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An evaluation of two independent populations for Formosan ferret-badgers sampling in Taiwan

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Abstract

Since the first outbreak of Formosan Ferret-badger (*Melogale moschata subaurantiaca*) rabies (FFB rabies) in the main island of Taiwan in July 2013, by the end of 2023, there had been 10 FFB rabies-endemic counties or cities in Taiwan. The Animal Health Agencies of various counties and cities in the whole country have sustainably implemented surveillance of rabies epidemics in FFBs and other carnivorous animals. This study aimed to investigate any possible difference between the distribution of wild FFBs sampled by the Animal Health Agencies in the 10 FFB rabies-endemic counties or cities and the sample distribution of roadkill FFBs recorded by the Taiwan Roadkill Observation Network (TaiRON) in the same counties and cities. The samples from the governmental Animal Health Agencies and from the nonprofit TaiRON were independent due to the lack of no administrative connection between them. Therefore, in this study, Mann Whitney U test was adopted to determine the difference between the stated two independent sampled populations. The result of the Mann-Whitney U test showed that there was no significant difference (p=0.20) between the distributions of FFBs sampled by Animal Health Agencies and TaiRON at different elevations at α=0.05. In conclusion, statistically, the sample distribution of FFBs collected by Animal Health Agencies for surveillance of FFB rabies and that by the TaiRON network had the same representativeness.

Keywords: Mann Whitney U test; Formosan Ferret-badgers; *Melogale moschata subaurantiaca*; Rabies; Road-kill; Taiwan

1. Introduction

The spotlight survey is a traditional method to estimate the population abundance of medium-sized nocturnal mammals [1-5]. In the United States, state agencies usually used the spotlight survey and/or road-kill survey methods to monitor populations of raccoons (*Procyon lotor*) [6]. Gehrt et al. [3] compared the survey data of Illinois Department of Natural Resources on the raccoon population for five consecutive years from 1989 to 1993. They found a high correlation between the long-term trends of spotlight indices and road-kill indices of raccoons in the State of Illinois (P < 0.001). Therefore, it is believed that both survey methods had the same statistical representativeness for the raccoon population over a large spatial scale [3].

In July 2013, the Formosan Ferret-badger (FFB; *Melogale moschata subaurantiaca*) rabies was detected in the FFB population as the sole reservoir species of FFB rabies [7-8]. Like raccoons, FFBs are also nocturnal carnivores. Ever since 2013, the Animal and Plant Health Inspection Agency (APHIA), the national competent authority for *animal and plant health inspection* agency, announced periodically the data of sustaining national surveillance program of FFB

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rabies. Animal samples were collected by various Animal Health Agencies of counties and cities of the whole country. Announced surveillance data of each animal sample included: collection date, coordinates of location, species, sex, estimated age, dead/alive, if any person or other animals were exposed, result of direct fluorescent antibody test (DFA). Animal samples were collected from: dead animals found on the roads, and dead animals or living animals with suspected rabies symptoms. All animal samples were submitted to the national Veterinary Research Institute (VRI). The VRI used the direct fluorescent antibody test (DFA) to perform the diagnostic test for rabies, according to the standard operating procedure in Chapter 3.1.17 of "OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals" [9]. For rabies in FFBs, the congeneric species transmission was common. Host-shift events, the sustained transmission within a non-reservoir species after a cross-species transmission event, were less common [10]. As at July 2024, there were only 13 FFB rabies cases of cross-species transmission ever since July 2013.

Prior studies indicated that FFBs were the species widely distributed in the forests at various altitudes in Taiwan [11-12]. Besides, in Taiwan, stray dogs were significantly more dominant than FFBs. This was because stray dogs were typically larger than FFBs and relied on humans for food [13-14]. Therefore, the FFB was not commonly found in areas where there were stray dogs [15-17]. Because the living conditions for the FFB had aforementioned variables, the authors were to assess whether the distribution of the collected FFB samples for the sustaining national surveillance program of FFB rabies was representative, using the existing sole metric of road-killed record from the long-established Taiwan Roadkill Observation Network (TaiRON) [18] as the standard reference.

The Taiwan Roadkill Observation Network (TaiRON) is a national citizen science project launched in August 2011 by the Taiwan Biodiversity Research Institute, Ministry of Agriculture, with the goals of: (1) reducing roadkill, (2) citizen science, (3) environmental education, and (4) cherishing life [19]. Chyn et al. [20] pointed out that the road density of Taiwan is twice that of the United States at 1.2 km/km^2 in Taiwan and 0.67 km/ km^2 in the United States. In addition, the TaiRON database is currently one of the most extensive roadkill databases in the world. Over the years, the TaiRON website has published large amount of data on the species distribution of representative wild animals following expert analyses [20-21].

The FFBs sampling method employed by the Animal Health Agencies of various counties and cities in Taiwan is different from the sampling method applied by the TaiRON. However, there is no administrative subordination between the two organizations, so the sample populations of FFBs collected by the two organizations were independent. Therefore, in this study, the Mann Whitney U test was used to compare the distribution of FFB samples collected by the two organizations for the years from 2013 to 2023.

2. Materials and Methods

2.1. Sampling Methods of Animal Health Agencies and TaiRON

The STATUTE FOR PREVENTION AND CONTROL OF INFECTIOUS ANIMAL DISEASES OF TAIWAN states that any suspected cases of animal rabies should be reported to the local Animal Health Agencies. The method of collecting animal samples in the sustaining national surveillance program of FFB rabies, has always been implemented in accordance with the aforementioned STATUTE. The animal samples collected included dead animals by the roads, and dead or living animals with suspected symptoms of rabies from other sources. Animals were sampled with the assistance of law enforcement officials from the municipal/prefectural competent authority for veterinarians, wildlife conservationists/researchers, and ordinary people [8,10,15-17,22-23]. This study utilized historical records of the animal samples for the years from 2013 to 2023.

Likewise, the number of road-killed FFB samples published on the TaiRON website for the years from 2013 to 2023, was downloaded [18]. In addition, the geographic information of different towns, including elevations and road areas, was downloaded from the Taiwan Geospatial One-Stop (TGOS) website, Ministry of Internal Affairs [24]. The data on resident populations of various counties and cities of Taiwan in 2021 were downloaded from the website of the Department of Household Registration, Ministry of Internal Affairs [25].

2.2. Statistical Analysis

The Mann-Whitney U test [26] is a non-parametric statistical test to compare the distribution of two independent groups, which aims to determine any significant difference between the distributions of two separate groups. It helps to identify if one group tends to exhibit higher or lower values compared to the other group by comparing and evaluating the ranks of the observations [27]. The data of FFBs collected by various Animal Health Agencies in FFB rabies-endemic cities/counties and similar data downloaded from the TaiRON website were from two independent parent FFB

populations distributed in the main island of Taiwan. The hypotheses for a Mann-Whitney U Test in this study were: (1) the null hypothesis (H_0): the distributions of FFBs sampled by Animal Health Agencies and by TaiRON at different elevations were equal, and (2) the alternative hypothesis (H_1): the distributions of FFBs sampled by Animal Health Agencies and TaiRON at different elevations were not equal.

3. Results and Discussion

3.1. 3.1 The Geographical Information of Formosan Ferret-badger Rabies-endemic Area

Table 1 listed the distribution of the towns, population, road area, geographical area, and population density at different elevations in the FFB rabies-endemic area in Taiwan in 2021. According to this table, the human population density decreases rapidly in the areas 600 m above sea level in Taiwan. The collection of FFBs samples might be biased due to the decrease in human population density; hence, in this study, the data from the areas below 600 m above sea level were used to conduct the Mann-Whitney U Test.

Table 1 Information of geography and human demography in FFB rabies-enzootic areas in Taiwan

Group	Elevation (meter)	Number of townships	Human population*	Road area (km²)	Land area (km²)	Human population density (people/km²)
1	0~100	134	7,883,498	437.22	6,021	1,309
2	100~200	23	1,260,925	65.10	1,632	773
3	200~300	12	698,104	47.06	1,352	516
4	300~400	12	335,224	35.08	1,654	203
5	400~500	3	67,054	27.23	562	119
6	500~600	9	128,994	13.32	1,487	87
7	>600	26	342,857	45.58	10,669	32
Total		219	10,716,656	670.58	23,377	458

*2021 Source.

3.2. Distribution of Formosan Ferret-badger samples collected by the Animal Health Agencies and downloaded from TaiRON website at different elevations

Table 2 showed the distribution of FFBs samples collected by the Animal Health Agencies and TaiRON at different elevations in the FFB rabies-endemic area of the main island of Taiwan for the years from 2013 to 2023.

Table 2 The FFB samples collected by Animal Health Agencies and TaiRON in FFB rabies-endemic areas in Taiwan at different elevations, from 2013 through 2023

	Elevation (meter)	FFBs sampled by Animal Health Agencies	FFBs sampled by TaiRON
1	0~100	124	86
2	100~200	103	55
3	200~300	173	91
4	300~400	337	202
5	400~500	149	204
6	500~600	167	149
Total		1,053	787

3.3. Statistical calculation of Formosan Ferret-badger samples collected from Animal Health Agencies and TaiRON

As shown in Table 3, the number of the FFB samples collected by Animal Health Agencies and TaiRON at different elevations were ranked from smallest to largest value, along with the summed ranks of each group. The ranks for each group were summed and the Mann-Whitney U test statistical result was selected as the smallest of the two following calculated U values; here, $n_1=6$ and $n_2=6$, were the number of samples, and $R_1=47$ and $R_2=31$, were the sums of the ranks in the two groups, respectively. For our analyzed data, $U_1=10$ and $U_2=26$. We therefore selected U=10 as the test statistic. As our $p=0.20$ (by IBM SPSS 20.0) was greater than the two-sided significance level of $\alpha=0.05$, we did not reject the null hypothesis. Therefore, the Mann-Whitney U test showed no significant difference between the distributions of FFBs sampled by Animal Health Agencies and those sampled by TaiRON at different elevations (p > 0.05).

Table 3 Numbers and ranks of the Formosan Ferret-badger samples collected by Animal Health Agencies and TaiRON

FFBs sampled by Animal Health Agencies	FFBs sampled by TaiRON	Rank (Animal Health Agencies)	Rank (TaiRON)
	48		1
	68		2
	83		3
91		4	
92		5	
	131		6
135		7	
	140		8
155		9	
159		10	
	184		11
323		12	
n1=6	n2=6	R1=47	R2=31
		U1=10	U2=26
		U=10 (*p*=0.20*)	

*p > 0.05

Figure 1 showed the distribution of the number of FFBs samples collected by the Animal Health Agencies and TaiRON in the FFB rabies-endemic area at different elevations for the years from 2013 to 2023.

In the past twenty years, the national public participation of citizen scientists in collecting road-killed data had been used to study the distribution of wild animals to monitor the impacts of roads over scales far beyond the limitations of traditionally regular professional observers in traditional survey studies, and had thus gradually replaced traditional survey methods. Additionally, since the total length of roads was anticipated to increase in the whole world, the contribution of roadkill survey methods to wildlife studies had sustainable growth [1,21,28-29]. The results of this study showed that no significant difference was observed between samples of FFBs collected by the Animal Health Agencies of various local governments from the FFB rabies-endemic area and road-killed FFBs samples collected by TaiRON from the same area for the years from 2013 to 2023, at different elevations. It indicated that the road-killed record was a reliable reference data concerning the population distribution of wildlife. In addition, this study also proved that the sample distribution of FFBs collected by Animal Health Agencies for surveillance of FFB rabies was representative.
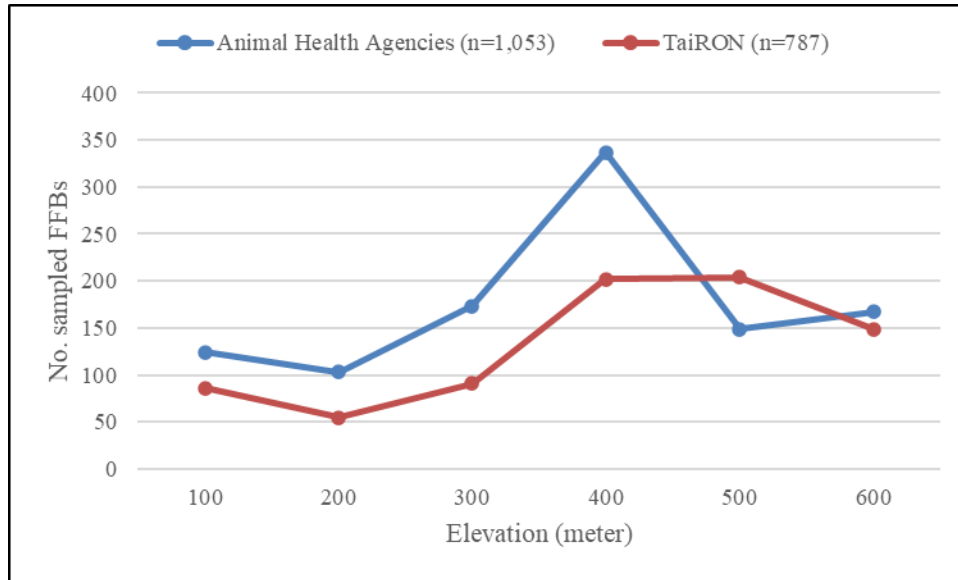
Figure 1 The distribution of Formosan Ferret-badger (FFBs) sampled by Animal Health Agencies and TaiRON at different elevations, for the years from 2013 to 2023 in the main island of Taiwan.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author Contributions

Conceptualization and Methodology C.H.L. and M.C.W. Statistical analysis M.C.W. Visualization C.H.L. Writing, review and editing, all authors. Funding C.H.L. All authors had read and agreed to the published version of the manuscript.

Statement of ethical approval

This study did not involve the use of nonhuman animals and did not need to submit to Institutional Animal Care and Use Committees (IACUCs) accordingly.

Statement of Informed Consent

An informed consent was taken from all the participants.

Data Availability Statement

The datasets analyzed during the current study are available from the R.O.C. (Taiwan) government's open website.

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