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The outbreaks and surveillance of animal rabies in Gangwon-do

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Abstract

Although human rabies deaths are rare, the disease remains a public health problem in Korea. Here we report the outbreaks and surveillance of animal rabies in Gangwon-do. Animal rabies infections were identified in 119 animals from 1993 to 2003. The 78% of all rabid animals were domestic species in Gangwon-do. Wild Korean raccoon dog (*N. p. koreensis*) continued to be the only reported rabid wildlife species. Outbreaks of rabies infections in Korean raccoon dogs are found in broad geographic regions across the northern Gangwon-do. The principal rabies hosts today are probably wild animals in Gangwon-do. The malaise, cerebral dysfunction, anxiety, confusion, agitation and abnormal behavior of the animals were the important symptoms of the disease. The Encephalitis, infiltration with lymphocytes and polymorphonuclear leukocytes and the inclusion bodies (Negri bodies) in neuronal cells were the specific histopathological signs. The results of indirect fluorescent antibody test (IFA) for animal rabies diagnosis were identical and the technique was useful to diagnose the disease. Preexposure vaccination is recommended for persons in high-risk groups, such as laboratory workers, veterinarians and certain animal handlers.

Key words : Rabies, Outbreaks, Negri bodies, Vaccination

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Introduction

Rabies is a highly fatal viral disease of mammals. Rabies virus belongs to the order *Mononegavirales*, viruses with nonsegmented, negative-stranded RNA genomes[1-5]. Rabies virus infects the central nervous system and causes acute encephalitis in all warm-blooded hosts including humans, and once clinical signs of rabies appear, the disease is nearly always fatal[2,6-8]. Dissemination of the virus within the central nervous system (CNS) is rapid, and includes early involvement of limbic system neurons[6,9,10,)]

Transmission of rabies virus usually begins when infected saliva of a host is passes to an uninfected animal. Various routes of transmission have been docu mented and include contamination of muc ous membranes (i.e., eyes, nose, and mouth), aerosol transmission, and corneal transplantations[1,9,11,12]. The most common mode of rabies virus transmission is through the bite and virus-containing saliva of an infected host. Investigations have shown both direct entry of virus into peripheral nerves at the site of infection and indirect entry after viral replication in nonnervous tissue (i.e., muscle cells). The incubation period is the time from exposure to onset of clinical signs of the disease.

The incubation period may vary from a few days to several years. Pathology of rabies virus infection is typically defined by encephalitis and myelitis. Perivascular infiltration with lymphocytes, polymorph onuclear leukocytes, and plasma cells can occur throughout the entire CNS. Rabies infection frequently causes cytoplasmic eosinophilic inclusion bodies (Negri bodies) in neuronal cells, especially pyramidal cells of the hippocampus and Purkinje cells of the cerebellum. The direct fluorescent antibody test (dFA) is the most frequently used technique to diagnose rabies in animals[2,9,13,14)]. This test requires brain tissue from animals suspected of being rabid. So this test can only be performed post-mortem (after the animal is dead) [2,10,14-16)]. Although all species of mammals are susceptible to rabies virus infection, only a few species are important as reservoirs for the disease.

In Korea, 49 cases of rabies from 1907 to 1910 and 13,859 cases from 1924 to 1940 have been reported in mammals including dogs, cats, cattle and Korean raccoon dogs[15)]. More than 40~80 cases were reported annually from 1950 through 1960 to the Government authorities. The number of rabies-related animal deaths in Korea was declined annually after the turn of the 1980's and there was no rabies outbreak report from 1985 to 1992. But unfortunately, one case of rabies has broken out unpredictably in September 1993 in Gangwon-do[15,16)]. After that, the rabies cases reported to the authorities each year have occurred in animals like dogs, cattle and Korean raccoon dogs. The principal rabies hosts in Korea are probably wild Korean raccoon dogs[15,17)].

This study was performed to define current epidemiologic patterns of animal rabies, and to investigate laboratory identi fication of positive rabies cases. We hope this report will help and provide appro priate information for the developm ent of rabies control programs.

Specimens tested

About 119 rabid dogs, cattle and wild Korean raccoon dogs in Gangwon-do were submitted from 8 counties (city). The Gangwon-do Veterinary Service Labor atory (GVSL) also examined these specimens. The symptoms of rabid 17 dogs, 43 cattle and 22 Korean raccoon dogs were observed during their lifetime (Table 1). The malaise, cerebral dysfun ction, anxiety, confusion, depression, agit ation, salivation and abnormal behavior of the animals such as were the point of the observations. All the animals observed were necropsied before or after death. During necropsy, gross lesions were observed in each organ.

To reproduce this disease in laboratory animals, the suspected animal brain samples were homogenized and injected into the cerebral meninges of mice.

Table 1. Clinical signs of rabid animals

Clinical signs	Rabid animals		
	Cattle	Dog	Raccoon dog
Malaise	43	13	2
Cerebral dysfunction	43	17	22
Anxiety	43	17	8
Agitation	8	12	1
Depression		6	22
Salivation	43	17	5

Histopathological examinations

Brain tissues were collected and fixed in buffered formalin. The fixed brain tissues were embedded in paraffin and sectioned (4 ㎛ in thickness) to make specimen slides. The slides were stained with hematoxylin-eosin solution and observed with microscope (magnifying power of 200~400). Rabies infection frequently causes cytoplasmic eosinophilic inclusion bodies (Negri bodies) in neuronal cells, especially pyramidal cells of the hippocampus and Purkinje cells of the cerebellum.

So the existence of inclusion bodies in neuronal cells (including in those of exper imentally inoculated mice) was positive criterion in this study. Encephalitis, infiltration with lymphocytes and polymor phonuclear leukocytes were also observed (Table 2).

Table 2. Histopathological signs of animal rabies observed

Histopathological signs	Rabid animals		
	Cattle	Dog	Raccoon dog
Encephalitis	41	48	23
Infiltration with lymphocytes	35	45	21
Infiltration with leukocytes	35	45	21
Negri bodies	42	50	26
No signs	1*		

* Negri bodies confirmed in mouse innoculation test.

Immunopathological tests

A part of brain samples were collected and sectioned (4 ㎛ in thickness) at frozen state for indirect fluorescent microscopic examinations. The thin frozen rabies-suspected brain tissue sections were put

on the slide glass and fixed with acetone for 10 minuets. The sections were mounted with anti-rabies monoclonal antibody solution and incubated at 37℃ for 45 minutes. After washed with cold phosphate buffered saline solution (PBS), the section were mounted again with fluorescent-labeled 2nd antibody solution (FITC conjugated goat anti-mouse IgG) and incubated at 37℃ for 45 minutes and washed three times for 5 minutes with cold PBS. Fluorescence microscopic observation was performed on the slide. The areas where antigen is present were visualized as fluorescent-bright-green using a fluorescence microscope (Fig 1).

Inoculation tests on laboratory animals

To reproduce this disease in laboratory animals, the suspected brain samples were homogenized and centrifuged and the suspensions were attenuated with saline. The 0.03 ㎖ of virus inoculums (attenuated suspensions) was injected into the cerebral meninges of 10 mice. The same quantity of virus free saline was also injected into the same region of 10 mice as control. The clinical signs on the mice were observed for 12~20 days. Most part of the inoculated mice were suffered from illness and the skin of the mice were rough after 7~10 days. Within 15 days,

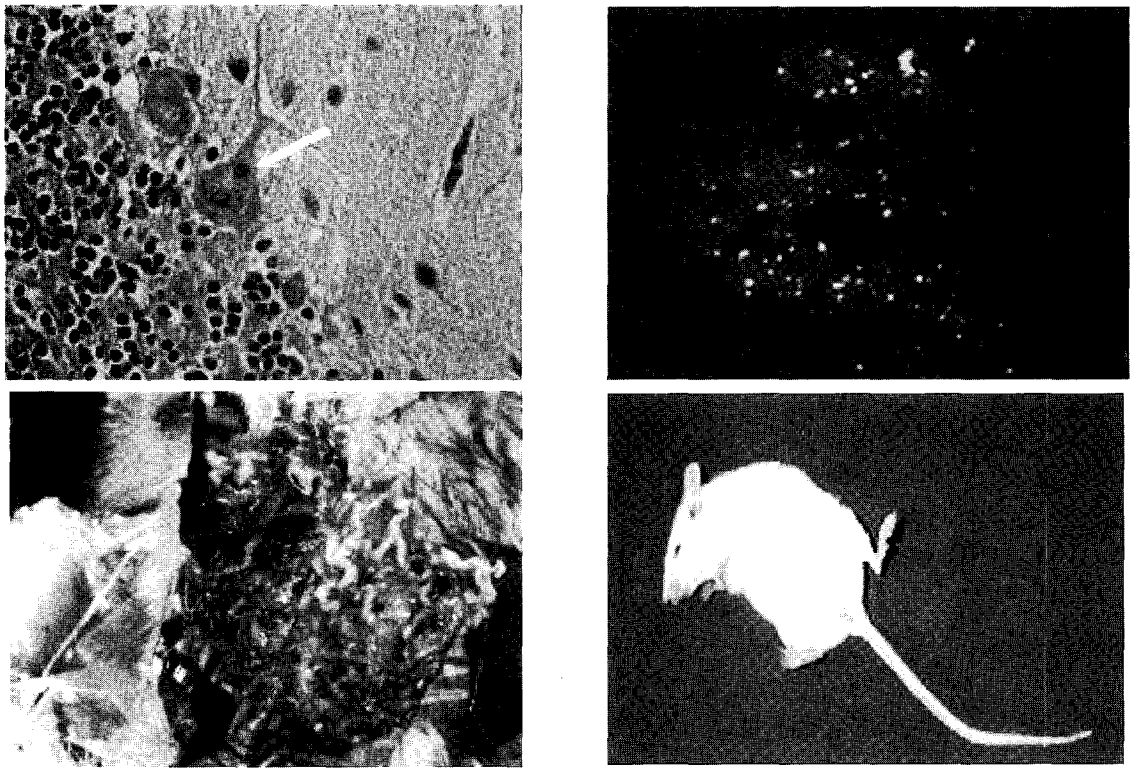

Fig 1. The clinical, histopathological and immunopathological signs of rabid animals.
Left above : Negri bodies in neuronal cells(in a rabid Korean raccoon dog, X400).
Left lower : Foreign bodies in the stomach of a rabid dog.
Right above : Fluorescent-green neuronal cells(in a rabid Korean raccoon dog, X200).
Right lower : Bent back and comatose state (in a experimentally inoculated mouse).

most part of the inoculated mice was dead. All dead experimental mice were necropsied to observe the lesions. The brain samples of the mice were also collected and fixed in buffered formalin. The following procedures of histopa thological examinations on the brain tissues were the same as in natural cases. Microscopic observation was performed and the existence of inclusion bodies in neuronal cells was positive criterion in this study (Fig 1).

Surveillance of rabies

Below (Table 3) is surveillance informa tion from 1993 to 2003, showing the distribution of rabies in Gangwon-do. Animal rabies infections were identified in 119 animals from 1993 to 2003 (Fig 2). The 78% of all rabid animals were domestic species in Gangwon-do. The number of reported cases of rabid domestic animals is 71% from 2002 to 2003. Rabid wildlife accounted for 22% of the total rabies cases from 1993 to 2003. Korean raccoon dogs continued to be the only reported rabid wildlife species. Outbreaks of rabies infections in Korean raccoon dogs are found in broad geographic regions across the northern

Table 3. Distribution of rabid animals in Gangwon-do from 1993 to 2003

Region / Year	Total 119	Cheolwon County 78	Hwacheon County 16	Injae County 4	Yanggu County 12	Sokcheo City 2	Yangyang County 2	Gosung County 1	Chuncheon City 4
Total	D:50 C:43 R:26	D:35 C:34 R: 9	D:8 C:3 R:5	D:3 C:1	D:2 C:5 R:5	R:2	D:1 R:1	D:1	R:4
1993	D:1	D:1							
1994	D:8 C:5	D:5 C:3	D:2	D:1 C:1	C:1				
1995	C:2 R:1				C:2 R:1				
1997	D:4 C:5 R:1	D:4 C:5 R:1							
1998	D:8 C:12	D:8 C:12							
1999	D:7 C:4 R:5	D:5 C:2 R:3	D:1 C:2 R:1			R:1	D:1		
2000	D:2 R:1	D:2			R:1				
2001	D:5 C:3 R:7	D:5 C:3 R:4			R:2	R:1			
2002	D:12 C:7 R:7	D:4 C:5 R:1	D:4 R:4	D:1	D:2 C:2 R:1		R:1	D:1	
2003	D:3 C:5 R:4	D:1 C:4	D:1 C:1	D:1					R:4

* Unit: head, D: Dog, C: Cattle, R: Korean raccoon dog

Gangwon-do (Fig 3). The epidemiology of rabies addresses several questions: what animals have rabies and where it breaks out, and what are the best strategies for preventing rabies in animals.

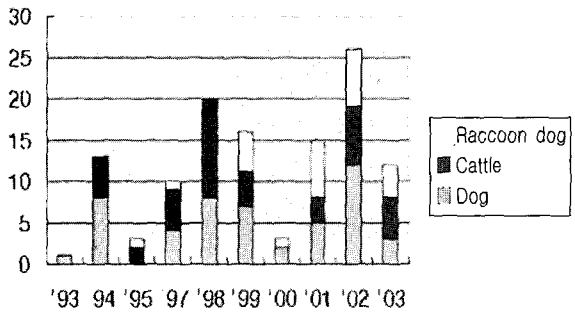

Fig 2. Number of animal rabies case in Gangwon-do (1993 to 2003).

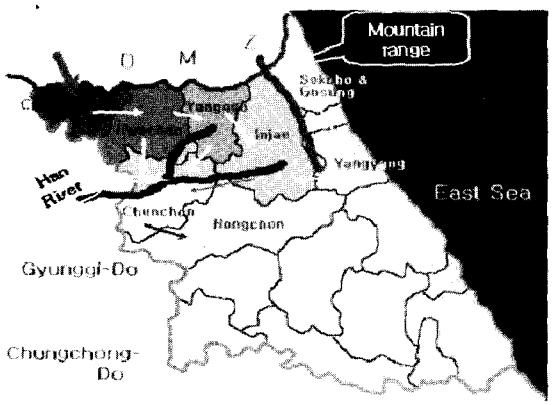

Fig 3. Spreading routes of animal rabies in Gangwon-do

Discussions

Rabies is not, in the natural sense, a disease of humans. The Human infection is incidental to the reservoir of the disease in wild and domestic animals. Although some countries (UK, Australia) are rabies free through vigorous con trol[1,7,14,18,19], rabies is endemic in wild animals in most parts of the world[3,13,14,18,20]. The wild animal cycle con stitutes the natural reservoir. Wild Korean raccoon dogs may bite and infect dome stic animals (cattle, horses, pigs, dogs and cats) which in turn may infect human. Occasionally wild animals may infect human directly. Despite the evid ence that control of dog rabies through programs of animal vaccination and elimination of stray dogs can reduce the incidence of animal rabies, exposure to rabid dogs is still the cause of over 90% of human exposures to rabies[7,12,18]. So the number and distribution of cases of rabies in domestic and wild animals are important for public health. The reservoirs of wildlife rabies, virtually unknown in Gangwon-do, especially in DMZ regions, are also potential sources of rabies infection for dogs[15,17]. Rabies remained a widely-distributed enzootic disease of terrestrial mammals in Gangwon-do (Fig 3).

To be a rabies free country, effective system of the disease surveillance must be in operation. Each year, scientists from the Gangwon-do Veterinary Service Labor atory (GVSL) collect information about cases of animal rabies and publish the information in a summary report[15,17]. The most recent report, entitled "Recent outbre aks of rabies in Gangwon-do" contains the epidemiologic information on rabies during 1997~2001[15]. From 2002 to 2003, 7 counties (or city) reported 38 cases of rabies in animals[15]. Chuncheon was the city that has not reported any rabies case animals but newly reported 4 cases in 2003. The geographical range of animal rabies outbreaks was extended to southern area of Gangwon-do. Various routes of transmission have been documented but

the most common mode of rabies virus transmission is through the bite and virus-containing saliva of an infected host. Several factors may affect the outcome of rabies exposure. These include the virus variant, the dose of virus inoculum, the route and location of exposure, as well as individual host factors, such as age and host immune defenses.

Rapid and accurate laboratory diagnose in animal rabies is essential for timely administration of postexposure prophylaxis. If a diagnostic laboratory can determine whether or not an animal is rabid within a few hours, the laboratory results may save a patient from unnecessary physical and psychological trauma. The rabies virus is a single antigenic entity but there are a large number of antigenically similar rabies-like viruses[3,7,18,20,21]. Saliva can be tested by virus isolation or reverse trans cription followed by polymerase chain reaction (RT-PCR)[2,6,9,10,16]. Serum and spinal fluid are tested for antibodies to rabies virus10[6,16]. Other tests for diagn osis and research, such as electron microscopy (EM), histologic examination, immunohistochemistry (IHC) and isolation in cell culture are useful tools for studying the virus structure, histopa thology, molecular typing, and virulence of rabies viruses. The direct fluorescent antibody test (dFA) is frequently used method to diagnose animal rabies2[11,13,16]. This test has been thoroughly evaluated for more than 40 years, and is recognized as the most rapid and reliable of all the tests available for routine use. All rabies laboratories in the United States perform this test (post-mortem) on animals suspected of having rabies[7,13,14,18]. Mean while in Korea, indirect fluorescent antibody test (IFA) is regarded a standard method for animal rabies[13,15]. This test has also been evaluated for years and more frequently used in Korea. The most important part of an IFA test is the 1st antibody (anti-rabies antibody). When anti-rabies antibody is incubated with rabies-suspect brain tissue, it will bind to rabies antigen. Unbound antibody can be washed away. The remains (unwashed antibody-antigen complex) will also bind to the fluorescent-labeled 2nd antibody when incubated with and unbound 2nd antibody can be also washed away. The areas where fluorescent-labeled antibody-antigen complex is present can be visua lized as fluorescent-bright-green areas through a fluorescence microscope[2,15,21]. The test also can only be performed post-mortem (after the animal is dead).

The epidemiology of rabies addresses several questions: what animals have rabies and in what regions, how many animals get rabies and from what animals, and what are the best strategies for preventing rabies in people and animals. Epidemiologic information is often presen ted as statistical data. For example, in 2003, 12 cases of animal rabies were reported in Gangwon-do. Korean raccoon dogs accounted for almost 34% among them[15]. Therefore, vaccinations for all dogs are the most important not only to keep dogs from getting rabies, but also to provide a barrier of protection for humans. If domestic animal is bitten by a rabid wild animal, successful vaccination (rabies vaccine and immune globulin) programs must be performed. More oral vaccines for raccoon dog rabies[4,20,22] must be scattered on the forest in northern part of the Gangwon-do aircrafts. Also oral rabies

vaccine in dogs must be developed with all means available[23]. Unfamiliar animals (wild or domestic, even if they appear friendly) must not be handled carelessly. Stray animals must be isolated (removed), because they may be unvaccinated and could be infected by the disease. Preex posure vaccination is recommended for persons in high-risk groups, such as laboratory workers, veterinarians and cert ain animal handlers. Other persons whose activities bring them into frequent contact with rabies virus or potentially rabid Korean raccoon dogs, dogs, or other species at risk of having rabies should also be considered for preexposure prop hylaxis.

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