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RABIES: FALSE, FORGOTTEN AND FRESH FINDINGS*

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> The man recovered of the bite,
> The dog it was that died.
>
> *Oliver Goldsmith, 1766*

Despite the universal fear of rabies, knowledge of its natural history and cause remained virtually static for centuries. The link between mad dogs and horrific human deaths was established in antiquity but all else was anecdotal. Change occurred during the period in the eighteenth century in Europe known as the Enlightenment when the concept of contagion espoused by an English physician, Dr S.A. Bardsley,[1] slowly emerged. Fifty years later William Youatt,[2] a well-known and respected British veterinarian, adopted Dr. Bardsley's views and postulated that the British Isles could be rid of rabies if every dog was quarantined for eight months. Three decades later, Louis Pasteur[3] began his successful search for a post-exposure treatment. Thereafter, with the notable exception of the discovery of vampire bat rabies in Brazil by Carini,[4] our understanding of rabies hardly rose until the middle of the twentieth century.

The consensus in the 1950s was recorded by Dr T.F. Sellers in 1954 in a chapter in the 2nd edition of Harrison's *Principles of Internal Medicine*.[5] He wrote that rabies in humans was invariably fatal and that no authentic instance of recovery had been established. The vaccines in vogue were still Pasteurian in character being based on attenuation of rabies-infected nervous tissue. Sellers warned that if a patient had earlier received anti-rabic 'vaccine' then iatrogenic paralysis must be considered.

The scientific database at the mid-century was collated by C.E. van Rooyen and A.J. Rhodes and published in their textbook, *Virus Diseases of Man* in 1948.[6] In seven chapters they recorded the then-known clinical and pathological features of rabies in humans and animals. They stressed that once rabic symptoms developed the outcome was uniformly fatal. They sketched a hazy pathogenesis and identified the need for more research. Thereafter the geographical distribution and methods of spread were discussed, backed with a table listing the animals and birds that had transmitted rabies to man. A short section of the general measures considered necessary for the control of the disease followed; it mentioned prophylactic vaccination of animals but not of man. Other chapters covered diagnosis, the differentiation between street and fixed strains, and cited preliminary evidence on antigenic variation. The Negri Body had a chapter to itself that ended: 'We can only conclude that the exact nature of the Negri Body is not yet known'. Immune mechanisms are broached. They noted that even among known susceptible species an occasional animal resists infection. At the time of writing they recorded that the role of passively acquired immunity was controversial. Likewise, they noted a divergence of opinion as to whether 'rabicidal' substances in serum indicated resistance to infection or not. Animals, however, could be immunised by injection of live or inactivated virus. The last sections detail various methods of human post-exposure anti-rabies treatment and the dangers encountered.

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In the second half of the twentieth century the information curve on rabies accelerated exponentially. Sadly, only the current availability of safer rabies vaccines appears to have been taken on board by our policy makers who appear to be stuck in the lore of the 1950s. Rabies is not unique; the aetiological agent is a typical vector-transmitted Rhabdovirus, albeit the vector is an animal rather than an insect.

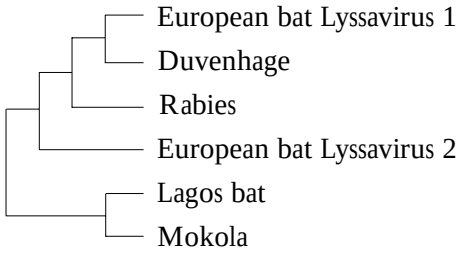
RHABDOVIRIDAE

The large Rhabdoviridae family of bullet-shaped, negative-sense ssRNA viruses has five genera, each of which contains several confirmed species and many tentative species. In addition, numerous other species have still to be assigned. Some multiply only in mammals and birds, fish, arthropods, or other invertebrates. Many infect plants and plant-feeding arthropods.[7] They replicate in the cytoplasm budding from intracytoplasmic membranes. The Negri Body is a cytoplasmic inclusion body specific for rabies virus. Defective RNAs that may contain functional genes are found in viral populations but they only replicate in the presence of homologous and, less commonly, heterologous, helper rhabdoviruses. The rhabdoviruses infecting domestic and wild animals belong to the Vesculovirus, Lyssavirus, and Ephemerovirus genera. A massive collaborative study by staff in laboratories routinely handling these viruses revealed that each genus has several serotypes, which cross-react to a greater or lesser degree irrespective of the genus; for example, 25 of 89 rhabdoviruses studied cross-reacted with rabies lyssavirus but it is not known whether infection with an ephemerovirus would provide protection against rabies.[8] The major mode of transmission is through the bite of an infected vector; animals transmit lyssaviruses, phlebotomine sandflies transmit vesciculoviruses and mosquitoes spread ephemeroviruses. All members of these genera may infect by other routes, e.g. animals grooming each other such that infected saliva is licked onto the mucosae of susceptibles,[9] aerosol deposition,[10] ingestion of infected carcasses,[11] transplacentally,[12] and, sadly, from transplanted corneal grafts.[13]

LYSSAVIRUS GENUS

Rabies virus is the prototype of the lyssavirus genus. There are six official serotypes (Table 1) that share amino acids, express nucleotide homology with rabies virus, and provoke clinical disease that mimics rabies encephalitis.[14]

TABLE 1
Amino-acid homology of Lyssavirus serotypes.[14]



The virions, like the virions of other rhabdoviruses, have five structural proteins.[7] They are differentiated by using monoclonal antibodies, neutralization tests with virus-specific sera, and genetic typing.[15,16] In addition to the officially recognised lyssavirus serotypes several other ill-defined candidates for the genus are suspected: for example, Oulou fato, a pathogen affecting dogs in West Africa[17] and myomorphic rabies in East European rodents.[18]

Host range

Humans are most at risk from infected domestic dogs in an urban environment. However, all warm-blooded animals are susceptible. A characteristic of rabies virus is its ability to adapt to a single sylvatic host species in a specific environment such that the virus as a species is maintained for ever within the specific species.[19] The only rabies virus detected in a wild free-living host in the United Kingdom this century was a bat virus isolated from a sick bat in Sussex in the summer of 1996. Nearly 2,000 other bats were screened with negative results. Rare overspills into other contact species may occur accidently but they are irrelevant from the point of view of survival of the virus as a parasitic organism. On at least two occasions, man has re-located an established sylvatic rabic niche; the rabies-viverrid niche identified on the Indian sub-continent was the origin of infection of mongooses that were imported to control the rodent populations on Caribbean islands in the mid-nineteenth century and introduced rabies that still exists.[20] More recently, raccoon rabies was introduced into the New England State from an established niche in the south-eastern United States; apparently thousands of raccoons are imported every year from the Southeast to private hunting clubs in the Northeast.[21]

The specific sylvatic rabies niches were delineated originally by epidemiological methods. The early work has been and is being confirmed by the detection of characteristic nucleotide substitutions that permit the identification of variant biotypes associated with specific niches.[14]

Cattle are the species most frequently infected accidently; many pundits assume that rabid cattle are dead-end hosts but many veterinarians have had to undergo unpleasant post-exposure treatment on the advice of their medical advisers after handling rabies-infected cattle. Other aberrantly infected ruminants, however, are not dead-end infections; in the nineteenth century, fallow deer in Richmond Park were wiped out by rabies that was transmitted between them by biting.[22] Kudu antelopes, initially infected with rabies by jackals in Namibia in 1977, created a novel epidemic niche in which 10,000 died; Kudus are apparently hyper-susceptible to rabies and shed enormous numbers of the virus in their saliva which is transferred to susceptible antelopes when they indulge in social grooming.[23,9]

Virus-host interaction

Although all mammals and several species of domestic and wild birds are susceptible to rabies not all are equally susceptible.[24] Species long classified as hyper-susceptible include the fox, coyote, jackal, wolf, ox, baboon, kangaroo and kudu. Dogs are considered to be moderately susceptible.[13,23]

Likewise, strains of rabies virus vary widely in their virulence for specific hosts. A viral glycoprotein is largely responsible for pathogenicity[25] and it has been proved possible to select non-virulent mutants using specific monoclonal antibodies.[26] Nevertheless, variants non-pathogenic for adult mice even when injected intracerebrally remained fully pathogenic in suckling mice showing that pathogenicity is not a feature

of the virus alone. A field demonstration of the dual roles of virus and host in their interaction occurred recently in Zimbabwe where an attempt to vaccinate jackals and leopards using baits seeded with live SAD vaccine had to be stopped because of deaths in scavenging baboons.[27]

Abortive infection

Rabies does not always ensue from a bite of a rabid animal. Dogs may or may not resist or succumb to an inoculation of virulent rabies virus. Those that resist do not develop antibodies.[28] This phenomenon of resistance to infection has yet to be explained.

Incubation

Incubation periods of rabies vary widely; the British Committee of Inquiry on Rabies in 1971 found that 50% of the clinical cases occurred within one month of the commencement of quarantine, 80% within four months, and the remainder after four months.[29] A low percentage of horses held in quarantine having been naturally infected with fox-mediated rabies develop the disease after release.[30] The consensus amongst veterinary practitioners is that the natural incubation period falls between 15 and 50 days of the biting episode.[31] There is some evidence that the incubation period is dose dependent.[32]

Latency

Prolonged incubation periods measured in years occur occasionally in humans and animals. The virus deposited in a bite wound penetrates striated muscle cells where either it multiplies or becomes sequestered as a sub-viral particle. The multiplying virus enters the peripheral nervous system initiating the disease whereas the sequestered particles lie dormant and do not stimulate the production of antibodies. The mechanism whereby the sequestered sub-units are rescued and so induce disease is not known.[33]

Clinical spectrum

In what has become a classic study of the responses of dogs to infection with rabies virus, Fekadu, Shaddock, and Baer[34] witnessed the following syndromes:

- Sudden death without premonitory signs.
- Typical clinical signs of prodromal behavioural changes leading into an excitative phase followed by a terminal paralytic phase.
- Typical clinical signs followed by recovery and the appearance of specific antibodies.
- Inapparent infections detected by the development of antibodies.

The same pattern occurs in other species with modifications. For example, cattle are more alert than placid and bellow constantly day and night. Horses become irritable, roll as if they had colic, shed copious volumes of virus-rich saliva, and become unmanageable. Kudus behave like the horses.

Immunity

Animals that survive the clinical disease possess protective antibodies but at the time of writing, we have no method of differentiating the antibodies stimulated by natural overt or inapparent infections from those induced by vaccination. One of the most exciting epidemiological findings of the past decade came from a survey of antibodies

to lyssaviruses in sera of stray dogs, pet dogs that had never been vaccinated, and the owners of the pet dogs. The survey was conducted in Nigeria by staff of Ahmadu Bello University under the guidance of Dr G.W. Beran, a former senior scientist at the Veterinary Faculty, Iowa State University, USA. Of 463 unvaccinated pet dogs, 142 (31%) had antibodies against rabies virus; 83 (39%) out of 212 apparently healthy stray dogs were serologically positive, and out of 350 human sera examined 100 (29%) were positive. The serological results were validated at the National Veterinary Services Laboratories at Ames and at the Wistar Institute, Philadelphia. The authors cite two possible explanations: either the sero-positive dogs and people were exposed to the virus, sickened, and recovered, or after exposure stayed healthy yet produced detectable antibodies.[35]

Veterinarians studying wildlife populations in rabies niches are familiar with 30-60% seropositive rates; some assume a rabies antibody prevalence of around 30% as evidence of endemicity of the infection; for example, 30% of raccoons in Florida,[36] and 38% of mongooses on Grenada.[37] The detection of antibodies in the sera of wild raptors, carrion-eaters and scaveging birds caught in mist nets has been used in the USA to assess the prevalence and/or incidence of rabies in terrestrial mammals. It works because rabid infections in most wild birds are inapparent.[38]

Carriers

Some (all?) recovered and inapparently infected dogs are carriers and intermittently shed virus in their saliva.[39,40] These animals pose a risk that is not recognized in our quarantine regulations.

Diagnosis

Intra-vitam diagnosis is possible from frozen sections of skin biopsies taken from hairy areas. It is recommended that the sections be stained by the rapid fluorescence focus inhibition test (RFFIT) which fluoresces antigen in nerves around the base of hair follicles.[41] Impression smears of corneal membranes stained similarly are not so sensitive.[42]

Post-mortem diagnosis is achieved by examining impression smears or frozen sections of sliced brain stained with RFFIT. An enzyme-linked immunosorbent assay (ELISA) designed for use in epidemiological surveys is also available although it is not as sensitive as RFFIT.

Virus should be isolated from the brains of index cases in neuroblastoma cells of murine origin. If viable virus isolation is not possible, amplification of DNA copies of the rabies RNA will enable the typing of the virus by phylogenetic sequencing.[43]

Neutralizing antibodies in serum or cerebrospinal fluid are quantified in neuroblastoma cell cultures by identifying free virus by RFFIT. There is also a liquid phase blocking ELISA for detecting antibodies.[44]

THE HUMAN FACTOR

Twenty-one years ago Colin Kaplan edited a slim book entitled *Rabies the Facts* that summarised the then-known dangers of rabies and the risk of its entry into Britain.[13] It acknowledged rabies had the highest case fatality rate of any known human infection but, nevertheless, was not a major cause of human morbidity and had had little impact on historical events. Seminal investigations in the past two decades have shown that rabies virus behaves like most viral pathogens infecting some of its hosts, including humans, inapparently and yet inducing the production of rabies-specific antibodies.[35,45,46] In the Nigerian investigation only 18 of the 350 dog owners with rabies-specific antibodies recalled being bitten by a dog.[35] How did the others acquire the atypical disease?

Antibodies in classic clinical cases of rabies emerge late in the pathogenesis of the disease occurring after extracellular virus is released in the final phase of the infection. The antibodies have little effect on the outcome.

The means by which rabies virus induces atypical inapparent infections is not known. Presumably the invading virus is exposed early to immunologically active cells and the resultant antibodies prevent it entering peripheral nerves. The antibody response in atypical cases mimics that induced by the live, attenuated, non-neurotropic Flury strain of rabies developed as a live vaccine for dogs.[47] Future epidemiological studies will have to embrace serological findings.

Incidence in humans

No human cases of rabies have been caused in the United Kingdom since 1902.[48] Nevertheless, 12 people since then have died from rabies in the United Kingdom, nine were infected in Asia and three in Africa.

Risk to Europeans

After the end of the Second World War a pandemic wave of rabies in red foxes spread out of Poland across mainland Europe. Early in the 1980s the western wave penetrated Italy and France. The World Health Organization co-ordinated a massive control programme based on the distribution of baits containing live rabies vaccine in forest and rural areas. The prolonged campaign was an enormous success; for example, the peak annual incidence of 24,373 proven cases of rabies in Europe occurred in 1989[49] whereas in the latest set of data issued in 1996[50] the incidence is 8,080 cases with less than 300 occurring in Western Europe and the rest in Eastern Europe. In 1989, 15,356 red foxes died and in 1996, 4,816. Significantly, the major aberrant hosts bitten by foxes were cattle, small farm ruminants and farm cats. In the same period no human beings were infected in Western Europe, whereas 56 died in Eastern Europe, the likely vector being infected dogs.

If fox rabies gained entry into the United Kingdom, the risk to humans would far exceed the low risk in mainland Europe. The red fox there is a forest and rural animal in contrast to the huge population of red foxes in urban Britain where they have nightly contact with millions of domestic dogs and cats.[51]

Clinical rabies in humans

A recent outstanding, practical review of overt rabies in humans is the chapter on Rhabdovirus Infections written by Mary and David Warrell in the book on *Exotic Viral Infections*.[52] They stress that incubation periods are very variable ranging from four days to 19 years or more. It is shortest when the site of infection is closest to the brain; for example, they quote 22-39 days in patients infected from corneal implants[53] and 66-69 days from bites on the extremities. An editorial in the *Lancet* discussed prolonged rabies incubation periods in 1991 without using the word 'latency'.[54]

The Warrells warn that the change of mood during the vague prodromal symptoms together with the intense itching at the site of the bite are common in both victims that remain healthy and those that develop encephalomyelitis. Overt disease in humans is either furious or dumb unlike the progressive evolution common in affected animals from furious to paralytic. In the furious syndrome, the brain stem, cranial nerves, limbic system and higher centres are involved whereas in the paralytic syndrome the medulla, spinal cord and spinal nerves malfunction. The latter syndrome is commoner in victims of vampire bat-transmitted rabies. Recovery occurs but it is rare.

Treatment of human rabies

Post-exposure prophylaxis should be instituted as soon as possible. The bite wound must be scrubbed with soap and water and then swabbed with globulin from a phial of rabies hyperimmune human plasma. Thereafter immediately inject 1.0ml of inactivated cell cultured-derived virus vaccine into the deltoid muscles repeating the injection on days 3, 7, 14, and 30. If the animal vector remains healthy for ten days or its brain proves to be negative for rabies stop the treatment. The Warrells avoid injection of the gluteal muscles because allegedly lower antibody levels result. The standard post-exposure vaccine regimen is very expensive and in poorer countries they recommend the use of intradermal doses of 0.1ml at eight sites on day 0, at four sites on day 7, and at single sites on days 28 and 90. They also claim that to date (1995) there is no report of rabies encephalitis in a patient who has received the recommended optimal post-exposure vaccine treatment on the day of the bite.

Pre-exposure vaccination should be given to high-risk personnel working in rabies laboratories, quarantine kennels, and veterinary clinics. Travellers to countries where rabies is endemic should also be vaccinated. The regimen is either three 1.0ml intramuscular doses or three 0.1ml intradermal doses.

Post-exposure prophylaxis in previously-vaccinated people includes thorough cleansing of the bite wound without swabbing with globulin and the administration of two 1.0ml booster doses on days 0 and 3.

DISCUSSION

An attempt has been made to appraise the natural history of rabies from the viewpoint of assessing the risks of importing the disease in a live animal into the United Kingdom. The risks are very real. The present Quarantine Regulations have safeguarded the country well but they do not cover all the risks. For example, the Waterhouse Committee wrongly recommended the removal of restrictions on the import of cattle and horses; cattle are the commonest aberrant host of sylvatic rabies and fox rabies is still active on the European mainland. Moreover, the belief that cattle become dead-end hosts is a myth. Rabid horses are fearsome animals and clinical cases are not uncommon in Europe; their status should be re-assessed.

Dogs, particularly dogs with rabies antibodies, pose the real threat. Despite statements to the contrary, rabies virus is a typical virus that infects about as many hosts inapparently as it does overtly. The full significance of the fact that surviving, naturally-infected dogs could act as Trojan horses has yet to be realised. Any preventative scheme has to take this on board. Dogs must be bled before vaccination as well as after. If the pre-vaccination serum is free of rabies antibodies, then all is well. If the first serum is positive, it will be necessary to demonstrate that they are not naturally acquired. If they are naturally acquired, it would be wise to consider euthanasia. A short term of a few weeks quarantine would suffice to run the tests. Before any action can be taken several answers are required:

- Can carriers always be identified at each PCR test?
- Can we devise a system that distinguishes vaccine antibodies from wild antibodies?
- Can we identify latency?
- Can we abolish the use of live vaccine which theoretically could rescue sequestered sub-units in latently infected animals?
- Can we develop better *in vivo* tests?
- Do we need to interfere with sylvatic rabies niches?

REFERENCES

[1] Bardsley SA. Miscellaneous observations on canine and spontaneous hydrophobia: to which is prefixed, the history of a case of hydrophobia, occurring twelve years after the bite of a supposed mad dog. *Mem Manchester Lit Phil Soc* 1793; 4:431.

[2] Youatt W. *The dog* London: Longmann, Brown, Green and Longmans, 1851; 145.

[3] Pasteur L. Méthode pour prévenir la rage après morsure. *C R Acad Sci Paris* 1885; 101:765.

[4] Carini A. Sur une grande epizootie de rage (vampire bat rabies). *Ann Inst Pasteur Paris* 1911; 25:843-6.

[5] Sellers TF. Rabies. In: Haddison TR (ed). Principles of internal medicine, 4th ed. London: Lewis, 1954; 1106.

[6] Van Rooyen CE, Rhodes AJ. *Virus diseases of Man* New York: Nelson, 1948; 792-806.

[7] Wunner WH, Calisher CH, Dietzgen RG *et al*. Rhabdoviridae. In: Murphy FA, Fauquet CM, Bishop DHL *et al* (eds). Virus taxonomy. classification and nomenclature of viruses. Vienna: Springer-Verlag, 1995; 275.

[8] Calisher CH, Karabatsos N, Zeller H *et al*. Antigenic relationships among rhabdoviruses from vertebrates and hematophagour arthropods. *Intervirol* 1989; 30:241.

[9] Barnard BJH, Hassel RH, Geyer H, De Koker WC. Non-bite transmission of rabies in Kudu (*Tragelaphus strepsiceros*). *Onderstepoort J Vet Res* 1982; 49:191-2.

[10] Constantine DG. *Rabies transmission by air in bat caves* Atlanta: Public Health Service Publication No. 1617, 1967.

[11] Soave OA. Transmission of rabies to mice by ingestion of infected tissue. *Amer J Vet Res* 1966; 27:44.

[12] Martell MA, Montes FC, Alcocer BR. Transplacental transmission of bovine rabies after natural infection. *J Infect Dis* 1973; 127:291.

[13] Kaplan C. (ed.) *Rabies the facts* Oxford: OUP, 1977.

[14] Smith JS. New aspects of rabies with emphasis on epidemiology, diagnosis, and prevention of the disease in the United States. *Clin Microbiol Rev* 1996; 9:166-76.

[15] Bourhy H, Kissi B, Tordo N. Molecular diversity of the Lyssavirus genus. *Virol* 1993; 194:70.

[16] Schneider LG, Cox JH. Bat lyssaviruses in Europe. In: Rupprecht CE, Dietzschold B, Koprowski H (eds). Lyssaviruses. Berlin: Springer-Verlag, 1994; 207.

[17] Thiery G. Particularités de la rage dans l'ouest Afrique. *Bull Epiz Dis Afr* 1959; 7:265.

[18] Sodja I, Lim D, Matouch O. Isolation of rabies-like virus from murine rodents. *J Hyg Epidem Microbiol Immunol* 1971; 15:220.

[19] Chalmers AW, Scott GR. Ecology of rabies. *Trop Anim Hlth Prod* 1969; 1:33.

[20] Johnson HN. Rabies virus. In: Horsfall EL, Tamm I (eds). Viral and rickettsial infections of Man, 4th ed. Philadelphia: Lippincott, 1965; 814.

[21] Rupprecht CE, Smith JH. Raccoon rabies - the re-emergence of an epizootic in a densely populated area. *Sem Virol* 1994; 5:155.

[22] Cope AC, Duguid W. Report on the diseases which have existed among animals in Great Britain and among animals in foreign countries in 1887. *An Rep Agric Dept Privy Council Off Contag Dis Inspect Trans Anim* 1888, 12.

[23] Barnard BJH, Hassel RH. Rabies in Kudus (*Tragelaphus strepsiceros*) in South-west Africa/Namibia. *J S Afr Vet Ass* 1981; 52:309-14.

[24] Scott GR. Rhabdoviruses. In: McFerran J McNulty Ms (eds). Virus infections of birds (Vol. 4, Virus infections of vertebrates). Amsterdam: Elsevier, 1993; 387.

[25] Dietzschold B, Wunner WH, Wiktor TJ *et al*. Characterisation of an antigenic determinant of the glycoprotein which correlates with pathogenicity of rabies virus. *Proc Nat Acad Sci USA* 1983; 80:70.

[26] Coulon P, Rollin P, Aubert M, Flamand M. Molecular basis of rabies virus virulence I. Selection of virulent mutants of the CVS strain with anti-G monoclonal antibodies. *J Gen Virol* 1982; 61:97.

[27] Bingham J, Foggin CM, Gerber H *et al*. Pathogenicity of SAD rabies vaccine given orally in chacma baboons (*Papio ursinus*). *Vet Rec* 1992; 131:55-6.

[28] Fekadu M, Chandler FW, Harrison AK. Pathogenesis of rabies in dogs inoculated with an Ethopian rabies virus strain. Immuno-fluorescence, histologic and ultrastructural studies of the central nervous system. *Arch Virol* 1982; 71:109.

[29] Waterhouse R. *Report of the Committee of Inquiry on rabies* London: HMSO, 1971.

[30] Aubert M. Discussion. In: Beynon PH, Edney ATB (eds). Rabies in a changing world. London: Brit Small Anim Vet Ass, 1995; 96.

[31] Anon. Rabies. In: Siegmund OH, Eaton LG (eds). The Merck Veterinary Manual, 3rd ed. Rahway: Merck Inc, 1967; 244.

[32] Fekadu, M. Canine rabies. *Onderstepoort J Vet Res* 1993; 60:421-7.

[33] Scott GR. Rabies. In: Sewell MMH, Brocklesby DW (eds). Animal diseases in the Tropics. 4th ed. London: Bailliere Tindall, 1990; 346-9.
[34] Fekadu M, Shaddock JH, Baer GM. Excretion of rabies virus in the saliva of dogs. *J Infect Dis* 1982; 45:715.
[35] Ogunkoya AB, Beran GW, Umoah JU *et al*. Serological evidence of infection of dogs and man in Nigeria by Lyssaviruses (family Rhabdoviridae). *Trans R Soc Trop Med Hyg* 1990; 84:842-5.
[36] McLean RG. Raccoon rabies. In: Baer GM (ed). Natural history of rabies, vol.2. New York: Academic Press, 1975; 53.
[37] Everard COR, Baer GM, Moore SA. Rabies serum neutralising antibody in mongooses from Grenada. *Trans R Soc Trop Med Hyg* 1981; 75:654.
[38] Gough PM, Jorgenson RD. Rabies antibodies in the sera of wild birds. *J Wildf Dis* 1976; 12:392.
[39] Fekadu M, Shaddock JH, Baer GM. Intermittent excretion of rabies virus in the saliva of a dog two and six months after it had recovered from experimental rabies. *Amer J Trop Med Hyg* 1981; 30:1113.
[40] Aghomo HO, Oduye OO, Tomori O, Ibe M. Isolation of rabies virus from clinically healthy and previously unvaccinated dogs. *Bull Anim Hlth Prod Afr* 1989; 37:131-6.
[41] Bryceson ADM, Greenwood BM, Warrell DA *et al*. Demonstration during life of rabies antigen in humans. *J Infect Dis* 1976; 131:71-4.
[42] Schneider LG. The cornea test; a new method for the *intra-vitam* diagnosis of rabies. *Zbl Vetmed* 1969; 16B:24-31.
[43] Bourhy H, Sureau P. *Laboratory methods for rabies diagnosis*. Paris: Commission des Laboratoires de reference et d'Expertise de l'Institut Pasteur, 1991; 197.
[44] Esterhuysen JJ, Thomson GR, Prehaud C. A liquid phase blocking ELISA for the detection of antibodies to rabies. *J Virol Meth* 1995; 51:31-42.
[45] Cleaveland S, Dye C. Maintenance of a microparasite infecting several host species: rabies in the Serengeti. *Parasit* 1995; S33-S47.
[46] Laurenson K, Esterhuysen J, Stander P, Van Heerden J. Aspects of rabies epidemiology in Tsumkwe District, Namibia. *Onderstepoort J Vet Res* 1997; 64:39-45.
[47] Koprowski H, Cox HR. Occurrence of chick-embryo-adapted rabies virus. *Proc Soc exp Biol NY* 1948; 68:612-5.
[48] Gardner S, King A. Rabies - recent developments in research and human prophylaxis. In: Capner PM (ed). Current topics in virology. Salisbury: PHLS Lavenham Press, 1991; 141-63.
[49] Schneider LG, Müller WW, Hohnsbeen KP. *Rabies Bulletin Europe 1989* 1990; 13(4):4.
[50] Müller WW, Cox JH, Hohnsbeen KP. *Rabies Bulletin Europe 1996*. 1997; 20(4):15.
[51] Macdonald DW. *Rabies and wildlife a biologist's perspective*. Oxford: OUP, 1980; 7.
[52] Warrell MJ, Warrell DA. Rhabdovirus infections of humans. In: Porterfield JS (ed). Exotic viral infections. London: Chapman and Hall, 1995; 343-83.
[53] Helmick CG, Tauxe RV, Vernon AA. Is there a risk to contacts of patients with rabies? *Rev Infect Dis* 1987; 9:511-8.
[54] Anon. Human rabies: strain identification reveals lengthy incubation. *Lancet* 1991; 337:822-3.