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Animal-Origin Viral Zoonoses

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ISSN 2662-4346 ISSN 2662-4354 (electronic)
Livestock Diseases and Management
ISBN 978-981-15-2650-3 ISBN 978-981-15-2651-0 (eBook)
https://doi.org/10.1007/978-981-15-2651-0

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

In the present context of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), causing Coronavirus Disease (COVID-19) nothing is crucial than the discussions over the emergence of human viral infections having natural or intermediate animal hosts. The ongoing COVID-19 pandemic has altered us for making future preparedness plans in tackling evolving and upcoming disease which could arise in days to come. Bats, as well as several other wild animals like palm civet and pangolins, have been linked with the emergence of zoonotic viruses with the accumulation of genetic changes. With reporting of COVID-19 in a pet dog from his infected owner has further unlocked a window of dialogue on reverse zoonoses (human to animal transmission). The ongoing pandemic of SARS-CoV-2, as well as previous epidemics of coronavirus SARS-CoV of 2002 and MERS-CoV of 2012, has socially and economically affected the world.

Furthermore, to name a few more, the viral diseases/infections such as Rift Valley fever, West Nile fever, avian influenza A (H5N1), Nipah virus, Zika virus, and swine influenza A (H1N1) are frightening adversely public health globally. Therefore, the viral infections having zoonotic links became the researcher's prime choice. The restricted availability of safe and inexpensive prophylactics and therapeutics forces us to depend mainly on the control and preventive measures for limiting the transmission of emerging zoonotic viral diseases. In the current scenario, utmost need realized is for developing the capacity building for detection and differentiation of the pathogen, developing rapid, sensitive, and cost-effective door-step assays/kits, and strengthening of regional and peripheral diagnostic laboratories and clinical and surveillance of the diseases in the susceptible and in-contact animal populations.

An up-to-date resource is essential for the public and research community to apprehend the latest information and trends in the field of emerging zoonotic viruses that might help to adopt corrective actions. In the current compilation on "*Animal-origin Zoonotic Viruses*," we intend to deliver a conversant resource in this area. The collection highlights the consequence of zoonotic viral diseases to the public and livestock industry using apposite examples. This book describes the precise and up-to-date information on zoonotic animal viral diseases which have emerged in the

recent past or are re-emerging due to several complex environmental factors. Decisively, the chapters delineate current day information on the emergence and circulation of zoonotic animal viral diseases with a focus on the virus, diseases, hosts, diagnostics, prophylactics, and therapeutics. The book discusses important viruses/viral infections of public health concern in various chapters authored by national and international experts. Moreover, the book provides the essential information in the form of tables and figures, with specific references at the end for readers to obtain further details on each topic.

In total, fifteen chapters in this book are covering important zoonotic animal viruses and wild animal's role in the spread of zoonoses, including drivers of emerging viral zoonoses. The first chapter (Chap. 1) by Dr Isloor and coworkers provides an overview on the oldest and most discussed zoonotic viral disease "*Rabies*," highlighting the significance of diseases, its current worldwide status and detection ways, whereas Chap. 2 on the *Monkeypox virus* by Nikola Sklenovska provides a brief overview of virus epidemiology, immunopathobiology, and diagnostics. Likewise, comprehensive information is provided on the *Nipah virus* in Chap. 3 by Dr Saxena. Calicivirus poses severe threat globally as a cause of acute gastroenteritis in young and adults. In Chap. 4, Dr Ghosh and colleagues discussed the progress on *Animal Caliciviruses*. Influenza disease, a century-old problem, still possess a threat to the public and livestock. An overview of the *Avian Influenza virus* is given in Chap. 5 by Dr Nagarajan and associates, and Dr Saxena's team have provided an overview of the *Pandemic Influenza A virus (pH1N1)* in Chap. 6.

The burden of poxviruses is tremendous in humans and animals. Dr Amit Kumar and team in Chap. 7 have provided a comprehensive overview of the *Buffalopox virus*. In Chap. 8, *Animal Rotaviruses*, which come under the family *Reoviridae,* is discussed by Dr Vlasova's team. This chapter mainly focuses on rotaviruses affecting different animal hosts, and a few of them are also zoonotic, explaining their epidemiology, diagnosis, and control. The next chapter (Chap. 9) by Dr Venkatesan's group elaborates the *Capripoxvirus and Orf virus*, giving its current situation globally. These two viruses are well known for their economic burden in small ruminants rearing countries. In the subsequent chapter (Chap. 10), Dr Vassilis Papatsiros has overviewed *Hepatitis E viruses* which have become a big problem during these days, having relevance to animals. Dr Hemida in Chap. 11 explains about MERS-CoV that affected humans and involved camels in their transmission cycle during the outbreaks occurred initially in 2012 in Saudi Arabia and nowadays well discussed during the ongoing pandemic of SARS-CoV-2. In the next chapter (Chap. 12), Dr Das and associates provide a detailed account of the *Japanese encephalitis virus,* the economically significant encephalitis disease, where swine acts as an amplifier host.

Dr Naveen's team has dealt with *Picobirnavirus*, a small newly identified virus, affecting several animal hosts as well as human beings in Chap. 13. It is now recognized as an emerging virus problem related with coinfections and immunocompromised individuals. A detailed account on *Drivers of Emerging Viral Zoonoses* is discussed in Chap. 14 by Dr Ghatak and team. The human–wildlife interface is considered highly significant on account of the emergence of different pathogens.

The last chapter (Chap. 15) is on *Viral Zoonoses: Wildlife Perspectives* by Dr Milton and colleagues.

We believe that owing to the in-depth knowledge of crucial zoonotic animal viruses with high-quality contributions by experts, the present book will be an excellent source of information for the readers. The information compiled would be useful for veterinary professionals, clinicians, public health experts, researchers, students/scholars, animal producers, faculty, and students. Further, it would help those who have an interest in virology, viral diseases, epidemiology of viral infections, viral zoonoses, and management of viral diseases and epidemics, for countering important animal viral diseases.

We, the Editors, would like to express our gratefulness to all the contributors for their support and hard work in making this book compilation a realism. We also extend special thanks to all the peer reviewers whose competent expertise and rigorous reviewing of the manuscripts helped the authors to improve their manuscript further to reach the publication phase. The Editors are grateful to the Springer Nature Publisher for accepting the book proposal. We extend our special thanks to Dr Bhavik Sawhney, Associate Editor, Biomedicine, Springer Nature for providing all the editorial help and high cooperation while processing the manuscripts for its successful publishing.

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World Society for Virology

"Animal-Origin Viral Zoonoses" a publication from World Society for Virology.

About World Society for Virology

World Society for Virology (WSV) is a non-profit organization, 501c3-ID No. 001303257 that was established in 2017 with the mission to strengthen virology research on different viral diseases of humans, animals, plants, and others.

The WSV main objectives include but not limited to:

1. Gather the virologists worldwide in the main society that does not require a fee for its membership [a great obstacle for many virologists in many countries] and provide help to all whenever possible.
2. Build up a network of scientific collaborations among virologists worldwide.
3. Build international bridges for virology laboratories worldwide.
4. Help virologists worldwide to advance their careers and obtain awards.
5. Provide educational resources free of charge and freely available to all members.
6. Help and facilitate getting scholarship and vacancies for virologists worldwide.
7. Build up databases of virologists based on their field of specialization for remote assistance and guide in case of the existence of any disease outbreak.

For details, visit www.ws-virology.org

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About the Editors

Yashpal Singh Malik is presently working as "ICAR National Fellow" at the premier Veterinary Institute of the country-Indian Veterinary Research Institute (IVRI), Izatnagar, India. His major research achievements include contributions in viral disease epidemiology, virus–host interactions, microbial biodiversity, characterization, and diagnosis. He acquired advanced training in Molecular Virology from the University of Minnesota, Saint Paul, USA; Division of Virology, Ontario Research Institute, University of Ottawa, Ontario, Canada, and Wuhan Institute of Virology, Wuhan, China. He is a recipient of several prestigious national, state, and academy awards/honors including ICAR Jawaharlal Nehru Award (2001); Young Scientist Award of the Association of Microbiologists of India (2000) and Uttarakhand Council of Science and Technology (2010). He is active member of noted scientific and professional societies of international and national repute. He has been bestowed with several honors in the form of distinguished Associateships/Membership viz. Associateship of National Academy of Agricultural Sciences (2010), Membership-National Academy of Veterinary Sciences (2010); CSIR-Senior Research Fellowship (1997–2000), ICAR-Junior Research Fellowship (1995–1997); Academic Merit Scholarship in bachelor's degree (1990–1995). He is elected Fellow of the Indian Virological Society, Indian Association of Veterinary Public Health Specialists, Indian Society for Veterinary Immunology and Biotechnology, and National Academy of Biological Sciences. Dr. Malik is

member International Committee on Taxonomy of Viruses (ICTV) on Birnaviridae and Picobirnaviridae study group and managing committee member of World Society for Virology. He has supervised 3 Ph. D. and 17 M.V.Sc. students. Over the years, he has developed several technologies and diagnostic kits and also has filed two national patents. He has authored 5 books, 25 book chapters, and published 2017 scientific research and review articles in peer-reviewed national/international journals of high impact factor. Dr. Malik has been the Editor-in-Chief of Journal of Immunology Immunopathology and also edited the special issues of several reputed journals.

Raj Kumar Singh is currently the Director-cum-Vice-Chancellor of the ICAR-Indian Veterinary Research Institute, Izatnagar. Dr. Singh is a noted scientist of high repute with specialization in veterinary microbiology, biotechnology, molecular epidemiology, diagnostics, and vaccinology. Dr. Singh has served as Head, Division of Virology, Station-in-Charge at IVRI, Mukteswar campus, Uttarakhand, and later Director, NRC on Equines and VTCC, Hisar. Dr. Singh has 10 national patents (granted-2 and filed-8), developed >8 live attenuated vaccines/vaccine candidates and >26 diagnostic tests/assays/kits. He has authored 2 books, 23 book chapters, and published over 245 scientific research papers, 52 reviews, 15 lead papers, and 24 guest editorials/compendium chapters. Dr. Singh has supervised 8 doctoral and 11 master's students. Dr. Singh received several prominent awards including prestigious ICAR Rafi Ahmed Kidwai Award and Team Research Award, DBT Tata Innovation Fellowship Award; Agriculture Research Leadership Award, FAO Fellowship for training at the University of California Davis, USA, and many others. He is serving as President ISVIB, besides having several distinguished Fellowships and life memberships of prestigious professional societies.

Kuldeep Dhama is presently working as Principal Scientist in the Division of Pathology at ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. He has research and teaching experience of more than 25 years in the field of microbiology, immunology, virology, public health, medicine, and biomedicine. He has developed several diagnostics, vaccines, and immunomodulatory modules to counter infectious diseases of animals and poultry. He has to his credit more than 600 publications, 6 books, and 65 book chapters. He has been recognized as an extremely productive researcher in the "Nature" journal publication. He is honored with 50 Best Paper Awards and other recognitions. He is NAAS (National Academy of Agricultural Sciences, India) Associate, worked as Nodal Officer—WTO, and Member—Wildlife Health Specialist Group (IUCN). He is actively serving as Editor-in-Chief, Co-EIC, Editor and Member, Editorial board of more than 20 scientific journals.

Chapter 1
Rabies

S. Isloor, R. Sharada, and S. Abdul Rahaman

Abstract Rabies is a viral disease of zoonotic importance, endemic in several countries in Asia, Africa, Western Europe and North and South America. The dog remains the most important source of infection in the countries of Asia, Africa and Latin America. Rabies is endemic in all the countries of the Indian subcontinent. This disease primarily affects the central nervous system producing abnormal behaviour and paralysis in most of the hosts it afflicts. DFA is most widely employed for post-mortem diagnosis of rabies. The development of dRIT is one of the most significant developments in the diagnosis of rabies. Further, LFA, an immunochromatography based tool, is a rapid test and highly useful for diagnosis of rabies at the field without the need for laboratory equipment. Recently, versions of RT-PCR and real-time PCRs including the LN 34 real-time PCR are becoming popular in molecular diagnosis and epidemiological studies. Further, the rabies virus neutralization tests (FAVN or RFFIT) are considered to be the gold standards to assess the anti-rabies vaccinal antibodies. As an alternative, quantitative ELISA is used. Rabies diagnosis in animals is revolutionized through recent OIE initiatives in India through twinning programme with a mandate of "Strengthening of diagnosis of rabies in animals in India". To achieve control of rabies in animals, particularly dogs, a co-ordinated multipronged approach involving various agencies is necessary. There is need to evolve the programme for vast rural India with emphasis on regular booster vaccination and seromonitoring vaccinal antibodies.

Keywords Rabies · Dogs · Diagnosis · DFA · dRIT · LFA · RFFIT · ELISA · OIE twinning · Control

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Y. S. Malik et al. (eds.), *Animal-Origin Viral Zoonoses*, Livestock Diseases and Management, https://doi.org/10.1007/978-981-15-2651-0_1

1.1 Prologue

Rabies derives its name from the Sanskrit word "Rabhas" meaning "to do violence" which describes the furious form of the disease. This neurotropic virus infects all warm-blooded animals causing a serious disease of fatality involving the central nervous system and has been documented for more than 4000 years (WHO 2018a, b). References about human deaths from bites of mad dogs exist in the Babylonian legal codes in 2300 BC. Democritus described the disease in dogs and domestic animals in 500 BC. The Iliad of Homer (eighth century) describes a character Homer, who suffered from rabies. In 1804, Zinke first demonstrated the infectious nature of saliva by inoculating it from infected animals to healthy dogs. In 1885, Louis Pasteur developed, tested and used the vaccine against rabies without knowing the nature of the virus which led to the era of prevention of diseases by vaccination. Remlinger first demonstrated the filterability of this infectious agent in 1904, and in the same year Negri demonstrated the intracytoplasmic inclusion bodies, later known as "Negri bodies" in brain tissues of rabid dogs. The rabies virus was adapted in cultures of non-neuronal cells by Kissling in 1958 which led to large scale propagation of virus in cell cultures for vaccine production (Sarma 2009).

Globally, the dog is the common source of exposure of human beings to rabies virus. Other mammals, especially wild carnivores and bats also pose a threat as they are reservoirs of the virus (OIE 2013; Birhane et al. 2017; Rupprecht et al. 2017). Wild animals such as jackals, foxes, mongoose, rats, squirrel, wolves, skunk, vampire bats act as natural reservoir hosts.

1.2 Epidemiology

Rabies occurs in all countries except Japan, UK, Ireland, Cyprus, New Zealand, Scandinavia, Hawaii islands, Caribbean islands, Australia and Switzerland. The disease is endemic in many countries of Africa, Asia, North and South America and Western Europe. Two epidemiological cycles are established in rabies, namely the urban and the sylvatic cycle. The urban cycle is maintained in dogs and transmitted to other species through bite of a rabid dog. The dog remains the most important source of infection in the developing countries of Africa, Asia and Latin America (Zee and MacLachlan 2005). The sylvatic cycle is maintained amongst wild animals which can result in spillover infections to the domestic animals and man. Based on susceptibility, the hosts are broadly classified into four categories; most susceptible are the fox, coyote, wolf, jackal and voles. Skunk, bat, mongoose and cattle are considered increasingly susceptible. The moderately susceptible hosts are dogs, sheep, goat and horses; birds are said to be least susceptible. Foxes are important reservoirs in Western Europe, portions of Canada, Alaska and the desert south-western regions of the USA, whereas skunks and raccoons act as reservoirs in regions of North America. In Asia and Africa, mongoose is the reservoirs. Recently,

the first case report of rabies in a wolf (*Canis lupus pallipes*) from India was documented based on laboratory evidence (Isloor et al. 2014). The first confirmed case of rabies in a sloth bear (*Melursus ursinus*) from India was reported by Patel et al. (2018) from Gujarat state. Cattle and equines are considered dead-end hosts. Rats and bandicoots are naturally susceptible. Laboratory animals such as mice, rabbits and guinea pigs can be infected experimentally (Sarma 2009).

1.2.1 Global Scenario

Globally, based on the prevalence of rabies, the countries have been classified as (a) countries with enzootic canine rabies—Asia, Latin America, Africa, (b) countries where canine rabies is under control and wildlife rabies is prevalent—Western Europe, Canada, the USA, (c) rabies free countries—most islands, Australia, England, Japan (De Serres et al. 2008). In America, various species of insectivorous and vampire bats harbour the rabies virus, and 30 different variants of the virus are identified in various species of bats in North America (Nadin-Davis et al. 2001; Nadin-Davis and Loza Rubio 2006) which can spill over to other species of animals in the wild. The rabies virus is maintained in domestic dogs, vampire bats and insectivorous bats in sylvatic cycles in South America (Favi et al. 2002; Kobayashi et al. 2005). The virus is also reported in monkeys, wolves, coyotes, skunks, foxes and mongooses (Belotto et al. 2005; Everard and Everard 1988) in South and Central America. The discovery of Australian bat lyssavirus (ABLV) in 1996 made Australia lose it "rabies-free" status. ABLV was isolated from insectivorous bat and four species of flying fox bats following the death of a ten-year-old girl with clinical signs of rabies (Gould et al. 2002). The last case of dog transmitted rabies was reported in 1867, and since then Australia was considered "rabies-free" though incidences of dog rabies were occasionally reported which were attributed to importation of the dogs (Warrilow 2005). In Europe, intense vaccination campaigns were successful in controlling dog rabies in 1940s, but wild canines escalated the incidences of rabies since then. Terrestrial rabies is under control in countries such as the United Kingdom, Finland and the Netherlands through strict vaccination campaigns (Bourhy et al. 2005). There was a paucity of information regarding rabies in the African continent until late twentieth century. Rabies occurs as scattered foci and is spread by dogs in sparsely populated countries spanning the Saharan desert; occasional cases in camels have been reported (Swanepoel 2004). The rabies-related viruses, viz. Mokola, Lagos bat and Duvenhage virus have been presumed to be in circulation since hundreds of years in the African continent (Nel and Rupprecht 2007). The World Health Organization (WHO) has reported 23,700 human rabies deaths per annum in Africa (WHO 2013) which is reported mostly in poor rural communities and children and is attributed to the inadequate and costly necessary resources for rabies prevention and treatment.

1.2.2 Rabies in the Indian Subcontinent

Rabies is endemic in all the countries of the Indian subcontinent comprising of Afghanistan, Bangladesh, Bhutan, India, Nepal, Maldives, Myanmar, Pakistan and Sri Lanka. It is estimated to have claimed the lives of about 59,000 people every year (Hampson et al. 2015) with an estimate of 45% of all deaths due to rabies reported in the Indian subcontinent (Gongal and Wright 2011). Of these, about 20,000 are in India as per the WHO survey report of 2004. However, in India, the current estimates of death in human beings due to rabies is ranging from 17,000 to unconfirmed 10,000. The high prevalence of rabies in the subcontinent is attributed to a lack of awareness of post-exposure preventive measures such as washing of wounds, vaccinations and administration of immunoglobulins. Furthermore, poor supply of anti-rabies vaccine and rabies immunoglobulins (RIGs), especially in rural health-care facilities and expensive vaccines and RIGs are other contributory factors. In Bangladesh, rabies is endemic and is third in the list of countries that are endemic for rabies (Hossain et al. 2011). Annually, more than 2000 deaths in human beings are reported (Hossain et al. 2012) with most of the victims reported being children of less than 15 years of age from the poor rural population (Hossain et al. 2011, 2012). A surveillance study during 2010–2012 reported deaths due to rabies in population of several domesticated animals including cattle (2845), goats (547) and sheep (Salahuddin et al. 2016; Mondal and Yamage 2014). Interestingly, this surveillance did not document the cases of rabies in dogs. In Bhutan, only one human death was reported in 2011 (Pelzang and Tshewang 2011). In Maldives, there are no dogs and rabies is not reported in either humans or animals. However, the cats and bats are the potential threats in future. There is an active rabies awareness programme instituted by the government. In Nepal also majority of the human rabies deaths are attributed to dog bites. The reported human deaths are 37 per annum (Singh and Shrestha 2011). In Afghanistan, during 2010, 40 rabies deaths were reported. However, estimates of human rabies deaths were reported to be higher in the provinces with poor vaccination coverage (Hidaythullah 2011). In Myanmar, annually, approximately 600,000 human beings are bitten with most of them being children and annually estimated 1000 deaths. In this process, each month 2500 dogs were killed by the Yangon City Development Committee. This drew criticism from residents and animal rights activists. In India, the prevalence of rabies is high. While the exact number of rabies deaths is unknown, with estimates ranging from 10,000 to 17,000 (Hampson and Meslin 2013) it accounts for 36% of the world's deaths. Incidence of rabies is higher in Indian rural areas (1.8 per 1000) compared to urban areas (1.4 per 1000), and thousands die every year from the disease (Sudarshan et al. 2007). In India, a significant reduction in the number of human deaths due to rabies could be achieved through targeting with preventive campaigns including preventive vaccination of animals and post-exposure vaccination of humans (Suraweera et al. 2012). In India, the Association for Prevention and Control of Rabies in India (APCRI) in collaboration with World Health Organization (WHO) conducted a study to assess the burden of human rabies in 2004. In the study, it was found that the annual

incidence of human rabies was estimated to be 17,137 and an additional 20% was added to this estimate to include atypical forms. The primary animal responsible for bites was dog (96.2%), most of which were stray (Sudarshan et al. 2007). As a part of recently completed WHO-APCRI Indian multicentric rabies survey in 2017 (Sudarshan et al. 2018), laboratory-based surveillance for the status of rabies in dogs/cats was carried out in the islands of Andaman/Nicobar and Lakshadweep. The islands of Andaman are historically free from rabies but have considerable dog population. Whereas the Lakshadweep islands are not only free of rabies but also dogs. However, there is sizeable population of cats in Lakshadweep. The initial dog's brains ($n = 4$) from Andaman and cat brain samples from Lakshadweep ($n = 5$) screened were negative for rabies.

1.3 Classification

Rabies is a disease caused by the rabies virus of the genus *lyssavirus* of the family *Rhabdoviridae*, order *Mononegavirales*. The lyssa viral species are divided into two phylogroups based on their genetic distance and serological cross-reactivity. The rabies virus belongs to the phylogroup1 (ICTV 2017; Rupprecht et al. 2002). The other lyssaviruses produce disease like rabies and bats are an important reservoir host of several of these viruses (Zee and MacLachlan 2005). Monoclonal antibody analysis has demonstrated considerable antigenic variation among virus isolates from outbreaks associated with different wildlife vectors or from different geographical areas (Debbie and Trimarchi 1992; Sarma 2009).

Highly virulent strains of rabies virus isolated from naturally occurring cases are referred to as "Street viruses". The attenuated laboratory strains are referred to as "Fixed viruses". These are street viruses adapted to the laboratory by passaging them in unnatural hosts like rabbits or in cell culture. These strains differ from each other in their biological properties in laboratory animals, for example, virulence, incubation period and distribution and nature of lesions in target tissues. The street viruses have a long incubation period, an affinity for salivary glands and produce intracytoplasmic inclusion bodies but do not produce any cytopathic effect in cell cultures. The fixed strains have short and defined incubation period of 5–7 days and are more neurotropic for rabbits but have no affinity for the salivary glands; they multiply faster and are stable viruses. They are comparatively less pathogenic and do not produce inclusion bodies, thus are used for vaccine production. These can infect only by the CNS route. Further, these are not seen in the saliva and other peripheral secretions. For example Flury strain, Street Alabama Dufferin (SAD), Vnukovo, Kelev. The antigenic variation between the strains can be distinguished by their reaction with monoclonal antibodies.

1.4 Virion Properties

Rabies virus is a bullet-shaped (80 nm × 180 nm), an enveloped, negative-sense—single-stranded RNA (11–15 kb) virus. The nucleocapsid is helically coiled and cylindrical in shape. The genome of rabies virus codes for five different viral proteins, namely nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA dependent RNA polymerase (L) in the order 3′–5′. The RNA exists as ribonucleoprotein (RNP) with the N protein tightly encasing the RNA. The N protein is present in abundance and is an important structural component of the viral ribonucleoprotein core which is required for propagation of virus. Furthermore, N protein is the primary target for diagnosis of rabies. The phosphoprotein is a cofactor component of the viral polymerase. The M protein facilitates virion budding by linking the nucleocapsid to the lipid envelope that contains the G glycoprotein. The glycoprotein is the major surface protein and is peplomers or spikes seen on the surface of the virion. These form approximately 400 trimeric spikes or peplomers of 6–7 nm in length which are closely aligned on the viral envelope. The G protein is highly antigenic and has the epitopes for vaccine-induced neutralizing antibodies and rabies immunoglobulins (RIGs). The G spike protein controls major aspects of host cell infection such as receptor binding, antigenicity and host adaptation. Moreover, it is involved in the trans-synaptic spread within the central nervous system. The nucleoprotein includes epitopes involved in cell-mediated immunity. The polymerase aids in transcription and replication of the virus (ICTV 2017).

The lipid component of virions is derived from the cell membranes of the host. The virus contains approximately 67% proteins, 26% lipid, 4% RNA and 3% carbohydrate and has a buoyant density of 1.19–1.20 g/cm^3 in caesium chloride and 1.17–1.19 g/cm in sucrose gradients. The sedimentation value ranges between 500 and 1000S (Sarma 2009; Chandra et al. 2015). It is heat-labile and inactivated by heating at 56 °C for 30 min. The virus is ether sensitive and readily inactivated by exposure to sunlight or UV radiation, formalin (1%), cresol (3%), beta-propiolactone (0.1%), mercuric chloride (0.1%), aqueous solutions of household bleach, quaternary ammonium compounds and hospital disinfectants. The virus persists in the infected brain tissue for up to 10 days at room temperature and several weeks at 4 °C but is relatively susceptible to disinfection. It can be preserved indefinitely at −70 °C and by freeze-drying. The virus is susceptible to pH below 7.0 and above 10 (Debbie and Trimarchi 1992; Zee and MacLachlan 2005; Chandra et al. 2015). Laboratory adapted strains haemagglutinates goose erythrocytes at 0–4 °C and pH 6.8 (Sarma 2009).

1.5 Viral Replication

The rabies virus infects all warm-blooded animals and replicates in them. Thus the virus can be propagated in chick embryo or duck embryo or various cell culture systems like baby hamster kidney cells (BHK21), human diploid cells (WI-26), mouse neuroblastoma cells (MNA) (Zee and MacLachlan 2005). The glycoproteins are essential in the entry of rabies virus into the susceptible cells through receptor mediation. The virus is taken in by pinocytosis via the clathrin-coated pits in vesicles of cytoplasm. The fusion of viral membrane with the endosomal membrane is dependent on pH after it is endocytosed by a process known as viropexis. This results in release of the ribonucleoproteins (RNPs) by uncoating (Roche and Gaudin 2004; Gaudin 2000). The RNA is synthesized by viral origin polymerases L and P through stuttering transcription. In the replication mode, complete length ribonucleoproteins are generated. Further, the structural components of the viral envelope, the matrix protein and glycoproteins are necessary for assembly and release of matured virus particles (Rose and Whitt 2001). The virions mature as they are released by the budding through the plasma membrane. The rabies virus buds through the intracytoplasmic membranes of the infected neurons or plasma membranes of the salivary glands and epithelial cells. The rabies virus replicates slowly and usually does not induce cytopathic effect (CPE) as it does not shut down the synthesis of host cell protein and nucleic acid synthesis. However, it results in formation of prominent inclusion bodies that are of diagnostic importance. The M protein is an important factor in budding of virus and a regulatory protein (Finke et al. 2003; Finke and Conzelmann 2003).

1.6 Clinical Features

The clinical signs of rabies are similar in most species but vary between individuals. The incubation period is prolonged, varying from 2 weeks to 6 months and even longer in some exceptional circumstances (WHO 2018a, b). The CNS disturbance manifests as behavioural changes like nervousness, irritability, hyperexcitability, ataxia, altered phonation. The affected animal prefers to be in isolation. A change in temperament of the animal is a common feature noticed in rabies infections wherein a docile animal becomes vicious or aggressive, and an aggressive animal turns docile. The clinical signs of the disease with minor exceptions for different species can split into three phases that could be overlapping at times. A prodromal phase exists which lasts for 1–3 days before the overt clinical disease which is normally overlooked. In this phase the animal shows only vague clinical signs which intensify rapidly after the onset of paralysis. The affected animal succumbs to death within 10 days of onset of clinical signs. Excitative phase referred to as the furious form of the disease follows the prodromal phase and is the most commonly encountered form. It is also referred to as the “Mad-Dog Syndrome”. The animal exhibits

restlessness with nervous signs and could be aggressive. It turns aggressive on slightest provocation (of sound or noise) and uses its teeth, claws, horns or hooves and thus bites at anything that gains its attention. The affected animal will exhibit an alert posture with dilated pupils and an anxiety expression. It loses the sense of fear, swallows foreign objects and shows hyperaesthesia. Carnivores tend to roam aimlessly attacking any moving objects or personnel on its way. As the disease progresses, furious symptoms reduce and paralytic signs sets in, incoordination and seizures are seen and the animal finally succumbs to death due to progressive paralysis within 2–14 days after the onset of clinical signs. Paralytic phase also termed as the dumb form manifests as hydrophobia and profuse salivation or inability to swallow due to paralysis of the pharyngeal muscles. Dropping of lower jaw is characteristic. The animal remains dumb and rarely bites, thus posing a risk of infection. The paralysis progresses rapidly resulting in coma and death within few hours.

1.6.1 Dogs

The incubation period in dogs vary from 10 days to 2 months, and the clinical signs attributable to the CNS are paramount in dogs. There may be hyperexcitability or lethargy, pharyngeal paralysis and thus frothing of saliva, posterior paresis or paralysis, sudden coma and death. Behavioural changes are common during the early phases of the disease when the dog behaves abnormally, hides in dark corners, shows unusual agitations, becomes restless. Fever, dilatation of the pupils and photophobia are sometimes present. The furious form follows the prodromal phase and the affected dogs may bite without any provocation. It may bite itself and inflict serious injuries. Some dogs exhibit only a paralytic stage with the characteristic dropped jaw and incoordination. Progressive paralysis begins with the muscles of the head and neck region. The tone of bark changes due to partial paralysis of vocal cords. Convulsions are seen in the terminal phase followed by incoordination and posterior paresis. Once the clinical signs set in, the disease progresses rapidly to the death of the animal due to respiratory failure generally within 3–8 days. It is during this clinical period and up to 5 days before recognition of clinical signs that the virus may be present in the saliva. This mandates the 14 days confinement and observation of a dog that has bitten a person or other animals (Zee and MacLachlan 2005). The excretion of virus in the saliva of infected dogs is intermittent and is variable (Hemachudha et al. 2013).

1.6.2 Cats

The clinical signs in cats are of a furious type and are similar to that in dogs, but the affected cats have a greater tendency to hide in secluded places and are more vicious

than dogs. The cat might strike in air with its forepaws. After 2–4 days of the excitation phase, the paralysis of posterior third of the body follows.

1.6.3 Cattle, Buffalo, Sheep and Goat

The incubation period may vary from 2 weeks to many months. In cattle, the prodromal signs may manifest as an animal being off feed and water and a drop in milk production which is of little diagnostic value. Lactation ceases abruptly, grinding of teeth, salivation or pharyngeal paralysis is often misdiagnosed as a choke. Cattle become aggressive with rabies infrequently. In furious form of the disease, the cattle loses its placid expression and becomes alert and restless. The eyes and ears of affected animal follow the sound and movement and thus butts moving objects, attacks man and animals nearby. Salivation, choking, absence of rumination, rectal straining and paralysis of hindquarters are noticed. There may be sexual excitement, and the animal starts bellowing abnormally due to vocal paralysis and intermittently until it succumbs to death within 12–24 h. Once the clinical signs are evident, the disease progresses rapidly to the death of the animal generally within 5–7 days. During this period and up to 5 days before recognition of clinical signs, the virus may be excreted in the saliva.

1.6.4 Horses and Mules

In horses, the signs are similar to tetanus. Initially, there is a weakness or lameness. The infected horses and mules appear distressed and agitated. They start rolling, which is normally confused with colic. The animal may bite or strike viciously and becomes unmanageable causing self-inflicted wounds. Tremors and spasms are noted in specific muscles. Difficulty in swallowing, progressive paralysis, stiffening of the hindquarters, ataxia and eventual death within 2–4 days.

1.6.5 Pigs

The symptoms are characterized by excitement, irritation, rooting up the ground or rubbing at the surface, aggressiveness, biting of hard objects, other animals and man followed by paralysis and death in 2–4 days.

1.6.6 Humans

Human infections are most commonly due to rabid dog bites. The early signs include headache, extreme thirst, vomiting and anorexia. Later, the painful spasms of the pharyngeal muscles when drinking (hydrophobia) is experienced. This is followed by excitement to sensory stimuli which progresses to generalized paralysis. Death is the inevitable outcome once clinical signs develop (Zee and MacLachlan 2005). The rabies virus can be detected especially in saliva, lacrimal secretions, urine and tissues of nervine origin, thus posing a risk when exposed to these secretions and excretions. Transmission among humans has been reported as a result of infected tissue or organ transplantation (Rupprecht et al. 2016; WHO 2018a, b). A single case of perinatal transmission has been reported (Aguèmon et al. 2016; Rupprecht et al. 2016).

1.6.7 Monkeys

In monkeys, clinical signs are similar to that exhibited in humans with hydrophobia, paralysis, anxiety. However, non-human primates do not play a major role in the transmission of rabies.

Herbivores do not transmit the disease. Exposure of the virus to mucous membranes and conjunctiva can result in infection, but infection through respiratory route is very rare. Inhalation of aerosols containing the virus in bat-infested caves can result in rabies. Transmission from bats is of increasing concern in the canine rabies-free areas since transmission can occur without any history of bite. The disease is also spread by frozen meat, urine and milk in bats. Intrauterine infections are reported in man, cattle, skunk, mice (Aguèmon et al. 2016; Rupprecht et al. 2016). Livestock is vulnerable victims of rabid carnivores and mongoose. In foxes, virus excretion is higher in urine and the nosing behaviour maybe a non-bite transmission mechanism in sylvatic rabies (Sarma 2009).

1.7 Pathogenesis

The rabies virus has an affinity to the nervous system (NS) (Wunner 1987) and reaching the CNS is critical for the virus to establish the infection. Various mechanisms are used by the virus to strategically evade the immune system of the host, but the detailed mechanism of evading the host and its pathogenesis during the early stages is not well understood. The glycoprotein and phosphoprotein have a major role in axonal transport. The virus cannot penetrate intact skin, and thus the disease transmission is most commonly by a bite. The susceptibility of the virus is less by oral route than the intramuscular route. Thus ingestion is not a common mode of infection. In the affected animals, the incubation period depends on various factors

like the location of bite (its proximity to the CNS), depth of the wound, load and virulence of the virus, susceptibility of the species of animal which is bitten, the stress condition and immune status of the animal all these play role in pathogenesis and course of the disease. The actual events that occur in the incubation period are not certain, but at the site where the viruses enter the host, the movement of the virus may get delayed. Hyaluronidase present in the saliva of biting animals particularly wild carnivores increases the permeability of the virus in tissues and eases the entry of virus. It is well proved that the nervous system is capable of sensing the attack of rabies virus and that it can also mount an immune response at the earliest.

The strains of rabies virus which cause acute infections escape the host innate immune response at least partially. The various mechanisms adopted by the virus for evading the immune surveillance explain why rabies remains one of the few infections with a mortality rate of almost 100%. (Jackson 2016). The virus enters the host through the bite wound or scratch (very rarely through mucous membranes). Following exposure, the virus is deposited and persists in the local muscle tissue for hours or days (3–9 weeks). The initial replication of virus occurs in the cytoplasm of the muscle cells or epithelial cells in the lower layers of epidermis near the bite. Since the replication is minimum at the site of bite, no detectable immune response is seen during the incubation period. Viremic phase does not exist since the virus does not move through the blood or lymphatics. On entry into the host, binding occurs at the postsynaptic muscle membrane to the nicotinic acetylcholine receptor (nAchR) which enriches the virus at the neuromuscular junction or synaptic cleft thus enabling an efficient infection of the motor neurons. Some studies indicate that infection of muscle cells might be aided by the nAchRs, suggesting initial virus replication in muscle cells. Further, the virus enters neurons through neural cell adhesion molecule (NCAM) and p75 neurotrophic receptor and is transported to the cell body through the axon in vesicles. Though two mechanisms are proposed for this movement of virus in vesicles as (1) whole virion or (2) only the virus capsid, the evidence favours intact virion transport in vesicles through the axons (Lentz et al. 1982; Dodding and Michael 2011). Once the virus enters the axon, antibodies cannot inhibit its transport, and the virus moves to the CNS at a rate of 12–100 mm per day by fast axonal transport (Kucera et al. 1985; Tsiang et al. 1991). The viral infection induces production of inflammatory cytokines and chemokines which in turn attracts activated lymphocytes leading to their migration through blood–brain barrier. Viral strains causing encephalitis tend to maintain the physical integrity of the neuronal network and the neurons to facilitate invasiveness right from the entry site to the site of exit producing a non-cytopathogenic kind of infection. The pathogenic strains are capable of inducing peripheral immunosuppression inhibiting an immune response and thus favouring its survival and invasion of the entire nervous system. The movement of the virus in the neurons involves the interaction of amino acid residues in the phosphoprotein at position 143 and 147 with the cytoplasmic dynein light chain (LC8) (Jacob et al. 2000; Raux et al. 2000; Poisson et al. 2001). The LC8, a component of myosin V and dynein is of 10 kDa. In the axons, this LC8 is associated with the actin-based vesicle transport and the microtubule directed organelle

transport. In the CNS, the rabies virus uses the axonal microtubules for its retrograde movement.

Furthermore, phosphoprotein (P) was identified to be responsible for inhibition of type I interferons. Experiments in mammalian cells have revealed that the P protein prevents the expression of interferon stimulating gene by interaction with STAT1 (Vidy et al. 2005). The virions ascend in the axons of nerve cells causing neuronal infection. Further replication or amplification of virus occurs in the dorsal root ganglion before its ascent to the brain from the spinal cord. The virions bud from the infected cell causing infection to the neighbouring cells. This centripetal passive movement causes ascending neuronal dysfunction and also forms the pathognomonic Negri bodies. The virions reach the CNS via the spinal cord and multiply extensively in the limbic system of the brain causing release of cortical control of behaviour resulting in fury and behavioural signs being exhibited. After proliferation in the brain, the virus disseminates within the brain. Multiplication of the viruses in the neocortex results in paralytic or dumb form. This is when the animal becomes anorexic, stays in the dark with profuse salivation. The virus can also persist in the brain of infected skunks, rats, raccoons, bats and foxes for many months without exhibiting any overt clinical signs. The virus further travels centrifugally through peripheral nerves to the salivary glands, retina, cornea, tonsils and nasal mucous membranes. The virus replicates rapidly in the salivary glands, and thus infected saliva is the major source of infection which precedes the clinical signs in some animals. The virus is excreted in all secretions and excretions.

The expression of neuronal dysfunction accounts for the clinical signs of rabies that causes the animal to attack (Debbie and Trimarchi 1992; Zee and MacLachlan 2005). The virus may be excreted through the milk of rabid animals, but its role in causing the disease has not been documented (WHO 2018a, b). If the cerebrum or cerebellum is not infected, it results in an abortive type of infections where no clinical signs are seen.

The ability of the rabies virus to induce very mild pathological changes in the CNS which indicates dysfunction and not the death of neurons is important in disease production though rabies is a neurological disease (Jackson 2002; Lafon 2011). The successful invasion of the CNS is attributed to the two important complementary characteristics of the virus (1) ability to escape from the host immune response and (2) ability to protect the infected neurons from apoptosis or premature destruction since the neurotropic viruses cause cell death by either apoptosis or necrosis (Griffin and Hardwick 1999; Allsopp and Fazakerley 2000; Fazakerley and Allsopp 2001). The glycoprotein (G) spikes present on the surface of viruses and its ability to bind to the receptors on cells determine the neuropathogenicity of the virus. The G proteins are also responsible for the induction of apoptosis. Thus the ability to avoid apoptosis improves the pathogenic potential of the virus and also correlates with the degree of attenuation of virus (Morimoto et al. 1999). The rate of replication of the virus and its glycoprotein expression levels correlates inversely with the pathogenesis, whereas the kinetics of virus uptake and its spread directly correlates to the pathogenesis.

The infected animal will have no antibodies when they first show the signs of illness. This virus does not induce the immune system and does not produce cytopathic effects. However, a detectable level of antibodies is seen in serum and cerebrospinal fluid (CSF) after 8–10 days of onset of clinical signs. Neutralizing antibodies are detected only during the terminal stages when the animal is about to succumb to death. Experimental studies have revealed that T lymphocytes from immunized animals are cytotoxic for rabies-infected cells and these rabies-infected cells are lysed by antibodies in the presence of complement (Sarma 2009). The T cell response resulting in antibody production is a crucial factor for clearance of virus from the nervous system and thus survival. The CD8+ T cells play a dual role by functioning together with the antibodies in controlling the infection by clearing viruses from the nervous system and also induce neuronal apoptosis. These cells thus initiate an immunopathological reaction associated with clinical paralysis (Jackson 2016).

1.8 Pathology

In general, gross pathological lesions are not visible in rabid dogs except fresh bite wounds and signs of self-mutilation. Microscopic changes are limited to CNS. The histopathology of brain tissue revealed moderate neuronal damage with encephalomyelitis and perivascular cuffing of lymphocytes, mononuclear infiltration and polymorphonuclear cells. Cytoplasmic eosinophilia, cytoplasmic vacuolation, pyknosis and karyorrhexis are more commonly seen in freshly fixed and adequately treated brain tissue. Acidophilic round or oval inclusions in the cytoplasm of infected neurons are seen with a clear halo around it which are referred to as Negri bodies. These are found in the pyramidal cells of Ammon's horns, Purkinje cells of the cerebellum and brain stem. A large number of small inclusions can also be demonstrated in the smears of brain tissue by immunofluorescence or immunoenzyme methods. Experimental inoculation of mice with fixed rabies virus may also show numerous virus particles without the formation of Negri body (Chandra et al. 2015).

1.9 Diagnosis

Accurate and rapid diagnosis is critical for initiating post-exposure prophylaxis and public health control strategies. Various methods are used for the diagnosis of the disease. However, proper collection and submission of post-mortem specimens with special reference to brain tissues from animals suspected for rabies constitute the basis for confirmatory diagnosis of rabies (Isloor et al. 2017).

1.9.1 Preliminary Safety

All individuals and laboratory personnel involved in the handling of rabies suspected cases should undergo pre-exposure immunization and regular boosters as required. These personnel are at risk of rabies infection through various means. Hence, personal protective equipment (PPE) must be used at all levels starting from necropsy procedure.

1.9.2 Agent Identification

As rabies virus tends to get rapidly inactivated, the specimens collected should be sent on ice to the laboratory by the fastest means available. Various techniques are employed to diagnose rabies and are particularly employed on brain tissue, but other organs such as salivary glands. For laboratory diagnosis, both cerebellum and brain stem are recommended to be collected since the virus will be present in abundance in these and aid laboratory diagnosis. These parts of the brain can be obtained after removing the entire brain through the skull open method during necropsy.

1.9.3 Collection of Samples

In a rabies-infected animal, the brain, spinal cord, saliva, salivary glands may contain the virus, and fresh, non-fixed tissue is acceptable for diagnosis of the disease. The brain tissue is the choice of specimen for rabies diagnosis, and thus the animal suspected for rabies should be euthanized in a manner such that the brain is not damaged. Animal heads are accepted for diagnosis; care should be taken so that the neck should be severed at the midpoint between the base of the skull and shoulders. Only veterinarians or animal control officers who have been vaccinated and perfectly trained should remove the animal heads. The post-mortem should be done in a ventilated area using protective gear. After opening the skull, appropriate samples like the brain stem and cerebellum are collected. This is a laborious task and hazardous too in field conditions or even when the prosector is not well trained. An alternate method of brain sample collection without the need to break open the skull has been developed and is referred to as the occipital foramen route of brain sampling.

Brain Sample Collection Through Occipital Foramen Route
The brain sample is collected through the occipital foramen by introducing a drinking straw of 5 mm or a disposable plastic pipette of about 2 mL or by using the artificial insemination sheath which is about 10 cm long into the foramen in the direction of the eye. Samples of brain stem and cerebellum can be collected from the juice straw or artificial insemination sheath (Fig. 1.1). This approach was reported to

Fig. 1.1 Collection of the brainstem from foramen magnum approach in dog

be user-friendly, rapid and risk-free for accurate diagnosis of rabies (Ghouse et al. 2018). This encourages collection and submission of more number of brain samples from the field for the laboratory confirmation.

1.9.4 Transportation of Samples to the Laboratory

The specimens collected shall be transported to the laboratory at the earliest either by post or by courier or by air as suitable. The specimens suspected for rabies should be shipped on ice in a leak-proof container to the laboratory so that it does not pose a threat of contamination. Any undue delay can wither away the cooling effect of ice especially in tropical climates enhancing putrefaction of sample making it unsuitable for diagnosis. If it is not possible to send the samples in a refrigerated condition, other preservation techniques may also be used. The preservative used shall be based on the tests to be employed for diagnosis. However, in most of the situations, either the brain sample may be packed as such without any preservatives or shipped in glycerol saline in refrigeration.

1.9.4.1 Transportation of Specimens without Preservatives

This is the most commonly used method of sending samples to a diagnostic laboratory. The suspected brain samples are first placed in a sealed, rigid container and then labelled. As soon as the head is separated from the body of the animal, it is placed in a small plastic bag. Before packaging, the specimen has to be cooled in a refrigerator. If only the cerebellum and brain stem are transported, these should be first placed in a small plastic container and then placed in a small plastic bag. The

entire head when collected should be first wrapped in absorbing paper and then placed in resistant plastic bag. The primary package is then placed in a secondary container which is also tightly sealed and further put in an insulated container preferably made of expanded polystyrene. Absorbing materials to prevent leakage and cooling materials are placed in this tertiary container and finally sealed with an adhesive tape. The information relevant to the sample is placed in an envelope and attached on the outer surface of the box. The box should be labelled clearly as "BEWARE! BIOLOGICAL SPECIMEN FOR RABIES DIAGNOSIS. INFECTIOUS HAZARD!"

1.9.4.2 Transport Using Preservative Solutions

Preservative solutions are used if transit time to the laboratory is long or if transportation on refrigerants is not possible. The laboratory technique that is used for diagnosis determines the preservative to be used.

- The use of formalin solution is safe since it inactivates the rabies virus, but the sample becomes unsuitable for isolation/inoculation tests. These specimens are suitable for FAT and histology.
- Specimens can also be transported in glycerine solution which does not inactivate the virus rapidly but is capable of inhibiting the growth of contaminants temporarily.

1.9.4.3 Preservative Solutions for Diagnosis

For transit over short distances, the specimens for diagnosis of rabies are sent on ice in wide-mouth leak-proof containers. If the transit time is longer, samples are placed in preservative solutions as described below

(a) One half of the brain in either 10% formal saline or Zenker's fluid and the other half in 50% glycerol saline.
(b) Salivary glands in 50% glycerol saline.
(c) CSF, saliva and urine are transported in tissue culture medium with 2% saline.
(d) Specimens for cytological tests or histopathological diagnosis are transported in 10% neutral buffer formalin or in Bouin's solution.

In packaging, the materials capable of causing injury shall be avoided.

1.9.4.4 Labelling of the Specimens

The specimen container should be properly labelled using permanent markers before dispatching it to the laboratory. The label should inform about the date of collection,