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Original Article

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Abstract

The World Health Organization declared Taiwan rabies-free in 1961. On July 17, 2013, Taiwan confirmed the detection of rabies virus in wild Formosan ferret-badgers. This study investigated the epidemiology of Formosan ferret-badger rabies from July 2013 to December 2019 on Taiwan Main Island. There were two objectives of this study: 1) to study the temporal dynamics of the epidemics during this period; 2) to assess the risk of human exposure to ferret-badger rabies. Results indicated that a total of 805 rabies-confirmed ferret-badgers and 9 cases of spillover infection in non-reservoir hosts. The temporal dynamics showed only the epidemic in Eastern Taiwan exhibited the typical initial epidemic growth pattern; while the epidemics in Western Taiwan and Southern Taiwan appeared to have subsided to enzootic levels as of December 2019. As for human exposure to ferret-badger rabies, all cases of human exposure at home appeared in only one ferret badger and usually occurred in the evening. During the exposures, if a dog were present, it would usually spot the rabid ferret-badger earlier than the attack of the rabid ferret-badger and rush to kill it. There were 48 cases indoors and 21 cases outdoors of human exposure. The relative risk (RR) for human exposure to rabid ferret-badgers without dogs around was 4.73 times that with dogs around indoors (n=214; $p < 0.0001$). The risk for human exposure to rabid ferret-badgers without dogs around was 12.63 times that with dogs around outdoors (n=62; $p < 0.05$). In conclusion, the study showed that keeping dogs could protect people from suddenly unprovoked attacks by rabid ferret-badgers. The distribution of epidemic cases indicated that ferret-badger rabies was still sequestered to the mountainous regions. Dogs and cats should be vaccinated to establish an immunological barrier to stop the spread of the disease.

Keywords: Formosan ferret-badger rabies, Taiwan, rabies, wildlife diseases, epidemiology

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Introduction

Rabies Virus (RABV) is the prototype virus of the genus *Lyssavirus* in the family Rhabdoviridae and is the causative agent of classic rabies in human and all mammals. Once the infection is established, RABV will cause a fatal encephalomyelitis. Rabies infection in terrestrial animals is maintained in two epidemiological cycles, one urban and one sylvatic. In the urban rabies cycle, dogs are the main reservoir host. In the sylvatic rabies cycle, reservoirs comprise foxes, skunks, mongooses, raccoons, coyotes, raccoon dogs etc. (WHO, 2013). The World Health Organization declared Taiwan rabies-free in 1961 (WHO, 1966). On July 17, 2013, Taiwan confirmed the detection of rabies virus in wild Formosan ferret-badgers (Chiou *et al.*, 2013; OIE, 2013). To date, ferret-badger rabies is known to occur in China and Taiwan; and there is no dog rabies in Taiwan (GOV.UK, 2020). Ferret-badger associated human rabies cases emerged in China in 1994 (Zhang *et al*. 2009). However, genetically, the RABV variant that is enzootic to the Taiwan ferret-badger is distinct from all other known rabies virus variants (Chiou *et al*., 2013), and no human rabies deaths have been associated with the Taiwan ferret-badger virus epizootic.

The ferret-badger is an omnivorous wildlife mustelid. The pelage is longish and coarse; the general color of the upper parts is chocolate brown; hairs of the dorsum are pale basally. White facial markings are variable, generally covering the sides of the face below the eyes and in front of the ears, and continuous with the yellowish-white of the lips, chin, throat and venter; there is a squarish white spot between the eyes in the median line and a narrow, whitish stripe runs medially from the occiput, extending up to but rarely beyond the shoulders (Sousan Zoo, 2020; Storz and Wozencraft, 1999). Important food sources are earthworms and insects (Chuang and Lee, 1997). Reports of mean body mass for adults are less than 2 kilograms (Storz and Wozencraft, 1999; Zhang *et al*., 2010). The animal has poor visual perception, small teeth and weak biting force and it is considered as a weak competitor, weaker than stray dogs, and not as commonly found in urbanized areas as raccoons (Hadidian *et al*., 2010; Harris *et al*., 2010; Pei, 2001; Shih *et al*. 2017[b]; Shih *et al*. 2018; Wang and Fuller, 2003). The main areas where rabid ferret-badgers appeared were in mountainous areas with low population density (Shih *et al*., 2017[a]). The ferret-badgers exhibit strong nocturnal activity patterns, being seen throughout southern China, Taiwan, Hainan, the northern portions of Vietnam, Laos, Thailand, Burma, Bangladesh and North Eastern India (Storz and Wozencraft, 1999). The Chinese ferret-badger, which dwells mainly in southeastern China, is a different subspecies from the badgers in Africa and Europe (Zhang *et al*., 2009). In addition, Chinese ferret-badger rabies has formed an independent transmission cycle and might serve as another important rabies reservoir independent of dog rabies in China (Zhang *et al*., 2013). There are several wildlife species carrying rabies in China, including bats, Chinese ferret-badgers and raccoon dogs, which might serve as reservoirs for rabies viruses and can play a role in human or livestock rabies (Wang *et al*., 2014).

Rabies vaccination has always been mandatory for dogs in Taiwan ever since 1961. From the outbreak of ferret-badger rabies, the vaccination rate of dogs and cats rose rapidly to 97% in epizootic mountainous villages. The vaccination rate in non-enzootic area was 62%, while the overall vaccination rate nationwide was 66% (Chang *et al*., 2015).

The Taiwan Main Island was the sole enzootic area of Formosan ferret-badger rabies (Chang et al., 2015). Phylogeny of nucleoprotein and glycoprotein genes from ferret-badger rabies virus revealed that the variant was separated into two distinct groups, consistent with the geographic segregation of the west and east by the Central Mountain Range (Chang *et al*., 2015; Tsai *et al*., 2016). The Central Mountain Range is the principal mountain range on Taiwan Main Island, which can block the spread of the epidemic on the left and right sides of the mountains (Chang *et al*., 2015; Hsu *et al*., 2001; Tsai *et al*., 2016). The non-enzootic area of ferret-badger rabies is geographically located to the north of Daan River and Heping River on Taiwan island, which is called Northern Taiwan in this study. The enzootic area is located south of the two Rivers with a total of 9 counties or cities, comprising the areas of Eastern Taiwan, Western Taiwan and Southern Taiwan in this study (Chang *et al*., 2015). The objectives of this study were: 1) to study the temporal dynamics of the epidemics during this period; 2) to assess the risk of human exposure to ferret-badger rabies.

Materials and Methods

***Animal samples collection and rabies virus antigen testing*:** Rabies tests were performed on brain tissue from all suspected animals, ferret-badgers, including road-kills, those exhibiting neurological signs, captured or found dead. They were collected and submitted by private citizens, animal control officers or law enforcement officers, veterinarians, etc., to the national Animal Health Research Institute (AHRI) for test. The test for rabies antigen was the direct fluorescent antibody test (FAT). For the direct FAT test, each brain sample was used to make two subsamples and these were conducted concurrently. In cases of inconclusive results from FAT, samples were further inoculated into an MNA (Murine Neuroblastoma) cell line to detect replication of the virus.

***The excel RABIES FACT SHEET*:** The RABIES FACT SHEET comprised the following information: A. The record day; B. Annual serial number; C. The county where the sample was collected; D. The township where the sample was collected; E. The address of the case location; F. Time of death for the collected example; G. Case description (As detailed as possible, always consisting of the following information: 1. For ferret-badgers: Gender, Age, Dead or alive? How to manipulate the animal, such as: Caged to send or Euthanized then frozen to mail. 2. For any civilian person exposed or involved: The telephone numbers of related persons, treatment for the persons (usually moved to the official medical institute and a record left on this sheet). 3. If there were dogs around: Was the dog vaccinated, the name, gender, occupation, address, age, iPhone number etc., of the owner); H.

Organization of submission; I. Date sample was submitted; J. Animal type (ferret-badger, gem-faced palm civet, gender, young or adult etc.); K. blank; L. Date when AHRI was received; M. Pathological no. of AHRI; N. Date to do the test; O. Result of the test; P. Date for result report. Q. Note; R. Whether people were bitten; S. Whether dogs were present; T. blank; U. Whether humans were bitten by ferret-badgers and whether dogs were involved; V. latitude of the sample to be collected; W. longitude of the sample to be collected.

***Statistical analysis*:** MedCalc Statistical Software version 19.2.6 (MedCalc Software Ltd, Ostend, Belgium) was used in this study. The multiple line graph was used to prepare the annual statistical graph of temporal dynamics of rabid ferret-badgers from July 2013 to December 2019. The Chi-squared tests for two-way tables were used to calculate the relative risk (RR) and confidence intervals (CI) of incidents occurring indoors or outdoors. The information of each case of human contact with the rabid ferret-badger was filled in excel sheets according to: "Whether human was exposed or not"; "Whether dogs were present or not"; and "Did incidents happen indoors or outdoors". Information was filled in the excel tables and then the format of the statistical program of MedCalc started. The number of people being exposed or not to rabid ferret-badgers was defined as dependent variables. Whether dogs were present or not at incident locations were defined as independent variables. The term "human exposure" always indicated that human had been exposed to a rabid ferret-badgers, either indoors or outdoors. Non-rabid ferret-badger contacts were not included in this study. For all analyses, $p < 0.05$ was the threshold for statistical significance.

Results

***Surveillance of ferret-badger rabies*:** From July 2013 to December 2019, a total of 9893 mammalian vertebrates, consisting of 4621 wildlife and 5272 pets, were tested for surveillance of ferret-badger rabies viral antigen. A total of 805 rabies-confirmed ferret-badgers and 9 cases of spillover infection was found. Among them, 7 out of the 9 spillover infections were gem-faced palm civets. No human rabies deaths were associated with the Formosan ferret-badger virus epizootic.

***Temporal dynamics of ferret-badger rabies in Taiwan*:** Table 1 shows the synthesized annual subtotal numbers of rabid ferret-badgers from July 2013 to December 2019 in the areas of Eastern, Western and Southern Taiwan. Figure 1 shows the temporal dynamics of rabid ferret-badgers in Table 1. Among them, only the epidemic in Eastern Taiwan exhibited the typical initial epidemic growth pattern in ferret-badger rabies; while the epidemics in Western Taiwan and Southern Taiwan appeared to have subsided to enzootic levels as of December 2019.

Table 1 Number of rabid ferret-badgers at four different areas in Taiwan island, 2013 ~ 2019 (n=805).

Year	Eastern Taiwan	Western Taiwan	Southern Taiwan
2013	3	146	127
2014	15	48	84
2015	16	56	16
2016	3	35	4
2017	18	43	9
2018	52	49	9
2019	20	45	7

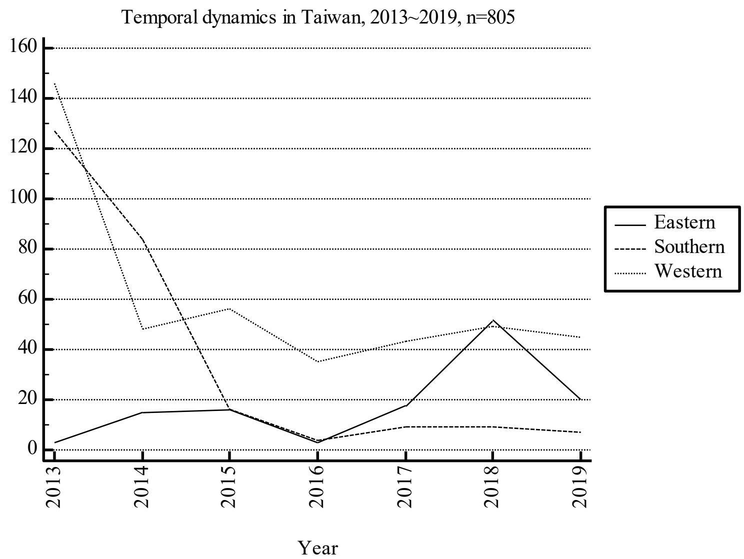
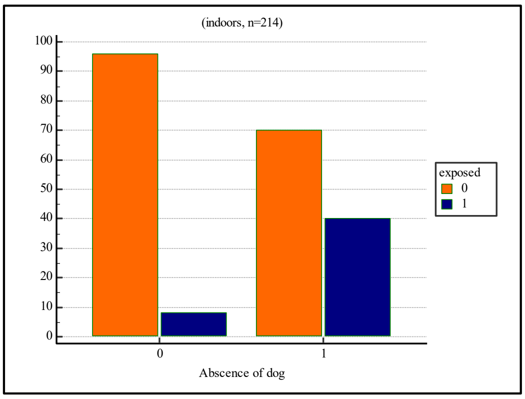


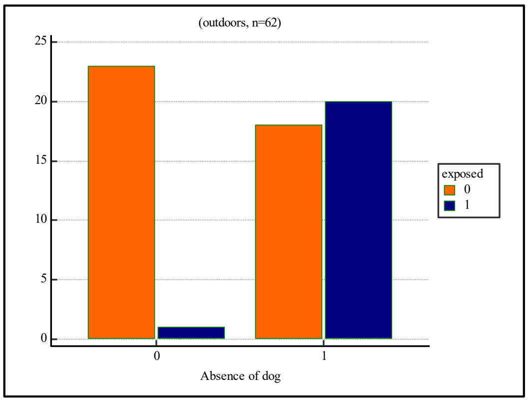
Figure 1 The temporal dynamics of rabid ferret-badgers at different areas in Taiwan island, from July 2013 to December 2019. Among them, only the epidemic in Eastern Taiwan exhibited the typical initial epidemic growth pattern in ferret-badger rabies; while the epidemics in Western Taiwan and Southern Taiwan appeared to have subsided to enzootic levels as of December 2019.

***Nature of human exposure*:** As for human exposure to rabid ferret-badgers, all cases of human exposures at home were only one ferret badger appearing and usually occurring in the evening. When incidents happened, usually people were resting or watching television in the room and the door of the house was always not closed. No one knew how and when the rabid ferret-badger entered the house. However, if there were a rabid ferret-badger, it always suddenly rushed to attack resident(s) from the dark part of the room. If there were a dog, or dogs, in the room, the dog(s) would spot it before the attack and would kill the ferret-badger. If there were no dog present and the resident was negligent, he/she was usually exposed to a rabid ferret-badger. Human exposures to rabid ferret-badgers outdoors always occur during the day on mountain roads or near mountain playgrounds. The rabid ferret-badger will suddenly rush out from the grass and attack people. If a dog is present, the dog (or stray dogs) will rush to kill the rabid ferret-badger before the attack. If there are no dogs present but the humans are alert enough, the rabid ferret-badger is often caught or killed by humans and then reported. From 2013 to 2019, A total of 276 out of 805 rabid ferret-badgers invaded human communities. Of these, 214 cases occurred indoors and 62 cases occurred outdoors. In these 214 indoor cases, the 96 rabid ferret-badgers that invaded human houses were killed by dogs. In these 62 outdoor cases, 23 rabid ferret-badgers were killed by stray dogs or guard dog(s). For human exposures, a total of 69 people was exposed to rabid ferret-badgers. Of these, 48 and 21 persons were exposed indoors and outdoors respectively. All people exposed to rabid ferret-badgers accepted post-exposure rabies prophylaxis as soon as possible. All owned dogs exposed to rabid ferret-badgers accepted rabies vaccination and 180 days quarantine. In these 276 incidents, a total of 128 dogs were exposed to ferret-badger rabies. Of them, 71 dogs had been vaccinated, 14 dogs had never been vaccinated and 43 dogs were not recorded in the RABIES FACT SHEET. To date, no cases of ferret-badger rabies, either in humans or dogs, have been reported.

***Relative risk (RR) analysis for human exposures to rabid ferret-badgers*:** Figure 2 shows the frequency for human exposure to ferret-badger rabies indoors and outdoors. Having a dog around and an indoor environment were risk factors for the value of RR was 4.73 ($p < 0.0001$), the 95% CI was 2.3237 to 9.6171, which indicated that the risk of humans to exposure to rabid ferret-badgers without dogs around was 4.73 times that of when dogs were around. Having a dog around and an outdoor environment were risk factors for the value of RR which was 12.63 ($p < 0.05$), the 95% CI was 1.8110 to 88.1022, which indicated that the risk of humans being exposed to rabid ferret-badgers without dogs around was 12.63 times that as when dogs around.



(a)



(b)

Figure 2 Frequency for human exposures to rabid ferret-badgers indoors (a) and outdoors (b).

Discussion

There were two objectives of this study: 1) to study the temporal dynamics of epidemics during this period, as shown in Table 1 and Figure 1; 2) to assess the risk for human exposure to ferret-badger rabies, as shown in Figure 2.

For spillover infection, Fisher *et al*., (2018) stated that spillover infections of one rabid biotype into the host range of the other are thought to occur from time to time. Concerning the ferret-badger rabies, Zhang *et al*., (2009) stated that spillover infections of ferret-badger rabies were maintained over a long time scale in new host species. These events were important for public health because they created new reservoirs for human exposure. Consequently, in sylvatic rabies epidemic areas, surveillance of spillover infection is important. In the last survey, data from January1999 to December 2014 was synthesized and 3 cases of spillover infection were found (Chang *et al*., 2015). In this survey, data from July 2013 to December 2019 was synthesized and 9 cases of spillover infection were found. Among them, 7 out of the 9 spillover infections were gem-faced palm civets, providing that the ferret-badger was related to the gem-faced palm civet ecologically. Chiang *et al*., (2012) used the canonical correspondence analysis (CCA) technique to analyze photographic rates and habitat niches of the 5 most common carnivores in Taiwan Main Island forests.

Data shows that among them, only ferret-badgers and gem-faced palm civets were nocturnal and both of them were found to be omnipresent in many areas across Taiwan forests. This might be associated with the causes of the 7 cases of spillover infection.

For the study of temporal dynamics, Figure 1 indicates that the epidemic in Eastern Taiwan appeared to be the pattern of the initially largest increasing epidemic size of rabies in the year 2018; while the epidemic sizes of Western Taiwan and Southern Taiwan show a pattern of diminishing epidemic size. Childs and Real (2007) described the development pattern of a rabies epidemic: "Rabies epidemics among wildlife reservoir host populations within defined regions frequently 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 within a local mammalian community. Following an initial epidemic of rabies, which is typically the largest of a possible series of epidemics that may emerge over time as wildlife rabies enters into a new region to infect previously naïve populations, a series of successively smaller epidemics may become indistinguishable against a background level of sporadic disease." According to this statement, Figure 1 indicates that the epidemic was too fast to be detected at the beginning of the epizootics in Western Taiwan and Southern Taiwan. While the course of the epidemics of Eastern Taiwan progressing more slowly than Western and Southern Taiwan. This was probably because the Central Mountain Range had blocked the way of the epizootic from Western Taiwan to Eastern Taiwan, ever since July 2013. In addition, Eastern Taiwan is a region of high mountains and highlands, while Western and Southern Taiwan consist mostly of basins, hills and plains (*National Taiwan Normal University, 2007*). However, the inference needs further scientific evidence to prove it.

For the risk of human exposure to ferret-badger rabies, this study shows that the dog was an important risk factor. The RABIES FACT SHEET recorded that when the rabid Formosan ferret-badger intruded human communities, large to medium-size dogs were able to kill it immediately, either indoors or outdoors (Shih *et al*. 2018). This fact explains the data of RR values in Figure 2, that when the rabid ferret-badger attacked humans, if there were no dogs around, the risk of people being bitten was higher than when there were dogs around. The major cause was that the ferret-badger is a small-toothed, weak biting force, with poor visual perception; a small-sized carnivore, whose body size and competitiveness are smaller than ordinary dogs (Duckworth *et al*., 2016; Pei, 2001; Shih *et al*. 2017[a]).

In this study, all humans exposed to rabid ferret-badgers accepted post-exposure rabies prophylaxis as soon as possible. To date, no human cases of ferret-badger rabies have been reported. Most deviations from the recommended protocol leading to death were from delays in seeking rabies prophylaxis; lack of or improper administration of rabies immunoglobulin; lack of or improper primary wound care and /or poor-quality rabies vaccine (WHO, 2013). To date, it seems that none of the abovementioned occurrences happened in Taiwan. Of the 128 dogs exposed to rabid ferret-badgers, either vaccinated or not, no dog was infected with rabies. This result relates to the issue of "What is a susceptible host?" Hanlon (2002) stating that "vaccine alone following severe exposure to dog rabies was unable to protect dogs from dog rabies." However, in this study, all the dogs were exposed to the ferret-badger virus instead of dog rabies. Hanlon (2013) also mentioned that "Due in part to innate immunity rather than adaptive immunity, multiple infectious units are required. A very susceptible species or individual host would be one in which only a small number of virus particles are required to result in an infection. Once an animal is exposed to rabies, the probability of infection depends upon the individual, the species, the rabies virus variant, the amount of virus, and the severity and route of the exposure." The above statement should be able to explain why none of these 128 dogs was infected with rabies. In conclusion, the progression of the epidemics in Western Taiwan as well as in Southern Taiwan was faster than in Eastern Taiwan. Consequently, the study of the spatio-temporal trend of ferret-badger rabies in Eastern Taiwan could be a possible objective to pursue in the future.

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