PATHOGENESIS OF RABIES VIRUS INFECTION IN DOGS:
DO DOGS RECOVER FROM CLINICAL RABIES?

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Introduction

Dogs are the principal transmitter of rabies to man and animals in most countries of Africa, Asia and South America, where they are responsible for over 95% of the human rabies cases reported world-wide (WHO, 1989). The clinical course of rabies in dogs and that it could be transmitted by bite has been known for over 4000 years (Tierkel, 1975). After the virus is introduced to the body by bite or inoculation, it is assumed to remain at the site of entry for a long time (Baer and Cleary, 1972). The virus has been shown to replicate locally, prior to its centripetal transport to the brain (Baer and Cleary, 1972; Murphy and Bauer, 1974; Baer et al., 1968). During the transport period, specific rabies antibody is not detected before signs of illness appear, apparently because insufficient viral antigen is presented to key organs to trigger the immune system.

Pathogenesis in the dog

Incubation period

The incubation period of canine rabies varies from 7 days to many months. The length of incubation period apparently depends on several factors, including the site of exposure (inoculation), the infecting dose and the virus strain (Fekadu et al., 1982).

In dogs experimentally infected with various doses of dog rabies virus strains to simulate natural infection and the incubation periods ranging between 7 and 125 days, depending on the dose and strain used, dogs were observed for at least 2 years to register unexpectedly long incubation periods (Fekadu et al., 1982). Some researchers reported that dogs died 8.5 months after challenge (Tierkel et al., 1975). Although these findings show that the incubation period is mainly dose-dependent, implying that the long incubation periods observed in naturally infected animals may be attributable to exposure to very small amounts of virus, the variation in incubation periods in naturally infected animals remains to be documented.

Clinical signs

1. Prodromal phase

During the prodromal phase, the dog's behaviour may change. Aggressive and high-strung dogs may become more affectionate than usual, and ordinarily friendly dogs may become shy and seek secluded areas or become snappy and irritable. The dog's temperature may rise slightly, the pupils may dilate and the nictating membrane may cover the eye. The dog may also salivate excessively.
2. **Excitable phase (furious rabies):**

Sighs of the disease are most easily recognised during this phase. The dog becomes severely agitated and restless and sometimes gets an urge to roam. The dog is most dangerous at this stage because of its urge to bite anything it encounters. In most cases, an altered phonation (a characteristic high pitched bark) develops, caused by paralysis of laryngeal muscles. The dog has difficulty swallowing because of spasms and paralysis of the pharyngeal muscle, causing the animal to drool. If the dog does not die during one of the characteristic convulsive seizures, the disease usually progresses to muscular incoordination, paralysis, coma, and death.

3. **Paralytic phase (dumb rabies):**

Dumb rabies occurs when the excitable phase is extremely short or absent. The most characteristic sign is the "dropped jaw" caused by paralysis of the masseter muscles. The animal often makes choking sounds as if a bone were stuck in its throat. Attempts to remove this "bone" often result in owners scratching their hands on the dog's teeth and being exposed to the disease.

The clinical signs of rabies in humans and animals have been known for centuries, but our knowledge of the pathogenesis of rabies in the principal vector, the dog, is still very limited. To elucidate some of the factors involved in the virus-host relationship in this animal, we inoculated dogs intramuscularly with varying doses of representative strains of street rabies virus from dog salivary gland suspension from rabies endemic areas of the Americas and Africa, to simulate natural infection (Fekadu et al., 1982; Fekadu and Shaddock, 1984).

The incubation and morbidity periods in these experimentally infected dogs, ranging from 9 - 125 days, depended on the dose rather than on the virus strains used. Seventy percent of the experimentally infected dogs developed the dumb form of rabies, 12 percent the furious form and 18 percent died without showing any signs of disease. In this experiment it was shown that the intramuscular lethal dose for a dog was 32 mouse intracranial lethal doses, not as high (86,000 MICLD50) as previously reported (Fekadu and Shaddock, 1984; Vaughn et al., 1965).

**Excretion of Virus**

The particular time of salivary virus excretion before sickness is crucial, since transmission may occur when the animal appears normal and no preventive measures are taken. The failure to appreciate the significance of such normally acting but infective animals can result in delayed diagnosis and possible fatal results in those persons exposed. Rabies virus is usually present in the saliva when clinical signs appear, but in some studies prior to 1970 rabies virus was also demonstrated in the saliva of dogs 3 - 6 days before clinical signs appeared (Vaughn et al., 1965).
Frequency of salivary gland infection

The frequency of salivary gland infection in experimentally or naturally infected rabid dogs has been reported to vary from 61 percent to 75 percent. In one experiment in 1982, however, in which dogs were inoculated peripherally with graded doses of canine virus (to simulate natural dog-to-dog transmission) the excretion of rabies virus in their saliva depended not only on the strain but also on the dose of inoculum (Fekadu et al., 1982). At necropsy, 67 - 83 percent of dogs with positive salivary glands excreted virus in their saliva before and during illness and 25 - 100 percent of the dogs in each group had virus in the salivary glands at death, depending primarily on the dose of inoculum.

Pre-symptomatic excretion time

The pre-symptomatic excretion time of the experimentally infected dogs ranged from 1 to 14 days. Of the 39 inoculated dogs that died, 37 percent excreted virus in their saliva 1 - 14 days before onset of illness, and an additional 10 percent excreted virus after onset. However, 64 percent of rabid dogs had virus in their salivary glands, depending on the incubation periods and dose of inoculum.

Almost all (95 - 100 percent) of the dogs inoculated with a lower dose (30 - 300 MICLD\textsubscript{50}) had virus in the salivary glands, while dogs inoculated with high doses rarely did. The canine rabies virus strains used in this experiment seem to be excreted long before onset, unlike the fox or dog strains used earlier (Vaughn et al., 1965; Fekadu et al., 1982).

The possible transmission of rabies from a rabid animal to a human usually depends on the presence of the virus in the salivary glands (at death or at sacrifice) and its possible excretion in the saliva. However, the failure to demonstrate virus in the salivary glands of rabid animals at death does not always exclude the possibility of virus having been excreted in the saliva; rabid animals may on rare occasions excrete virus in the saliva, yet have no virus in the salivary gland and brain at death (Fekadu et al., 1983).

Carrier state

Apparently healthy dogs in the field have been reported to intermittently excrete rabies virus in saliva. These observations in naturally infected dogs have recently been confirmed in an experimentally inoculated dog that recovered without any supportive treatment, then intermittently excreted virus in its saliva for up to 305 days after recovery (Fekadu et al., 1981). The excretion of virus in the saliva of such apparently healthy dogs may play a role in perpetuating the virus in nature and transmitting the disease (Fekadu et al., 1983). Tonsils may also play an important role as the sequestration site and source of excretion.
**PATHOLOGY**

1. **Light microscopy**

   Despite the striking clinical manifestations of rabies in both humans and animals, rabies shows little or no grossly visible pathologic changes. Meningeal vessel congestion is often the only visible abnormality and it may be quite marked, but subarachnoidal haemorrhage is rarely seen. Cerebral oedema, common in most viral encephalitides, may be mild or absent.

   The most common changes observed by light microscopy are mild to severe perivascular infiltration (cuffing) with lymphocytes, few plasma cells, and macrophages; focal and diffuse lymphocytic and granulocytic infiltration of meninges, as well as focal concentrations of lymphocytes and plasma cells beneath the ependyma and in the stroma of choroid plexus. Neuronal degeneration varies from minimal to severe; satellitosis and neurophagia of isolated neurons as evidence of early necrosis of these cells are rarely observed (Baer, 1975; Jubb and Kennedy, 1963; Tangchal et al., 1970).

   These changes are common in many viral encephalitides, however, and are not specific to rabies. The only pathognomonic lesion characteristic of rabies is the cytoplasmic inclusion body described by Adelchi Negri (Negri, 1903); through the immunofluorescent technique (Goldwasser and Kissling, 1958), the Negri body was documented to contain rabies nucleocapsid antigen.

   The degree of inflammation of the brain and, less commonly, the spinal cord, is directly proportional to the length of the incubation and morbidity periods. Intracytoplasmic inclusion bodies are present in the pyramidal cells of the hippocampus and occasionally in the Purkinje cells of the cerebellum. Other areas where Negri bodies are readily found are the brain stem, pons, cerebral cortex and cerebral part of the spinal cord. Sixty to 100 percent of experimentally infected dogs have Negri bodies, depending upon the virus strain and the dose of inoculum (Fekadu et al., 1982).

2. **Electron microscopy**

   At the ultrastructural level the pathological changes in the CNS also vary, ranging from moderate to severe. Inflammatory infiltrates and focal disruption of myelin are common. Some neurons contain from small, granular, finely fibrillar, viral matrices to numerous matrices, varying in size and shape. These are accompanied by prolific numbers of virus particles budding from membranes of the rough endoplasmic reticulum and occasionally from the outer lamella of the nuclear envelope and the plasma membrane (Fekadu et al., 1982).

   The eosinophilic ground substance (inner body) of the Negri body observed by light microscopy was also shown to be identical to the matrix seen by electron microscopy, corresponding to the site of virus replication (Matsumoto and Miyamoto, 1966; Matsumoto, 1975).
3. **Peripheral distribution**

Rabies virus spreads from the site of infection to the CNS and back to the peripheral organs via the nerves. The dissemination of virus in peripheral tissues depends on the dose of inoculum and the length of incubation periods. A large inoculum produces a short incubation period and a rapid course of illness, leading to death before spread of virus throughout the brain. After long incubation periods, virus is distributed in many parts of the body.

The ultimate distribution of viral infection depends upon the virus strain and the dose of inoculum used. The amount of antigen demonstrable in tissues varies markedly, depending on the dose of inoculum. Virtually every organ examined may have viral antigen, confirming previous reports in experimentally infected rodents. Viral antigen was occasionally demonstrated in most internal organs including the kidney, intestine, and bladder. Virus from the gastrointestinal mucosa, pancreas, or liver, may possibly be excreted but would most likely be inactivated by digestive enzymes. The most important source for rabies transmission is, therefore, the saliva and the oro-nasal secretions (Fekadu and Shaddock, 1984).

More specifically, virus was detected in salivary glands in only 25 percent to 40 percent of dogs inoculated with a large dose, compared with almost all dogs inoculated with a small dose. In naturally infected dogs the presence of virus in salivary glands ranges from 75 percent to 100 percent, suggesting that the amount of virus introduced by bite is low.

The most important factor in the transmission of rabies is the presence of virus in the saliva and salivary glands, especially during the period before detectable signs of disease appear. Previously, virus excretion in the saliva of dogs was considered to occur during or just before the appearance of signs, but in a recent experiment, we reported that dogs inoculated intramuscularly with canine strains of street rabies excreted virus in their saliva up to 14 days before signs appeared. In one dog that recovered after inoculation with an Ethiopian street rabies virus strain, rabies virus was excreted intermittently in its saliva for 305 days after recovery (Fekadu et al., 1981; Fekadu and Baer, 1980).

Excretion of virus in saliva depends on its presence in the salivary glands. Although tonsils have been shown to play an important role as the sequestration site and source for rabies virus excretion, in one dog that intermittently excreted virus in its saliva for months after recovery, the only tissue from which live virus was isolated at necropsy was the tonsils.

Tonsils from other animals experimentally infected with various isolates of rabies virus were examined for the presence of viral antigen in the tonsils in relation to salivary glands and brain. The findings show that tonsils are infected at higher frequency than the salivary glands (Table 1). In dogs inoculated with either insectivorous bat or with dog street virus strains, up to 20 percent recovered without supportive treatment; these recoveries were neither dose nor virus strain dependent (Fekadu, 1988).
Table 1.

Detection of rabies virus in tonsils compared with other tissues in different animals inoculated with street rabies virus strains

<table>
<thead>
<tr>
<th>Animal</th>
<th>Virus</th>
<th>Positive Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>No.</td>
<td>Strain</td>
</tr>
<tr>
<td>Dogs</td>
<td>14</td>
<td>Dog</td>
</tr>
<tr>
<td>Foxes</td>
<td>12</td>
<td>Fox</td>
</tr>
<tr>
<td>Raccoons</td>
<td>10</td>
<td>Raccoon</td>
</tr>
<tr>
<td>Skunks</td>
<td>6</td>
<td>Skunk</td>
</tr>
<tr>
<td>Monkeys</td>
<td>16</td>
<td>Fox</td>
</tr>
</tbody>
</table>

* NT = not tested

Resistance to challenge

Dogs that resisted street rabies virus challenge without signs of disease and failed to develop a virus neutralising antibody (VNA) titre, were challenged after a 2 year observation period. They developed a rather high anamnestic serum VNA response and resisted challenge, indicating that factors other than VNA induced by rabies G protein play a role in protecting dogs from rabies infection (Fekadu et al., 1992). It has also been reported that animals with WA titres succumbed when challenged, whereas others which had no detectable amounts of antibody survived (Lodmell et al., 1969).

In addition, the role of cell-mediated immunity has been noted in recovery from attenuated rabies virus infection in mice, in which T cells were stimulated by both rabies G and N proteins (Miller et al., 1978). VNA, therefore, may not be the only factor involved in recovery from rabies.

The mechanism of such recoveries, however, is still not well understood. Recovery from rabies has been reported in only a limited number of humans and animals since Pasteur's time. Pasteur was the first to report that dogs occasionally recovered from rabies, and he considered subsequent resistance of these dogs to reinfection as a strong indication of previous abortive infection.

The most commonly used criterion for detecting non-fatal rabies infection is the isolation of virus from saliva, brain, or other tissues of animals that recover after sickness. Brain biopsy specimens have even been taken from humans for virus isolation tests to confirm a diagnosis of rabies when apparent recovery from rabies had occurred.

However, a high VNA titre in the brain or the CSF has been shown to be the only definitive diagnostic test for demonstrating recovery from CNS rabies infection in humans and animals (Bell et al., 1966).
Sickness and recovery

In order to elucidate the role, especially in sickness and recovery, of the different rabies virus proteins in the pathogenesis of rabies in dogs, we inoculated dogs with preparations of various rabies virus proteins. Dogs were vaccinated with the rabies G-protein alone, a combination of G and the rabies nucleoprotein N, or the N protein alone, prior to peripheral challenge with a street rabies virus (Fekadu et al., 1992).

All dogs vaccinated with rabies G protein or G plus N proteins developed VNA titres. Dogs vaccinated with N protein alone, however, had no detectable VNA titre prior to challenge, and the levels of antibody directed against N protein were low (Tables 2 and 3).

Table 2

| Serum VNA titres of dogs vaccinated i.d. with either Vaccinia virus recombinant expressing rabies N protein, G protein or G plus N proteins or Vaccinia virus |
|---|---|---|---|---|---|---|---|
| Serum MA titre (WIC) at a given week post vaccination* |
| Group | 1 | 2 | 5 | 8** | 9 | 10 | 12 |
| I (N) | <0.1 | <0.1 | <0.1 | <0.1 | 0.3*** | 1.5 | 1.5 |
| II (G) | 0.3 | 1.5 | 1.5 | 1.5 | 37.0 | 37.0 | 37.0 |
| III (G + N) | <0.1 | 1.5 | 1.5 | 1.5 | 37.0 | 37.0 | 37.0 |
| IV (Vaccinia virus) | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | 0.3 NT**** |

* = all titres were <0.1 at day 0.  ** = challenge date  *** - one of the seven dogs in Group I had VNA 1 week after challenge  **** - Not tested.

Table 3

<p>| Rabies virus N antibody titres in dogs vaccinated with a vaccinia virus recombinant expressing the rabies virus N protein determined by ELISA against rabies R protein |
|---|---|---|
| Absorbency determined at 1:100 serum dilution |</p>
<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Pre-challenge</th>
<th>Post-challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>0.639</td>
<td>2.391</td>
</tr>
<tr>
<td>84</td>
<td>0.573</td>
<td>2.141</td>
</tr>
<tr>
<td>366</td>
<td>0.555</td>
<td>1.436</td>
</tr>
<tr>
<td>24</td>
<td>0.490</td>
<td>1.891</td>
</tr>
<tr>
<td>36</td>
<td>0.660</td>
<td>1.586</td>
</tr>
<tr>
<td>40</td>
<td>0.651</td>
<td>1.875</td>
</tr>
<tr>
<td>65</td>
<td>0.490</td>
<td>0.761</td>
</tr>
</tbody>
</table>

Five of seven dogs vaccinated with N protein alone developed clinical rabies 11 to 14 days after challenge. The incubation periods in these dogs were 3 to 7 days shorter than those of the control dogs (Table 4).
Table 4

Sickness and recovery in dogs vaccinated i.d. with Vaccinia recombinants expressing either rabies virus N protein, G protein, G and N proteins simultaneously or vaccinia virus.

<table>
<thead>
<tr>
<th>Group</th>
<th>Incubation period (days)</th>
<th>Sickness survival</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (N)</td>
<td>11-14</td>
<td>5/7</td>
<td>2/5</td>
</tr>
<tr>
<td>II (G)</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>III (G + N)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>IV vaccinia virus</td>
<td>14-21</td>
<td>6/6</td>
<td>6/6</td>
</tr>
</tbody>
</table>

The difference in incubation periods between controls and those vaccinated with N protein was statistically significant (p <0.02). All control dogs died. of the seven dogs vaccinated with N protein, only two died of rabies (although five had sickened). Viral antigen was demonstrated by FA in the brain tissue of all the dogs that died. The remaining three dogs in group 1 gradually recovered without supportive treatment, after a morbidity period ranging from 7 to 12 days. Recovery was confirmed by the presence of VRA titres in the CSF collected after the disappearance of clinical signs (Table 5), whereas none of the dogs vaccinated with the G protein, G + N proteins or the controls developed any detectable amounts of VNA in the CSF.

All dogs that were vaccinated with G or G + N had a high booster response 5 days after challenge, but only one of the dogs vaccinated with N developed a VNA titre 8 days after challenge. A similar VNA titre was also detected in one of the unvaccinated controls (Table 5).

Table 5.

MA titres of dogs vaccinated with vaccinia virus recombinant expressing rabies virus N protein and then challenged with street rabies virus

<table>
<thead>
<tr>
<th>EMA titres (IU/ml)</th>
<th>Post-challenge</th>
<th>Post-recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog No.</td>
<td>serum</td>
<td>serum</td>
</tr>
<tr>
<td>27</td>
<td>8.2</td>
<td>14.1</td>
</tr>
<tr>
<td>84</td>
<td>0.1</td>
<td>7.3</td>
</tr>
<tr>
<td>366</td>
<td>0.6</td>
<td>12.9</td>
</tr>
<tr>
<td>24</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>40</td>
<td>0.6³</td>
<td>NT³</td>
</tr>
<tr>
<td>65</td>
<td>&lt;0.1²</td>
<td>NT</td>
</tr>
</tbody>
</table>

1=Pre- and post- vaccination serum titres were <0.1 in all cases
2=Dogs that died
3NT - not tested

Protection, sickness or survival of dogs vaccinated with the N protein was not dependent on the titre of VNA or antibody against N protein prior to challenge, as dogs with detectable amounts of antibody died whereas others that had no VNA survived challenge (Table 5).
Our study shows that all dogs vaccinated with G protein were fully protected and developed a high booster response after challenge. Five (71 percent) of the seven dogs vaccinated with the N protein survived challenge with or without signs of rabies. Two (28.5 percent) of the seven N-protein vaccinated dogs that did not sicken failed to develop VNA after challenge, unlike dogs vaccinated with G protein, indicating that priming dogs with N protein may not induce higher VNA response, contrary to previous reports (Dietzschold et al., 1987).

Three of five N-protein vaccinated dogs (60 percent) sickened and recovered without supportive treatment and developed high VNA titres both in the serum and CSF, indicating that N protein may not only be involved in induction of T cell response (Miller et al., 1978; Mifune et al., 1981), but also in sickness and recovery (Fekadu et al., 1992).

The specific role of N protein in sickness and recovery in dogs is not clear. The mechanism of protection against rabies challenge in the absence of virus neutralising antibody could be attributed to the induction of cytolytic T cells as well as T helper cells that support the activity of virus-neutralising antibody-producing B cells; or by promoting the attachment of anti-N antibody via Fe receptor to phagocytic cells, which are then stimulated by the infecting (challenge) virus to produce cytokines that inhibit viral replication.

Conclusions

Most dogs experimentally infected with street rabies virus developed clinical signs of rabies before death, but up to 18 percent of our dogs died without showing detectable signs of illness. In those showing signs, rabies was not invariably fatal. Up to 20 percent of dogs recovered without any supportive treatment.

The pathological changes observed depended mainly on the inoculum dose; small virus doses produced longer incubation periods and resulted in more pathological changes.

Some dogs inoculated with dog strains excreted virus in their saliva up to 14 days before signs appeared. There was no relationship between the time of virus excretion in the saliva and the morbidity period, or the titre of virus in the salivary glands at death.

One dog that recovered from clinical rabies intermittently excreted rabies virus in its saliva for 305 days after recovery. The carrier state in rabies may possibly play a role in perpetuation and survival of the virus and may become a source for rabies outbreaks whenever a new population of rabies-susceptibles reaches the critical density.

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**REFERENCES**


