted life years (DALYs). Majority of these deaths occur in Asia (59.6%) and Africa (36.4%) (Knobel *et al*., 2005). Dog mediated rabies affected direct and indirect economic costs (PEP, animal tests, dog vaccination, and livestock losses) amounting to 8.6 billion US dollars per year. Livestock deaths alone amounted to 512 million USD per year, with major losses in African countries with livestock-dependent economies such as Ethiopia, Sudan and Tanzania; and in more populous countries in Asia *viz*., China, India, Bangladesh and Pakistan (Hampson *et al*., 2015). In addition, the effects also included the costs associated with the risk of human mortality and a global cost for canine rabies of US dollar 120 billion (Gemechu, 2017). World Health Organization (WHO) survey showed losses in productivity due to premature deaths (55% of total costs), the cost of PEP (20%) and direct costs to the medical sector and bite victims (20%) (WHO, 2018).

Rabies in India

The disease is one of the major public health concerns in India and is responsible for approximately 20,000 deaths per year, contributing about 60% of human rabies mortality in Asia and 35% of global mortality. In India, dogs are the main host for transmission and maintenance of rabies virus. However, in the absence of proper surveillance system, data on the role of wild animalsin rabies transmission and maintenance is nearly non-existent or scanty.

Rabies is an endemic disease in most parts of India with the exception of the islands-Lakshadweep, Andaman and Nicobar. As per the recent WHO supported India multicentric survey conducted by Association for Prevention and Control of Rabies in India (APCRI), none of the cat brain samples from Lakshadweep and dog brain samples from Andaman and Nicobar were found positive for rabies viral inclusions/ antigen (Isloor *et al.,* 2019).

In 2012–2013, the National Rabies Control Programme (NRCP) was launched with the National Centre for Disease Control (NCDC), New Delhi and the Animal Welfare Board of India as the nodal agency for both the human and animal components, respectively. The official website of NRCP (https://www.nhp.gov.in/national-rabies-control-programme_pg) and National Ministry of Health and Family Welfare (https://www.mohfs.gov.in) provide information on the achievements of the program (Radhakrishnan *et al*., 2020). The APCRI conducted WHO-funded Indian multi centric rabies survey (IMRS) to develop and provide a draft policy paper to the Director General of Health Services (DGHS), Government of India, New Delhi.

Presently, rabies is not a notifiable disease in animals in India (Rahman & Isloor, 2018). As per the recent notification from Department of Health and Family Welfare (order no: D.O.No.2283429/NRCP/DZDP-NCDC/DGHS; dated:20-09-2021), the human rabies has been declared as notifiable disease under Clinical Establishment Registration and Regulation Act, 2010 or other relevant Acts. Though, the States / Union territories are accountable to report rabies to the Central Bureau of Health Intelligence, Government of India, New Delhi. However, the lack of structured national or regional surveillance system is resulting in poor availability of both human and animal rabies pestilence data (Mani & Willoughby, 2017). Nevertheless, rabies is still not included in the list of diseases for routine surveillance by the states to be reported under Integrated Disease Surveillance Project (IDSP, 2020) of the Indian Ministry of Health and Family Welfare. Therefore, due to the limited reporting and imprecise estimates, rabies is still a major concern and burden, particularly for the poorest rural section of the community. More recently, the Ministry of Health and Family Welfare along with the Ministry of Fisheries, Animal Husbandry and Dairying, Government of India has come out as "Joint Inter-Ministerial Declaration Support Statement" in the form of national action plan for the elimination of dog mediated rabies (NAPRE) by 2030 through the One Health approach.

Epidemiology and Pathogenesis

Rabies is a fatal zoonotic disease yet it is preventable by vaccination in animals and humans. In spite of numerous rabies related deaths, the disease remains a neglected zoonosis and is grossly under-reported. According to APCRI and world rabies survey by World Health Organization, more than 99% of the estimated 59,000 human deaths worldwide of which approx. 15% victims are of children below the age of 15 years. These deaths are attributed mainly due to dog mediated rabies (WHO, 2018).

Molecular epidemiological analysis of suspected samples from wildlife revealed that genetically similar rabies virus of Phylogroup I are circulating within wild and domestic animals, highlighting the pivotal role of wild animals in the transmission and maintenance of rabies (Reddy *et al*., 2019, 2018). However, exceptional host shifts can continue to occur in unpredicted ways (Fisher *et al*., 2018). Recently, researchers from National Centre for Biological Sciences (NCBS) and National Institute for Mental Health and Neurosciences (NIMHANS), a WHO collaborating Centre for Reference and Research on Rabies, reported detection of rabies neutralizing antibodies in samples of fruit bats and insect bats from 6 villages in Nagaland in India (Mani *et al*., 2017). Detection of a naturally acquired rare Indian subcontinent lineage rabies virus infection has also been reported for the first time in domestic fowl in India after being bitten by a stray dog (Baby *et al*., 2015) as well as an increase in reported rabid rodents and lagomorphs (Fitzpatrick *et al*., 2014). Such revelation about the occurrence (although rare) of rabies infection in poultry and rodents indicates that spillover of infection is possible in highly endemic areas. Unusual occurrence of rabies in these species reiterates the importance of diagnostic testing and reconsidering for post-exposure prophylaxis for such possible exposures of susceptible animals and humans.

Rabies is also considered as a serious threat in livestock because livestock and other working animals, such as cattle, buffaloes, sheep, goats, horses, donkeys and camels can become infected with the rabies virus (Sunil kumar *et al.,* 2019). The epidemiology of rabies in livestock and working animals varies geographically. Changing land use and reforestation has expanded disease boundaries making livestock more vulnerable to come in contact with rabies infected bats and wildlife. Vampire bat rabies especially impacts livestock production in Latin America while in Eastern Europe and Asia, livestock and working animals are more readily infected by other wildlife such as foxes, jackals and wolves. In Puerto Rico, mongooses have been implicated in up to 70% of reported animal rabies cases (Berentsen *et al*., 2015). In Africa, dogs play an important role in the transmission of the disease to transport animals especially in urban and peri-urban areas (Gemechu, 2017).

The lack of surveillance and reliable data on the number of rabies cases/deaths in livestock and working animals (including human exposure) remains a major bottleneck to assess an economic impact of rabies on the local and national economies. Besides, the expenses related to transport and expensive PEP for rabies exposed family or community members may often lead to the distress sale of production animals and livelihoods assets, further having a bearing on food and economic security. Improved surveillance and elucidating explicit picture of the economic and public health impacts of rabies on the national and local economies would facilitate more appropriate allocation of resources and creation of much needed political will (Gemechu, 2017).

Transmission of the disease is most commonly through rabid animal bite/ carrier bats; however, infection can also be contracted from non-bite exposure to the virus such as, inhalation of aerosolized virus, organ or corneal transplants, scratches, abrasions, or open wounds contaminated with saliva or other potentially infectious material from a rabid animal. Veterinarians, medical staffs, laboratory personnel, butchers etc. handling virus infected animals, bite wounds, high risk laboratory samples and contaminated meat, respectively are occupationally at higher risk of contracting the disease (Singh *et al.*, 2017).

Although, incubation period of rabies virus is highly variable from 2 weeks to 6 years based on the site of inoculation of the virus, density of innervation and concentration of the virus deposited at the site, disease occurrence days or even 20 years after exposure has been documented in humans (Shankar *et al*., 2012; Rupprecht *et al.*, 2002). Bites on the head, face, neck and hands, finger tips with bleeding have the highest risk due to high density of neurons. Shorter the distance from the brain, shorter is the incubation period. The virus causes slow and progressive central nervous infection of the host and can result in death within 7-10 days of showing clinical signs without intensive care/treatment (Hemachudha *et al*., 2013). In cattle, clinical signs and death usually occur post 14 days of exposure to the rabies virus.

Current Scenario of Rabies Diagnosis

Diagnosis of rabies always require adequate epidemiological scrutiny and laboratory confirmation and should not be based only on history and clinical symptoms due to the fact that infection with certain (other) neurotropic pathogens, animals may exhibit almost similar neurological signs (e.g., cerebral malaria in malaria-endemic regions in humans). Rabies should be included in the differential diagnosis of all patients presented with unexplained, acute, progressive neurological signs, even in areas where the disease is rare, as it can occur locally in wildlife (such as bats, mongooses, foxes and jackals).

Rapid and accurate laboratory diagnosis of rabies in humans and other animals is essential as a short time lag between suspicion and confirmation of a rabies case is important for timely administration of PEP and cost-saving in case of a negative diagnostic result. Finally, improper transport of samples over long distances in climatically warm settings increases the risk of poor sample quality, which adversely affects fluorescent antibody test (FAT) results (Léchenne *et al*., 2016). Confirmation of positive rabies cases may also aid in defining current epidemiologic patterns of disease and provide appropriate information for the development of rabies control programs.

Ante-mortem Diagnosis

Ante-mortem diagnosis is challenging as it depends on extensive dissemination of virus through the nervous system and is strongly discouraged for rabies diagnosis in animals. Sensitivity of ante-mortem tests varies widely according to the stage of the disease, immunological status, intermittent viral excretion and training of the technical staff.

Secretions, excretions and biological fluids (such as saliva, CSF, tears, serum, urine, milk) and tissue samples such as skin biopsy samples, including hair follicles at the nuchal region can be used for ante-mortem diagnosis of rabies. The samples with the highest ante-mortem diagnostic sensitivity include three saliva samples, taken at intervals of 3-6 hrs, and skin biopsies (including hair follicles). Skin biopsy punch is used to collect skin biopsy samples of diameter, ~ 4 mm; total volume of 20 mm^3 (Dacheux *et al.*, 2008) or 1cm^2 from nuchal region of suspected animal (Macedo *et al*., 2006) or, a 5-6 mm diameter section of skin may be taken from the nuchal area (CDC, 1999). The biopsy specimen should contain a minimum of 10 hair follicles and be of sufficient depth to include the cutaneous nerves at the base of the follicle (Singh, 2019). Saliva samples (although risky to collect) can be collected from suspected animal using cotton swabs directly from oral cavity of rabies suspected animal (Johnson *et al.*, 2008; Shankar *et al.*, 2004). Oropharyngeal swabs and Sponge-tipped applicators are used for swabbing the anterior surface of tongue and cheek mucosa (Jackson *et al.*, 2008). Aspiration technique (Crepin *et al*., 1998) is used for collection of saliva samples for *intravitam* diagnosis of rabies. Use of sterile eye dropper pipette and syringe for collection of saliva sample directly from the mouth of animal has also been suggested. Liquid saliva sample (200μl) is preferred over saliva swab (Wacharapluesadee & Hemachudha, 2010; Dacheux *et al.*, 2008; Nagaraj & Paul, 2006). Virus secretion/shedding is intermittent in saliva, urine and even CSF, hence the serial successive sampling without addition of any preservative is recommended. It is pertinent to mention that the rabies virus appears in saliva of dogs long before appearance of clinical signs and subsequently during the clinical course (CDC, 2017; Tepsumethanon *et al*., 2004).

For a reliable ante-mortem diagnosis, molecular techniques such as PCR, DNA microarrays, nucleic acid probe are used. The most sensitive molecular technique for ante-mortem diagnosis is Real time RT-PCR (RT-qPCR) which has been proven to be 10 times more sensitive, rapid and specific for the detection of rabies virus RNA in brain biopsy (Orlowska *et al*., 2008; Hughes *et al.,* 2004). Imaging methods such as MRI, diffusion-weighted imaging, diffusion tensor imaging and biomarker detection have also been used (Singh *et al*., 2017). The recommended diagnostic test by World Organization for Animal Health (OIE) and World Health Organization (WHO) considered the gold standard test for detection of rabies antigen from fresh brain biopsy of suspected dogs/animals is direct fluorescent antibody test (dFAT). However, the sensitivity of detection of Rabies with FAT decreases significantly when used on non-nervous tissues, secretions and/or excretions of live animals (Singh, 2019). Paired samples of CSF at 2 weeks interval can be tested against rabies virus neutralizing antibody (RVNA) titre by rapid fluorescent focus inhibition test (RFFIT) to confirm the diagnosis of rabies encephalitis (Mani *et al*., 2016).

Fluorescent antibody virus neutralization (FAVN) test and Rapid Fluorescent Focus Inhibition Test (RFFIT) measure the host response to infection/vaccination and do not detect the presence of infectious virus/antigen directly. FAVN and RFFIT measure the level of neutralizing antibody. The test may lack sensitivity and specificity and interpretation of the test results may be difficult as the host response to infection varies substantially between individuals (Fooks *et al*., 2009; Feyssaguet *et al*., 2007).

A field level test that is rapid, performance friendly and low-cost method to detect rabies virus (RABV) will eliminate constraints in sampling (brain biopsy) and subsequent transportation to reference laboratory. It will contribute positively to surveillance and consequently to accurate data reporting, which is presently missing in the majority of rabies endemic countries (Léchenne *et al*., 2016; Mshelbwala *et al*., 2015). Rapid immune-diagnostictest (RIDT) based on lateral flow principle under reference laboratory conditions, give specificity and sensitivity of 93.3% and 95.3%, respectively compared to the gold standard FAT test. Under field laboratory conditions, the RIDT yielded a higher reliability than the FAT test particularly on fresh and decomposed samples and viral RNA can be extracted directly from the test filter paper and further used successfully for sequencing and genotyping.

Post-mortem Diagnosis

The most widely used test for postmortem rabies diagnosis is fluorescent antibody test/direct fluorescent antibody Assay which is considered as the gold standard test for rabies diagnosis and is recommended by both WHO and OIE. However, various rapid screening and inexpensive field test based on one-step Immuno-chromatographic lateral flow strip test such as Direct Rapid Immunohistochemical Test (dRIT) and Rapid Immunodiagnostic Test (RIDT) have been developed and are available commercially. These tests offer useful method for rabies diagnosis without the need for laboratory equipment. This immuno-chromatographic lateral flow strip test is a one-step test that facilitates low-cost, rapid identification of viral antigen. Such lateral flow assay can be used as a rapid screening test in animals.

Conventional histopathology with traditional staining methods such as Seller's stain does not always detect positive rabies cases in the absence of Negri bodies. Therefore, FAT/DFA are used to detect lesions or viral antigen in frozen tissue section (Isloor *et al*., 2020) and immunoperoxidase test especially with avidin-biotin complex in early diagnosis of rabiesin fixed tissue specimen (Hamir & Moser, 1994).

Mouse Inoculation Test (MIT) and Rabies Tissue Culture Infection Test (RTCIT) are two tests based on the propagation and isolation of the virus using laboratory animals (3-4 weeks old mice or 2 days old new born mouse) and established cell-lines such as neuro2a/CCL131, BHK-21/C13, Vero cell and McCoy cell, respectively (Fooks *et al*., 2009). They are considered as the reliable methods for the post-mortem diagnosis of rabies. In comparison to FAT, MIT was found to be superior and has been used by the New York State Laboratories as a routine diagnostic test (Singh *et al*., 2017). The inoculation of mouse or cell culture for diagnosis is a slow process and require longer turnaround times, *i.e.,* 4 days and 28 days, respectively, and may endanger the patient; however, these are employed as confirmatory tests.

Prevention and Control

Eliminating rabies from dog population significantly reduces human exposure to the disease and is considered to be the single most cost-effective intervention to control and eliminate prevalence of canine rabies (WHO 2013). In India, 99% of human rabies cases are due to dog bites and this can be prevented by mass canine rabies vaccination campaigns. Provision of post-exposure prophylaxis (PEP) and pre-exposure prophylaxis (PrEP) to patients with bites from suspected rabid animals and those in the high-risk group of occupation, respectively is integral in reducing human burden of rabies. Vaccination of dog is recognized as the most cost-effective solution to rabies prevention, provided vaccination of ≥70% of the dog population is achieved with that the least maintenance of the same level of herd immunity for 3-7 years for elimination (Gibson *et al*., 2019). Despite many success stories from Latin America (Vigilato *et al*., 2013) and other countries, endemic rabies in Africa and parts of Asia remains largely uncontrolled (WHO 2018). In Asia, even though significant investments have been made in PEP and dog vaccination, human deaths and livestock losses still remain high indicating the pitfall in prevention strategy efforts.

Even though, a strong body of theoretical and empirical is an innuendo towards vaccination of at least 70% of dog population to sufficiently control rabies outbreak, it is also important determinant to assess an ideal time for primary anti-rabies vaccination and booster dosage by proper assessment of humoral immune response in vaccinated dogs and to decipher the correct route and site of inoculation (Aliye & Seyyal, 2011; Seghaier *et al*., 1999; Smith *et al*., 1973). As per WHO recommendation, neutralizing antibody titre should be higher than 0.5 IU/ml in vaccinated dogs to exert the protective immune response level. The studies have shown that dogs with more than one successive vaccination against rabies had better antibody titres than those with a single anti-rabies vaccination (Smith *et al*., 2019; Pimburage *et al*., 2017; Tk S *et al.*, 2007; Gunatilake *et al*., 2003; Cleaveland *et al*., 2003; Sage *et al*., 1993) and a study carried out by Gunatilake and co-workers (2003) showed passive maternal immunity in puppies of vaccinated dams at the age of 3 months is well below the ideal protective level. Therefore, the recommendation for achieving ideal protective neutralizing antibody level is to give 2 doses of vaccines 1 to 3 months apart with annual boosters to all newly acquired pet dogs and cats in canine rabies endemic region (Suzuki *et* *al*., 2008).

Oral rabies vaccination (ORV) has been successfully used to control the disease in certain wildlife reservoir species (Maki *et al*., 2017; Freuling *et al.*, 2013; Cliquet *et al*., 2012). Countries should assess the need for both ORV of dogs and parenteral vaccination in their rabies control strategy. Field trials have shown the potential benefit of OVD in different countries and socio-economical settings, such as Tunisia, Turkey, Sri Lanka, the Philippines, the Republic of South Africa, and Morocco (Cliquet *et al*., 2018, Cliquet *et al*., 2007; Darkaoui *et al*., 2014; Estrada *et al*., 2001; Hammami *et al*., 1999; Vos & Aylan, 1999). However, parenteral vaccination of dogs should remain the foundation of mass vaccination campaigns. Apart from mass parenteral vaccination (carried out concurrently or sequentially), the use of oral vaccination, especially in free-roaming and inaccessible dogs, taking into account structure and accessibility of the dog population, should represent a complementary measure for the improvement of the overall vaccination coverage in dog rabies control programs (WHO, 2013). For ORV of dogs, the hand-out and retrieve model should be used. An optimal individual or combination vaccination strategy for both vaccination of wildlife (ORV with or without Trap- Vaccinate-Release) and dogs (Central Point Vaccination, House-to-House vaccination, with or without ORV) should be determined by taking the size of the target species population into account. The oral rabies virus vaccine bait candidates should be selected based on efficacy and safety profiles. Monitoring of human exposure to oral rabies virus vaccines and risk management should be undertaken.

Even though the crucial prevention efforts *viz*., vaccination and prophylaxis are available, however, the lack of proper surveillance result in incorrect estimation of the level of vaccination achieved/required in the canine and wildlife population; missing data on disease incidence, imprecise estimates of the economic impact and a general underestimation of the true worldwide burden of rabies (Anderson *et al*., 2015; Hampson *et al*., 2015; Nel *et al*., 2013). Currently, rabies is believed to be underreported at the extent of 1:60 in humans and much higher for animal rabies incidence (WHO, 2013; Léchenne *et al*., 2016). Poor surveillance is primarily a result of lack of political commitment and resource attribution towards rabies control, reinforced by the disregard of disease control in domestic dogs which constitute negligible economic value and the fact that rabies affects largely marginalized communities with difficulty in healthcare access (Léchenne *et al*., 2016).

Ministry of Health and Family Welfare, Government of India has initiated "National Rabies Control Programme" with the objective to prevent human deaths due to rabies and to prevent transmission of rabies through canine rabies control. The programme stresses on creating public awareness on rabies, its prevention and the importance of post bite prophylaxis/ therapeutic through education. Guidelines for animal bite management have been drafted for healthcare providers to minimize and avoid improper pre- and post-bite prophylaxis in humans (NRCP, 2019). India has an estimated 25-30 million stray dog populations compelling the control of stray dog population for effective control of dog mediated rabies. Fitzpatrick and co-workers (2016) reported that the highly feasible strategies adopted by Government of Tamil Nadu, India that included focusing on vaccination of stray dogs even as few as 7% of dogs annually, resulted in cost-effectively reducing human rabies deaths by 70% within 5 years, whereas a modest expansion to vaccinating 13% of stray dogs could cost-effectively reduce human rabies by almost 90%. This strategy can also be adapted to other stray animal populations in India thus, curbing the circulation of virus.

Controlling wildlife rabies is an important aspect in maintenance and circulation of rabies virus in the country that can be controlled by vaccination using parenteral/or oral baited vaccines and supported by an organized surveillance system to record proper data. Proof-of-concept programmes on the elimination of rabies have shown that simplifying and streamlining canine rabies vaccination campaigns will ensure successful implementation and high coverage which can also be adopted for streamlining wildlife vaccination (WHO, 2014).

Methods for Controlling Rabies in Dogs

In December 2015, the global rabies meeting in Geneva, Switzerland, developed a framework for the elimination of dog-mediated human rabies. The strategic vision of this framework is to reach zero human deaths from dog rabies by 2030. The framework was developed by the OIE, WHO, FAO and the Global Alliance for Rabies Control. The frameworkrevolves around 6 pillars, abbreviated as: **R-STOP-R**. (Canine Rabies Blueprint, www.caninerabiesblueprint.org):

R: Registration of Pet animals

S: Socio-cultural. It greatly influences the perceptions about rabies and dog-keeping practices being followed by at-risk populations. Activities include many aspects viz., level of awareness of rabies, responsibilities of dog ownership, precautions to ward off dog bites, post bite treatment, and community engagement.

T: Technical. The activities included in this pillar are: efficacious vaccines and vaccination programmes/strategies, logistical facilities, diagnostics support and effective disease surveillance.

O: Organization. Fighting rabies can be a very good model that is fit for One Health (OH) activities and easily help in the promotion of OH concept through multisectoral coordination, governance, monitoring and evaluation.

P: Political. For the elimination of rabies political will and support are critical. Activities include international support, legal frameworks and regional engagement.

R: Resources. For the ultimate elimination of rabies, a sustained long-term support is necessary. Under this framework the investment in rabies control, as well as the development of a work plan that encourages the investments in the elimination programmes of rabies for overall global public good should be sustained.

Elements of Dog Rabies Control Program- A Road Map

- ***Establish a national strategy*** with focal points and committees to prepare, implement and monitor long-term plans for rabies eradication based on understanding of the local epidemiological characteristics, education and awareness campaigns, mass dog vaccination and provision of PEP or PrEP to populations at risk.
- ***Enhance inter-sectoral cooperation*** amongst veterinary services, public health and wildlife management to design an evidence-based approaches to the eradication of human and animal rabies.
- ***Symbiotic Support integration of rabies control activities*** into all levels of the health service, and align them with other public health or animal disease control programmes in One Health framework.
- ***Integrated delivery of rabies control measures*** may have wider benefits in terms of strengthening health and veterinary service delivery, particularly in remote areas and neglected communities, improving inter sectoral collaboration and building community trust.
- ***Stimulate cooperation*** with the pharmaceutical industry and institutions for the provision of vaccines, both human and veterinary, and technical cooperation ensuring correct storage, prompt delivery and appropriate administration of high-quality available vaccines.
- ***Seek monetary support*** from government, bilateral, multilateral, public and private agencies and other donors in the framework of technical cooperation or humanitarian aid, with provision of auditable accounts.
- ***Conduct campaigns and education programmes*** to increase awareness of the benefitsof responsible dog ownership, basic care of bites from animals with suspected rabies and avoiding exposure to animals, to keep them updated with latest advancements in disease control.
- ***Strengthen surveillance and diagnostic capacity*** to include rapid diagnostic tests and rabies notification systems.
- ***Institute effective cross-border*** collaboration for rabies control and elimination, for checking trans-boundary disease spread.

Need for Co-ordinated Efforts in Rabies Management

Rabies a vaccine preventable disease, still remains a challenge to be managed in India, despite, the availability of effective interventions, exhaustive researches and public awareness of the danger. For effective management on a nation-wide scale to achieve "Zero Rabies Death by 2030" a WHO campaign, coordinated effort from the central, state and local government bodies, medical and veterinary communities and the public becomes paramount. National dog vaccination programmes with better PEP require consistent and sustained commitment that may yield widespread health benefits, particularly for the rural poor through dedicated para-medical staff. By adopting 'One Health' approach, the cost of vaccination to control canine induced rabies in India and trans-continental can be reduced.

Currently, India has designated **Rabies Diagnostic Laboratories** namely

- Rabies Research Laboratory, Central research Institute, Kasauli
- Rabies Research-cum-Diagnostic Laboratory, GADVASU, Ludhiana
- National Centre for Disease Control (NCDC), New Delhi
- All India Institute for Medical Sciences (AIIMS), Jodhpur
- WHO Collaborating Centre for Rabies, Dept.of Neurovirology, NIMHANS, Bengaluru
- Pasteur Institute of India, Coonoor, Tamil Nadu
- KVAFSU- CVA-Rabies Diagnostic Lab, OIE Reference Laboratory for rabies, Veterinary College, KVAFSU, Bengaluru
- Institute of Animal Health and Veterinary Biologicals, Bengaluru
- ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Yelahanka, Bengaluru
- Zoonoses Research Laboratory and Rabies Diagnostic Laboratory, Madras Veterinary College, TANUVAS, Chennai
- State Institute for Animal Diseases (Department of Animal Husbandry, Kerala), Thiruvananthapuram
- College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur
- College of Veterinary and Animal Sciences, Kerala Veterinary and animal Sciences University, Pookode, Wayanad
- Mission Rabies Office, Veterinary Hospital Complex, Tonca, Miramar, Panaji;
- Bombay Veterinary College, Parel, Mumbai
- College of Veterinary Science & A. H., Anand Agricultural University, Gujarat
- ICAR- Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly

Recent designation of OIE Reference laboratory for rabies at Veterinary college, KVAFSU, Bengaluru is an epitome of success. During 2019-2020, this laboratory was twinned under the OIE twinning programme with the CDC, Atlanta, US and APHA, Weybridge, UK on ‘Strengthening diagnosis of rabies’. As a part of this programme, several national and international training programmes were conducted for harmonization of rabies diagnosis and surveillance in animals with special reference to dogs. Furthermore, the laboratory is involved in providing scientific excellence and technical inputs to South Asian and South East Asian countries. A network of animal rabies diagnostic laboratories is already in place in India under this OIE twinning programme.

Increasing the number of certified and/or accredited diagnostic laboratories for rabies can definitely help in accelerating the speed of screening and confirmatory process of diagnosis to kick start control/prevention strategies to be advocated.

Recommendations

A. Regulatory Framework and Program Management

- Inclusion of human rabies as a nationwide notifiable disease among animals in India
- Strict implementation of the legal provisions for licensing and regular vaccination of pet dogs.
- Assessing rabies titre in pre and post vaccination for deciding in dose-regimen to cut down cost and assess the efficacy.
- Control of stray dog population through effective implementation of animal birth control (ABC) programs and mass vaccination.
- Effective enumeration of stray dog population regarding vaccination and herd immunity.
- Campaigns and education programs to increase awareness on responsible dog ownership, basic cares of animal bites and avoidance of unnecessary contact with unknown/stray animals.

B. Surveillance and Diagnosis

- State of the art surveillance system and diagnosis of wildlife, livestock and human rabies in the country to identify and control the source of entry of virus.
- Introduction of point of care diagnostics at the field level for early and immediate diagnosis.
- Information on dog ecology and population dynamics is also needed in order to decide on whether to include oral vaccination into the rabies control programme and, if that is the case, to develop effective means of bait delivery.
- This includes acquisitions of data from proposed rabies control areas relative to the proportion of owned dogs in the entire dog population and their levels of confinement (from totally restricted to permanently free-ranging).
- The proportion of unowned dogs and where they are cohabiting.

- The annual dog population turn-over so that along with vaccination rates, the frequency of campaigns for parenteral vaccination and/or vaccine bait application can be determined.
- The size of dog populations and the logistics associated with means of transport, movement and numbers of available vaccination teams, baits, and other supplies will also determine the efficacy of vaccination, both parenterally and orally, or in combination.
- Diagnostic assays must be evaluated to identify vaccinated animals, to identify vaccinated animals with protective immunity, and to identify free-ranging wild animals previously exposed to rabies infection.
- Characterization of the circulating virus for effective prevention and control.
- Specificity (based on the vaccine antigen and assay antigen relationship), sensitivity, accuracy (including linear range) and precision of different assays may vary among species. Standardization and quality control of reagents and procedures, whether kits or manual procedures are absolutely essential. These criteria should be evaluated before assigning the assay as fit for purpose.
- Timing of sampling for antibody response to oral vaccine bait uptake should be optimized to target the initial sero-conversion peak period, because this point predicts (given the appropriate cutoff and assay for the target species) survival as well as or even better than the level measured just before challenge.

C. Vaccination Strategy

- In order to develop a dynamic model to estimate the effectiveness of a health intervention in a dog population, partitioning of the process of canine vaccination into five elements namely, allocation of vaccines, and accessibility of dog populations, vaccine efficacy, vaccination effectiveness and summation of distributed vaccines will aid in estimating the effectiveness of a user-defined vaccination campaign.
- Reinforcing the on-going awareness programs for the public, veterinarians and medical community regarding dangers of inadequate management of animal bites and the importance of proper wound care and post-exposure vaccination with modern tissue-culture vaccines and the administration of human rabies immunoglobulin.
- Easy access to cost-effective modern tissue culture vaccines and popularization of intradermal PEP (human) and oral bait vaccines to reduce the cost and amount of vaccine dosage requirement for the poorer section of the society, livestock and pet or unowned animals that constitutes a major portion of high-risk victims and reservoirs.
- Continuous supply chain of rabies immunoglobulin and vaccines for PEP in humans and animals, with adequate cold chain facilities.
- Quality control of commercially available vaccines to avoid vaccination failure in both

animals and humans.

- In-depth research is required to identify and understand how rabies antigens are initially presented to influence the subsequent development of antibody responses. This can help to identify ways in which the response to prophylactic vaccination can be enhanced and how the natural immune response to infection can be boosted to prevent/reduce neuro-invasion.
- Virus strains used in oral mass vaccination of dogs should be identifiable by standard laboratory methods.
- Major carnivores for which oral vaccination techniques are desired will include: dog, red fox, wolf raccoon, raccoon dog, mongoose and jackal species etc.
- Baiting system guidelines will vary widely depending upon climate, target species ethology, target and non-target species characteristics, and urban versus rural environments, to be advocated with caution.
- Inclusion of pre-exposure vaccination to the routine childhood and/or susceptible livestock immunization schedule should be considered.
- Guiding about proper dose-regimens to be holistically followed.

Conclusion(s)

Rabies control programmes hinges on involvement of multiple public health agencies and sectors, so that an effective inter-sectoral cooperation using "One Health" approach can offer practical evidence-based solutions. To achieve control and eventual elimination of rabies requires vaccination coverage of at least 70% susceptible population to maintain the required level of herd immunity despite huge dog population turnover (births, deaths, animal movement) in the period between the campaigns. Mass parenteral canine vaccination campaigns (preferably annual) with vaccines meeting international standards are the mainstay of dog-mediated rabies control. Oral rabies vaccination (ORV) of dogs may be considered as a complementary measure to improve overall vaccination coverage when restraining of dogs is difficult. Evaluation of the coverage in all target areas should be carried out routinely supported by appropriate epidemiological counselling ensuring achievement of the envisioned goals. It is important that rabies vaccination programmes adequately accommodate responses to changes in epidemiological conditions. Greater community awareness, engagement and mobilization can improve/influence the turn- out for vaccination campaigns, their cost-effectiveness and sustainability and the surveillance and management of rabies cases. Service of professional dog handlers (who are properly vaccinated) to catch and restrain dogs humanely for vaccination can offer better coverage.

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