

Cross-species infections of Formosan ferret-badger (*Melogale moschata subaurantiaca*) rabies from 2013 to 2021 in Taiwan

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Abstract

This study is the first survey about the cross-species transmission of Formosan ferret-badger rabies worldwide. Only the Chinese mainland and Taiwan are the epidemic areas for this disease. In Taiwan, this disease first surfaced in 2013. There were 12 cases of cross-species transmission from 2013 to the end of 2021. These cases involved four species including one Asian house shrew (*Suncus murinus*), one 6-week-old puppy, nine masked palm civets (*Paguma larvata*), and one yellow-throated marten (*Martes flavigula*). Among the 12 cases of cross-species transmission, only the puppy, yellow-throated marten, and two masked palm civets had health feature records. Ticks and maggots parasitized the yellow-throated marten, and no other animals were found to be ectoparasites infested. To sum up, the animals with health features manifested emaciated and/or immature features. Additionally, the 12 cases of cross-species transmission occurred in the mountainous area or the rural area rather than the urban area. The risks of cross-species infection of Formosan ferret-badger rabies were discussed by quoting related research reports in recent years.

Keywords: cross-species transmission, Formosan ferret-badger, Taiwan, rabies, wildlife diseases

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Introduction

Rabies is a kind of acute, progressive, and incurable viral encephalitis. The pathogen is the neurotropic RNA viruses of the family Rhabdoviridae and genus Lyssavirus. The mammalian hosts include mainly Carnivora and Chiroptera. The virus is transmitted mainly by animal bites; once it is deposited in the peripheral wounds of injured tissues, it will be transmitted to the central nervous system. When the virus reaches the brain, it will cause lethal encephalomyelitis (Rupprecht *et al.*, 2002). The rabies virus of terrestrial animals remains in two epidemiological cycles; one is the urban cycle, and the other is the sylvatic cycle. In the urban cycle of rabies, dogs are the foremost hosts. In the sylvatic cycle of rabies, the reservoir hosts include foxes, skunks, raccoons, wolves, raccoon dogs, and ferret-badgers etc. (Zhang *et al.*, 2009; WHO, 2013). On July 17, 2013, Taiwan found rabies in wild ferret-badgers and reported it to the World Organization for Animal Health (OIE, 2013) in an immediate notification format. That was the first case of rabies in Taiwan since the World Health Organization announced that Taiwan was not an epidemic area of rabies in 1961 (WHO, 1966; Chiou *et al.*, 2013; Chang *et al.*, 2016). During the burst period of the Formosan ferret-badger rabies epidemic, many ferret-badgers were found dead by the road. The citizens were occasionally attacked by rabid ferret-badgers causing latent exposure (Shih *et al.*, 2018; Tu *et al.*, 2020). The Formosan ferret-badger rabies has occurred only in the Chinese mainland and Taiwan (Zhang *et al.*, 2009; Chang *et al.*, 2016). The ferret-badger is a kind of omnivorous mustelids with its upper body being chocolate brown and the base of the dorsal body setae being white. There are diversified white markings on its face. These markings extend to the part below the eyes and the front of the ears on both sides of the face. There is a quadrate white spot between the eyes on the central line of the face (Storz and Wozencraft, 1999). Earthworms, insects, and berries are the primary food sources of ferret-badgers (Chung and Lee, 1997). The average weight of an adult ferret-badgers is lower than 2 kg (Storz and Wozencraft, 1999; Zhang *et al.*, 2010). The ferret-badgers have very bad vision, small teeth, weak biting force, and lower viability than dogs. It is found that the ferret-badgers cannot survive in the living areas of stray dogs (Yen *et al.*, 2019). Additionally, the ferret-badgers do not go to cities to hunt for food and live on the ceilings or under the floors as raccoons do (Harris *et al.*, 2010). Therefore, the Formosan ferret-badger rabies is not a big trouble for residents in urban areas in Taiwan (Shih *et al.*, 2017b).

According to the Communicable Disease Control Act of Taiwan, the Taiwan government raised rabies to the first category of National Notifiable Diseases since 1999 and established stricter surveillance policies. Since 1999, the Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ) has established animal rabies surveillance programs and created the bat rabies and wild medium-sized carnivore zoonosis surveillance programs in 2008 and 2013, respectively (Chang *et al.*, 2016). Up to the end of 2021, 4,072 wildlife samples of Carnivora were tested, including 2,563

ferret-badger samples and 868 positive ferret-badger rabies cases. In the United States, the long-term mandatory dog vaccination programs successfully exterminated dog-mediated rabies in 2007 (CDC_US, 2007). However, about 66 dogs and 245 cats were bitten by rabid bats, rabid raccoons, or rabid skunks annually, causing cross-species transmission of rabies (Ma *et al.*, 2021). Cross-species transmission is not high (Gordon *et al.*, 2004; Kim *et al.*, 2014; Wallace *et al.*, 2014), but it is a threat to public health. Therefore, the U.S. government still requires pet owners to vaccinate their dogs, cats, and ferrets against rabies (CDC_US, 2022). Like the U.S., Taiwan is now free of dog-mediated rabies, and the Formosan ferret-badger is the unique rabies reservoir in Taiwan (Chang *et al.*, 2016). Considering public health, this study surveyed the cases of cross-species transmission of Formosan ferret-badger rabies in Taiwan.

Materials and Methods

Animal samples collection and rabies virus antigen testing: According to the Communicable Disease Control Act of Taiwan, any suspected cases of rabies must be notified to health authorities or animal epidemic prevention authorities or directly dial the 24-hour notification hotline. In this survey, all the submitted animal brain tissues were diagnosed with rabies. The samples included dead animals by the roads, and dead animals or living animals with suspected rabies symptoms from other sources. The animals were collected with the assistance of law enforcement officials from the municipal/prefectural competent authority for veterinarians, wildlife conservationist / researcher and ordinary people. According to the standard operating procedure in Chapter 3.1.17 of "OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2021", the Animal Health Research Institute (AHRI) used the direct fluorescent antibody test (DFA) to perform the diagnostic test for rabies (OIE, 2021). Each submitted brain tissue sample was tested synchronously with two repeated subsamples in the DFA test method. The sample was inoculated into the Murine Neuroblastoma cell line for rabies variant identification when the DFA test result was uncertain, as stated by Tu *et al.* (2020).

National rabies case report information: All the submitted animal samples had the following data: A. sample collection date; B. serial number of sample; C. sample collection address and collection coordinates; D. date of death of sample; E. sample description, the content must contain the species, sex, estimated age, dead/alive of animal samples, and the sample transportation method; F. if a person is bitten or not; G. if a dog was present and fighting with the ferret-badger and exposed; H. the age and vaccination record of the dog on the scene, the dog owners data and contact information; I. date of reception by testing authority, rabies testing completion date; and K. rabies testing results, as stated by Tu *et al.* (2020).

Results and Discussion

Animal Specimens: Table 1 showed the sample size of wild carnivores in this survey. Among the cross-species transmission cases of the Formosan ferret-badger rabies from 2013 to 2021 in Taiwan, the Formosan ferret-badger (reservoir), masked palm civet, yellow-throated marten and dog were carnivores, except for the Asian house shrew. The

Asian house shrew is a species found in a very wide variety of habitats, including forests, grasslands, and urban areas etc. In addition, the Asian house shrew is also a human commensal (Hutterer *et al.*, 2016). This species is frequently seen in Taiwan, mainly living in the drain ditches in human communities. It is classified as a pest by the Centers for Disease Control (CDC) in Taiwan. However, no data is available regarding their population and distribution in Taiwan.

Table 1 Sample size of wild carnivores surveyed in Taiwan, 2013 to 2021.

Year	Subtotal	Ferret-badger	Masked palm civet	Crab-eating mongoose	Small Indian civet	Siberian weasel	Otter	Taiwan black bear	Leopard cat	Marten
2013	1,019	831	161	17	3	4	0	0	0	3
2014	306	243	54	7	1	1	0	0	0	0
2015	321	204	91	14	1	0	9	0	0	2
2016	223	138	78	4	1	0	0	1	1	0
2017	374	236	110	11	5	1	8	0	2	1
2018	496	282	170	36	0	0	0	0	3	5
2019	456	257	146	33	7	0	0	0	13	0
2020	513	235	206	33	8	1	4	1	22	3
2021	364	137	160	29	5	2	4	0	24	3
Total	4,072	2,563	1,176	184	31	9	25	2	65	17

Like the Asian house shrew, no data is available regarding the population and distribution of wild carnivores in Taiwan either. Therefore, this survey referred to the study of Schwartz *et al.* (2020) using the road-killed wild carnivores as the main sample source. In table 1, the top two wild carnivores in descending order were ferret-badger 62.9% (2,563/4,072) and masked palm civet 28.9% (1,176/4,072). They accounted for 91.8% (3,739/4,072) of the total sampled carnivores; species other than the top two species only accounted for 8.18% (333/4,072). Prior studies in Taiwan indicated that the masked palm civet and crab-eating mongoose were distributed over the low-to-mid elevation forests, while the yellow-throated marten and Siberian weasel were distributed over the mid-to-high elevation forests. However, the ferret-badger was the only species widely distributed along with various elevation forests. Additionally, the ferret-badger, masked palm civet, and Siberian weasel were nocturnal animals. The yellow-throated marten and crab-eating mongoose were diurnal animals (Chen *et al.*, 2009; Chiang *et al.*, 2012).

Cross-species transmission of Formosan ferret-badger rabies: The transmission mode by which the reservoir species infects the species other than the reservoir species is known as interspecies transmission, cross-species transmission, or spillover. The data in Table 2 were the cases of cross-species transmission of Formosan ferret-badger rabies in Taiwan from 2013 to 2021. There were 12 cases of cross-species transmission in this period, involving four species, including one Asian house shrew (*Suncus murinus*), one 6-week-old puppy, nine masked palm civets (*Paguma larvata*), and one yellow-throated marten (*Martes flavigula*). The circumstance column of Table 2 showed the animal health features of these 12 cases of cross-species transmission. The rabid Asian house shrew was the first case of spillover infection, which was found in a rural house in Taitung County in 2013. The ferret-badger rabies in Taitung County, meanwhile, was just

in the initial pattern of rabies epizootic and lots of rabid ferret-badgers fled everywhere (Tu *et al.*, 2020). This could be the reason for the spillover infection of this case in human communities. The puppy of Serial no. 2 was "a 6-week-old puppy". The yellow-throated marten of Serial no. 12 had the health features of "emaciated, pale mucous membranes, infested with ticks and maggots, showed weakness". Additionally, these 12 cases of cross-species transmission occurred in the mountainous area or the rural area rather than the urban area.

Factors of cross-species transmission of rabies: The risk factors for cross-species transmission of rabies are very complicated. Wallace *et al.* (2014) indicated that the possible factors which induced the cross-species transmission of rabies among terrestrial animals included animal susceptibility to the virus, animal population densities, animal behavior, niche overlap, landscape characteristics, human population distribution, and environmental conditions. Prior research findings showed that cross-species transmission of rabies was very low (Gordon *et al.*, 2004; Kim *et al.*, 2014; Wallace *et al.*, 2014). Gordon *et al.* (2004) studied the risk factors of raccoon-mediated rabies in the cross-species infection of cats and found that the first epizootic period of raccoon-mediated rabies was the highest risk period of cross-species transmission (OR=7.4, $P<0.01$). It was followed by the inter-epizootic period (OR=5.1, $P<0.01$). Other similar studies also found that the number of rabid raccoons positively correlated with spillover infection of non-reservoir animals (Fogelman *et al.*, 1993; Wilson *et al.*, 1997). Mitmoonpitak *et al.* (1998) analyzed the epidemic record of dog-mediated rabies from 1987 to 1996 by The Queen Saovabha Memorial Institute, subordinated to the Thai Red Cross Society. They found that when the number of rabid dogs increased, the number of rabid cats increased accordingly. Data showed a strong positive correlation ($R=0.9919$, $P<0.0001$). The above epidemiological data

consistently indicated that the case load of cross-species infection of non-reservoir animals which contacted the reservoir animals increased with the number of reservoir animals infected with rabies. The truth validated the findings of Anderson *et al.* (1981):

the transmission of rabies depended on direct contact, so it was influenced by the population density of susceptible animals and the contact rate of rabid animals.

Table 2 Information of 12 cases of spillover from Formosan ferret-badger rabies in Taiwan, 2013~2021

Serial no.	Sampling date	City/County	Animal	Elevation (meters)	Circumstance
1	2013-07-25	Taitung	Asian house shrew	35	After being found at home, one Asian house shrew was killed and sent to the county's Veterinary Department.
2	2013-09-08	Taitung	Domestic dog	35	One 6-week-old puppy, bitten by a rabid ferret-badger.
3	2014-12-24	Pingtung	Masked palm civet	88	Found inside a house with convulsion and snout injury, died the next day.
4	2015-01-21	Taitung	Masked palm civet	375	Found dead on road.
5	2015-01-27	Taitung	Masked palm civet	245	Found dead on road.
6	2015-02-03	Pingtung	Masked palm civet	88	Found dead on road.
7	2015-05-06	Yunlin	Masked palm civet	397	Found dead on road.
8	2015-12-09	Pingtung	Masked palm civet	88	Found dead on road, adult.
9	2019-10-27	Kaohsiung	Masked palm civet	513	Found on the road with convulsion, snout injured, emaciated, adult, euthanized.
10	2020-01-09	Hualien	Masked palm civet	26	Found inside a house with a mournful cry, convulsion, emaciated, immature, and euthanized.
11	2020-02-12	Hualien	Masked palm civet	163	Found dead on road, adult.
12	2021-06-11	Taitung	Yellow-throated marten	1,769	Male, adult, emaciated, pale mucous membranes, infested with ticks and maggots, and showed weakness.

In recent years, comparative phylogenetic studies had further found that the host barrier characterizes the cross-species transmission of rabies, and this barrier had to be overcome after viral evolution. In other words, the longer the phylogenetic distance between the reservoir host and the novel host was, the lower the occurrence frequency of cross-species transmission was (Streicker *et al.*, 2010; Mollentze *et al.*, 2020). It suggested that the species contact rate was the precondition of cross-species transmission. In terms of phylogenetic comparison, the physiological or immunological barriers resulting from the differences in the classification of different hosts were very important for the successful occurrence of cross-species transmission (Fisher *et al.*, 2018). Therefore, if a non-reservoir animal was exposed to the rabies virus, it was not sure that it would be infected with rabies. Additionally, the research by the U.S. Centers for Disease Control and Prevention indicates that if a dog that had not been inoculated with a rabies vaccine was exposed to dog-mediated rabies (even if it was immediately inoculated with a rabies vaccine afterward), there would be no protective effect (Hanlon, 2002). This research showed that the susceptibility of the rabid reservoir to viral variant was hugely different from that of the non-reservoir.

Cross-species transmission of rabies in this survey:

Rabies epidemics among reservoir host populations follow a distinct course. Intervals of increased disease activity (epidemics) are separated by intervals (inter-epidemics) in which rabies may seem to disappear or reach undetectable levels in reservoir host populations (Childs and Real, 2007). To date, the Formosan ferret-

badger is the unique reservoir of rabies in Taiwan (Chang *et al.*, 2016). The oscillations in incidence data of the ferret-badger rabies in Hualien County showed the typical pattern of rabies epidemics among reservoir host populations (Tu *et al.*, 2020). However, in table 2, all animals spilled over from ferret-badger rabies variants, were free of the abovementioned typical pattern of rabies epidemics of reservoir host populations. Every case in Table 2 occurred individually, which implied that they were infected by rabid ferret-badgers.

The 6-week-old puppy of Serial no. 2 of Table 2 was bitten by a rabid ferret-badger and died of spillover infection of rabies. The dog's immune system is not mature until six months old (Pereira *et al.*, 2019), consequently, the innate or adaptive immunity of this puppy was underdeveloped. It was undoubtedly infected with rabies for weak resistance. On the contrary, in Taiwan in 2013, there were 14 adult household dogs not inoculated with the rabies vaccine. They fought with the in breaking rabid ferret-badgers at home and were exposed to the rabies variant. These 14 adult dogs were free of rabies in 7 years (Tu *et al.*, 2020). Scott and Nel (2016) indicated that age could influence the susceptibility of mammals to RABV infections. When young and senile individuals were exposed to rabies variants, they were more susceptible to being infected than mature ones. This was possibly because the pathogenicity of rabies to the host and the host's immune responses to rabies would be different due to different ages.

As stated earlier, since the ferret-badger and masked palm civet were nocturnal animals with large population sizes, it might be one of the causes of a high

spillover infection rate of masked palm civet. The yellow-throated marten and crab-eating mongoose were diurnal animals. They were unlikely to come into contact with the ferret-badger, so they were unlikely to have spillover infection. As for the unique yellow-throated marten of spillover infection found in this survey, it might have encountered a rabid ferret-badger in the forest and was too weak to escape infection from the rabid ferret-badger due to its suffering from serious myiasis and tick infestations (Obanda et al., 2013; Philip et al., 2014).

These 12 cases of cross-species transmission in Table 2 occurred in remote areas. Yen et al. (2019) indicated that in Taiwan stray dogs were mainly dependent on humans to feed on. An adult ferret-badger weighs about 2 kg, and a survivable stray dog usually weighs about 10 kg. As soon as there were stray dogs in human communities, the ferret-badgers were not able to survive. On the other hand, Harris et al. (2010) indicated that ferret-badgers were not adapted to the environmental conditions of human communities. As they did not like human food, and there were no setts for ferret-badgers in human communities, consequently, the cities were not attractive to ferret-badgers. Shih et al. (2017a) found a highly significant difference in the incidence of ferret-badger rabies between mountain townships and urbanized townships ($P < 0.001$) in the Chi-squared test. Shih et al. (2017b) also found that the cases of Formosan ferret-badger rabies mainly occurred in mountainous areas at an elevation of 300~600 meter. Besides the factor of stray dogs, lack of food and habitats for ferret-badgers in urbanized townships were other causes. The abundant food of ferret-badgers was mainly supplied by the forests, including important earthworms and insects and berries (Chung and Lee, 1997). The above findings may explain why the cases of spillover of Formosan ferret-badger rabies occurred in remote areas rather than densely populated cities.

In conclusion, researches on the cross-species transmission of rabies are rarely seen. This study proposed the cases of cross-species infections of Formosan ferret-badger rabies over the past nine years in Taiwan. Dogs and cats are pets and are closely bonded with humans. When pets are exposed to rabies variant, human health will be threatened. Therefore, governments of various countries require rabies vaccination for dogs and cats. As for wildlife rabies, the rabies prevention and control program were implemented only for the reservoir host which was a threat to human health, such as raccoon-mediated rabies and fox-mediated rabies. The former (raccoon) thrived in human settlements ecologically; the latter (fox) was taxonomically close to dog's consanguinity (Maki et al., 2017; Johnson et al., 2021; Ma et al., 2021). The WHO (1987) indicated that cats, although closely associated with dogs in urban ecosystems and often infected by rabid dogs, did not play an important role in the maintenance of chains of infection. In general cat rabies disappeared as the dog rabies was brought under control. However, cats played a significant role as transmitters of rabies from the reservoir in dogs to man. Therefore, prevention and control measures should be applied to cats wherever possible.

Institutional Review Board Statement: This study did not perform any invasive and painful experiments on animals and did not need to submit to *Institutional Animal Care and Use Committees* (IACUCs) accordingly.

Data Availability Statement: The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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