ความสัมพันธ์ระหว่างอาการทางคลินิกและผลการตรวจวินิจฉัยโรคพิษสุนัขบ้าในสุนัข ด้วยวิธี direct fluorescent antibody test

Association between clinical findings and diagnosis of rabies in dogs by direct fluorescent antibody test

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Abstract

Backgrounds: Rabies virus, a deadly virus infecting central nervous system, causing a disease that has been a major public health concern in Thailand. Rabid dogs manifest a wide range of different clinical signs. The aim of the study is to investigate the association between clinical findings and diagnosis of rabies in dogs by direct fluorescent antibody (DFA) test.

Methods: This study was conducted in all regions of Thailand from January to May 2018. Clinical characteristics and DFA results were obtained from 8 Department of Livestock Development (DLD) laboratories, including 1228 rabies submitted cases. Univariable and multivariable logistic regression were analyzed to define the association between clinical signs and DFA results.

Results: Among 650 DFA-confirmed rabid dogs (52.93%, 650/1228), most rabies cases were owned, older than one year of age and unvaccinated. From ten variables, univariable logistic regression indicate seven significant variables with $p < 0.05$. The multivariable logistic regression model showed four variables associated with rabies including aggression (OR = 5.07, 95%CI = 3.82, 6.72), running without apparent reason (OR = 2.63, 95%CI = 1.89, 3.64), animal/human or cage biting (OR = 1.51, 95%CI =1.00, 2.29) and abnormal barking (OR = 1.72, 95%CI = 1.04, 2.82).

Conclusions: Aggression, running without apparent reason, animal/human or cage biting and abnormal barking are highly associated with positive DFA results. Nevertheless, other non-significant clinical signs still need to be aware of the rabies. DLD officers should educate specimen collectors, dog owners, small animal practitioners and other relevant persons to be aware of dogs with clinal signs mentioned above. Further study should focus on experimental design with definite number of study population to address the association between rabies clinical signs in dogs and DFA results more precisely and completely.

Keywords: Clinical findings, Diagnosis of rabies, Direct fluorescent antibody test

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บทคัดย่อ

ที่มาของการศึกษา: โรคพิษสุนัขบ้าเป็นโรคติดเชื้อไวรัสในระบบประสาทส่วนกลาง ส่งผลให้เกิดอันตรายถึงชีวิต และเป็นปัญหาที่มีความสำคัญทางสาธารณสุขของประเทศไทย มีรายงานอาการที่หลากหลายในสุนัขที่เป็นโรคพิษสุนัขบ้า การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความสัมพันธ์ระหว่างอาการในสุนัขกับผลการตรวจโรคพิษสุนัขบ้าทางห้องปฏิบัติการด้วยวิธี direct fluorescent antibody test (DFA)

วิธีการ: ดำเนินการศึกษาทุกภูมิภาคของประเทศไทยระหว่างเดือนมกราคม-พฤษภาคม 2561 โดยการรวบรวมข้อมูลอาการทางคลินิก และผลการตรวจวินิจฉัยด้วยวิธี DFA จากห้องปฏิบัติการของกรมปศุสัตว์ 8 แห่ง จำนวน 1,228 ตัวอย่าง ดำเนินการวิเคราะห์สถิติเชิงพรรณนา และวิเคราะห์ความสัมพันธ์โดยใช้สถิติ univariable logistic regression และ multivariable logistic regression

ผล: พบสุนัขที่ให้ผลบวกต่อการตรวจโรคพิษสุนัขบ้า 52.93% (650/1228) โดยพบว่าส่วนใหญ่เป็นสุนัขมีเจ้าของ อายุมากกว่า 1 ปี และไม่เคยฉีดวัคซีน เมื่อทำการวิเคราะห์ความสัมพันธ์โดยใช้สถิติ univariable logistic regression พบอาการที่สัมพันธ์กับผลการตรวจโรคพิษสุนัขบ้าจำนวน 7 อาการ จากอาการทั้งหมด 10 อาการ ($p < 0.05$) จากนั้นใช้สถิติ multivariable logistic regression พบอาการที่สัมพันธ์กับผลการตรวจโรคพิษสุนัขบ้า จำนวน 4 อาการ ประกอบด้วย อาการดุร้าย (OR = 5.07, 95%CI = 3.82, 6.72), อาการวิ่งพล่าน (OR = 2.63, 95%CI = 1.89, 3.64), อาการกัดสัตว์กัดคน หรือกัดกรง (OR = 1.51, 95%CI =1.00, 2.29) และอาการเห่าร้องผิดปกติไปจากเดิม (OR = 1.72, 95%CI = 1.04, 2.82)

สรุป: อาการดุร้าย อาการวิ่งพล่าน อาการกัดสัตว์กัดคน หรือกัดกรง และอาการเห่าร้องผิดปกติไปจากเดิม มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับผลบวกต่อการตรวจโรคพิษสุนัขบ้าโดยวิธี DFA อย่างไรก็ตาม ควรมีการเฝ้าระวัง แม้ว่าสัตว์จะแสดงอาการที่ไม่สัมพันธ์กับการเป็นโรคพิษสุนัขบ้าอย่างมีนัยสำคัญทางสถิติ เจ้าหน้าที่ของกรมปศุสัตว์ควรให้ความรู้แก่ผู้ส่งตัวอย่าง เจ้าของสัตว์ ผู้ปฏิบัติงานทางคลินิก และผู้เกี่ยวข้องให้มีความตระหนักเมื่อพบสุนัขที่แสดงอาการเบื้องต้นดังกล่าว ทั้งนี้ในอนาคตหากมีการศึกษาความสัมพันธ์ของอาการทางคลินิกกับผลการตรวจวินิจฉัยโรคในสัตว์ เสนอแนะให้ศึกษาในรูปแบบการทดลอง มีการกำหนดเป้าหมายและกลุ่มประชากรให้ชัดเจน เพื่อให้ได้ข้อมูลที่ถูกต้อง และครบถ้วนยิ่งขึ้น

คำสำคัญ: อาการทางคลินิก ผลการตรวจวินิจฉัยโรคพิษสุนัขบ้า direct fluorescent antibody test

Introduction

Rabies is one of the most serious zoonotic diseases that can potentially affect humans and animals in many parts of the world. The etiologic agent belongs to the genus *Lyssavirus* of the family *Rhabdoviridae*. The transmission is predominantly occurred when broken skin or mucous membrane exposed with infected saliva (Knobel *et al*., 2005; OIE, 2021). According to the World Health Organization (WHO), rabies annually causes one percent deaths. Up to 99% of rabies human cases are caused by rabid dog exposure (WHO, 2021). Dogs are defined as the main reservoir of the disease, particularly in Asia and Africa (Taylor, 2013). Therefore, controlling and eliminating rabies in dogs, especially free-roaming dogs, is important for rabies prevention in human (WHO, 2013).

In Thailand, there were 16 human rabies cases in 2018 (Department of Disease Control, 2019). Although the number of human rabies had gradually declined, the trend of animal rabies had been moderately increasing from 250 cases in 2014 to 1,105 cases in 2018 (Department of Livestock Development, 2020). Canine rabies is

manifested in two clinical forms, furious and paralytic forms (Shuangshoti *et al*., 2013). Furious form is typically characterized by extreme behavioral changes including aggression, depraved appetite, and excitable behaviors. Paralytic rabies involves weakness, loss of coordination, inability to swallow and hypersalivation (OIE, 2009). Paralytic rabies plays an important role in misdiagnosis of rabies particularly when a history of animal bites is not included. Observation of clinical signs is essential as delay in diagnosis causes increased numbers of contact patients or animals and resulting in higher medical costs. Previous studies have been conducted on the clinical observation of rabies in animals. Association was demonstrated between positive direct fluorescent antibody test (DFA) results and biting in dogs, which is one of the six criteria for early presumptive diagnosis. The criteria help the physician for prioritizing the treatment and indicating appropriate laboratory examination (Tepsumethanon *et al*., 2005, Minougou *et al*., 2021, Tialla, 2021).

Multivariable logistic regression is a statistical method for analyzing binary and categorical data. The model can be used to assess the association between factors and outcome and to predict the probability of an outcome for particular cases especially in medical and animal research, for example influenza (Monto *et al*., 2000), foot and mouth disease (Sansamur *et al.,* 2020). However, the association between clinical findings and rabies diagnosis of dogs in Thailand using variable logistic analysis has never been reported. Therefore, the objective of this study is to investigate the association between clinical findings and diagnosis of rabies in dogs by DFA test. The findings of this study could be used to educate dog owners, specimen collectors, small animal practitioners and other relevant persons to be aware of rabies significant clinical signs.

Materials and Methods

Study area

This study was conducted in all regions of Thailand from January to May 2018. Data was retrospectively obtained from 8 Department of Livestock Development (DLD) laboratories which consist of National Institute of Animal Health (NIAH), Bangkok and other 7 Regional Veterinary Research and Development Centers located in Eastern, Western, Upper Northeastern, Lower Northeastern, Upper Northern, Lower Northern and Upper Southern region.

Data collection

One thousand five hundred and seventy-six rabies-suspected dogs were submitted for rabies diagnosis during January to May 2018. Three hundred and forty-eight cases were excluded due to no clinical sign data recorded. This study compiled clinical characteristics records of 1,228 rabies-suspected dogs in rabies submission forms and DFA results (OIE, 2021). Positive DFA result would be referred as positive case.

Specimens of dogs with suspicious behaviors were submitted from provincial livestock officers and sent to DLD laboratories. The information of demographic and clinical characteristics were retrieved. Demographic characteristics included name and address of the owner or sender, submission date, species, breed, sex, age, ownership status, vaccination status. Clinical characteristics

consisted of (1) drooping jaw, flaccid tongue, and excessive salivation (2) aggression (3) paralysis (4) muscle stiffness (5) running without apparent reason (6) difficulty swallowing (7) animal, human or cages biting (8) abnormal barking (9) regurgitation (10) depression and hiding in the dark place. The data was analyzed by Microsoft Excel version 2019 (Microsoft Corporation, 2019).

Disease mapping

Rabies submitted cases and positive cases in Thailand was illustrated in geographical distribution map constructed using QGIS version 2.18.28 (Open Source Geospatial Foundation Project, Switzerland).

Statistical analysis

Descriptive statistics was used to describe demographic features of animals. The samples were classified as positive or negative results using DFA. Statistical analysis was examined in 2 steps using Stata/IC Version 15.0, USA (Statacop, 2017). Firstly, univariable logistic regression was used to perform the relationship between clinical signs and DFA results towards rabies. Furthermore, categorical variables were expressed as odds ratio (OR) with a 95% confidence interval (CI) were estimated. Clinical signs with a significance level of *p*-value < 0.05 were selected and checked intervariable correlations for multicollinearity using the chi-square test. In the event of multicollinearity (*p*-value<0.05) was retained for multivariable logistic regression. Secondly, the multivariable logistic regression with the remaining significant clinical signs was performed to use the following equation:

$$\ln\left(\frac{P_i}{1-P_i}\right) = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k$$

where Pi is the probability of a positive DFA test in rabies-suspected case i (i = 1,.., 1228), Xk is a set of clinical signs (Xk = 1,.., k) and βk is the estimated coefficient for the clinical signs (βk = 1,..,k).

The model was compared with the full model using likelihood ratio test with the null hypothesis being βs of the dropped clinical signs equal to 0. Accepting the null hypothesis means the non-significant clinical signs from the full model can be dropped without a dramatic loss of fit. The quality of statistical model was estimated for prediction error based on the Akaike Information Criterion (AIC). The model with the lowest AIC adjusted and included in final model that was tested for model fitting by using the Hosmer–Lemeshow test (Bewick *et al*., 2005). Moreover, variables were expressed as adjusted odd ratio with a 95% CI. Clinical signs with a significant level of *p*-value < 0.05 were highly associated with positive DFA results. The characteristic of the model to discriminate between rabies and non-rabies was identified using the Receiver Operating Characteristic (ROC) method. The general rules of the area under the ROC curve (AUC) were : AUC = 0.5 (no discrimination), 0.5 < AUC < 0.6 (poor discrimination), 0.6 ≤ AUC < 0.7 (fair discrimination), 0.7 ≤ AUC < 0.8 (acceptable discrimination), 0.8 ≤ AUC < 0.9 (excellent discrimination) and AUC ≥ 0.9 (outstanding discrimination) (Hosmer and Lemeshow, 2000).

Results and discussion

A total of 1,228 submitted cases was analyzed in this study collected from 71 provinces of Thailand during January to May 2018 (Fig. 1). The highest number of submitted cases was from Surin (n=107), followed by Nakhon Ratchasima (n=102) and Songkhla province (n=73). There was no submitted case from 6 provinces including Ang Thong, Sa Kaeo, Mae Hong Son, Phuket, Phangnga and Yala province. Top five ranking provinces with the highest positive cases were Surin (n=69), by Songkhla (n=50), Nakhon Ratchasima (n=47), Yasothon (n=41), and Roi et province (n=34), respectively (Table 1S).

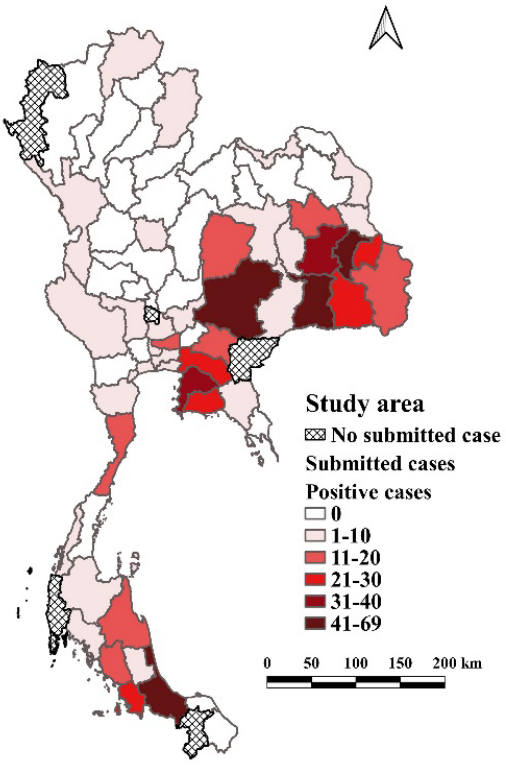

Fig. 1 Geographic distribution of submitted cases, rabies-positive cases in Thailand during January-May 2018

Regarding rabies status, demographic characteristics of rabid dogs were shown in Table 1. Our study found that 52.93% (650/1228) of samples were positive for rabies. Interestingly, most of the rabies cases were owned. It was possible that owners were more preferably to confirm whether their dogs got rabies or not. Most rabies cases were more than one year of age (47.23%, 307/650) and unvaccinated (26.62%, 173/650). It was possible that owned dogs were mostly vaccinated once but the vaccine were not boosted annually. Moreover, some owned dogs were not raised indoor that they had chances to contact other dogs especially stray ones. The World Health Organization recommended vaccinating rabies in puppies especially less than 3 months old for rabies endemic countries (WHO, 2013). At least 70% of the dog population should be vaccinated and continuously vaccinated to prevent rabies outbreaks (Hampson *et al*., 2009; Davlin and Vonville, 2012; WHO, 2018).

Some characteristics in this study were recorded as unidentified such as age (20.31%, 132/650), dog ownership (15.85%, 103/650) and rabies vaccination status (57.69%, 375/650) because rabies submission forms were not filled completely or DLD officers were carelessly approved those forms. Therefore, DLD officers should work with specimen collectors to complete the forms carefully as this important information is useful in rabies outbreak control.

Table 1 Demographic characteristics of rabid dogs diagnosed at DLD laboratories, during January to May 2018

Characteristics	Percentage (Proportion of rabid dogs)
Age	
1-6 months old	21.23% (138/650)
6 months old to 1 year old	11.23% (73/650)
> 1 year old	47.23% (307/650)
Unidentified	20.31% (132/650)
Dog ownership	
Owned	48.30% (314/650)
Ownerless	35.85% (233/650)
Unidentified	15.85% (103/650)
Rabies vaccination status	
Vaccinated within 1 year	10.30% (67/650)
Vaccinated > 1 year	5.38% (35/650)
Unvaccinated	26.61% (173/650)
Unidentified	57.69% (375/650)
Contact history	
Biting human	27.38% (178/650)
Biting animal	36.46% (237/650)
Contact saliva	16.15% (105/650)
Unidentified	20.00% (130/650)

Variables associated with rabies positive cases

Univariable logistic regression

Univariable logistic regression showed seven significant variables with $p < 0.05$ (Table 2). All intervariable correlations between significant variables were showing no potential redundancies ($p < 0.05$). All significant variables from univariable logistic regression were performed for multivariable logistic regression.

Table 2 Variables with rabies positive case based on univariable logistic regression

No.	Independent Variables	Level	Positive Case	Negative Case	*p*-value	OR (95%CI)
1	Aggression*	Yes No	390 260	103 475	<0.001	6.9 (5.26, 9.10)
2	Drooping jaw, flaccid tongue, excessive salivation*	Yes No	179 471	118 460	0.003	1.48 (1.12, 1.95)
3	Running without apparent reason*	Yes No	253 397	68 510	<0.001	4.77 (3.51, 6.53)
4	Animal, human or cage biting*	Yes No	126 524	43 535	<0.001	2.98 (2.05, 4.42)
5	Abnormal barking*	Yes No	61 589	32 546	0.01	1.76 (1.11, 2.84)
6	Regurgitation*	Yes No	47 603	63 515	0.02	0.63 (0.41, 0.96)
7	Depression and hiding in the dark place*	Yes No	101 549	147 431	<0.001	0.53 (0.40, 0.72)
8	Paralysis	Yes No	21 629	19 559	0.95	0.98 (0.49, 1.95)
9	Stiffness	Yes No	108 542	79 499	0.15	1.25 (0.90, 1.74)
10	Difficulty swallowing	Yes No	113 537	87 491	0.26	1.18 (0.86, 1.63)

* significant variable ($p < 0.05$)

Multivariable logistic regression

The final model was compared with the full model using likelihood ratio test (p = 0.22). Based on the AIC criteria, the final model included four variables that shown in Table 3. The final model achieved a good fit using Hosmer–Lemeshow test (p = 0.28). The accuracy of the final model was assessed by ROC and showed acceptable discrimination (AUC = 0.7547, Fig. 2).

Our study found that aggression was the most significant variables. The dogs with aggression had 5.07 times higher odd of having a positive DFA result than dogs without the sign of aggression (OR = 5.07, 95%CI = 3.82, 6.72). Moreover, this study showed that dog with running without apparent reason (OR = 2.63, 95%CI = 1.89, 3.64), animal, human or cage biting (OR = 1.51, 95%CI =1.00, 2.29) and abnormal barking (OR = 1.72, 95%CI = 1.04, 2.82) had higher odds of having a positive DFA result than dogs without these clinical signs. This finding was consistent with a previous study by Thiptara *et al.* (2011) which showed that aggressive dog had 11 times higher odd more likely to be rabid than non-aggressive dog. However, our study partially disagreed with Thiptara *et al.* (2011) who found pharyngeal paralysis and body paralysis were associated with rabies. This disagreement is probably explained by difference of sample size and duration of study. However, our cases were clinical furious rabies, possibly because the typical signs are simpler to be observed compared to those in paralytic rabies. Hence, in case of paralytic rabies, clinical cases are more likely to be underreported. In terms of the limitation of this study, as descriptive data were obtained through rabies submission forms filled by different specimen collectors who may not be aware of abnormal changes in the animals, thus the clinical signs in these dogs tend to be under-recorded.

The result of this study would be useful in public health information and bring awareness to the person who closely contact with rabies suspicious dogs with the significant clinical signs. Moreover, veterinary authorities should focus on regular rabies vaccination campaign and encourage the specimen collectors to fill the submission form completely.

Fig. 2 Predicted probabilities of final model based on the ROC method

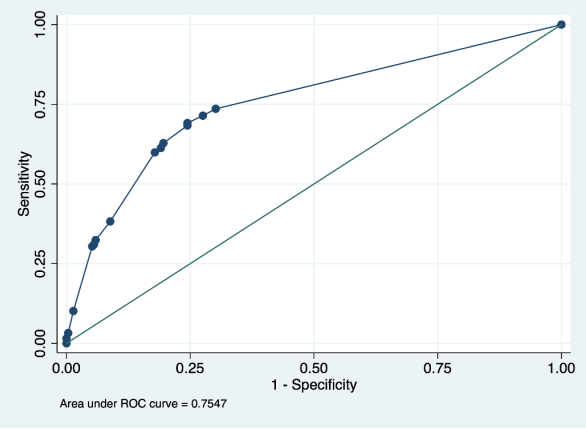

Table 3 Variables from the final logistic regression model for rabies

No	Variables	Adjusted OR (95%CI)	*p*-value
1	Aggression	5.07 (3.82, 6.72)	<0.001
2	Running without apparent reason	2.63 (1.89, 3.64)	<0.001
3	Animal, human or cage biting	1.51 (1.00, 2.29)	0.047
4	Abnormal barking	1.72 (1.04, 2.82)	0.032

Likelihood ratio test = 9.53 (p = 0.22), Hosmer–Lemeshow test = 5.05 (p = 0.28), AIC = 1422.23 (df = 5)

Conclusion and suggestions

This analytical study investigated association of clinical findings with rabies diagnosis in dogs by DFA. The results of this study suggested that aggression, running without apparent reason, animal, human or cage biting and abnormal barking were strongly associated with rabies. Nevertheless, other non-significant clinical signs still needed to be aware of the rabies. DLD officers should educate specimen collectors, dog owners, small animal practitioners and other relevant persons to strengthen their awareness of these clinical signs. Further study should focus on experimental design with definite number of study population to address the association between rabies clinical signs in dogs and results from DFA more precisely and completely.

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Annex 1

Table 1 S Positive DFA results in submitted cases by province of Thailand, January–May 2018

No.	Province	Positive cases	Total submitted cases
1	Amnat Charoen	28	32
2	Bangkok	6	15
3	Bueng Kan	0	1
4	Buriram	5	10
5	Chachoengsao	24	48
6	Chai Nat	0	1
7	Chaiyaphum	14	38
8	Chanthaburi	6	13
9	Chiang Mai	0	10
10	Chiang Rai	6	17
11	Chonburi	39	71
12	Chumphon	0	2
13	Kalasin	17	37
14	Kamphaeng Phet	0	5
15	Kanchanaburi	1	7
16	Khon Kaen	8	21
17	Krabi	7	19

No.	Provinces	Positive cases	Total submitted cases
18	Lampang	0	4
19	Lamphun	0	2
20	Loei	0	2
21	Lopburi	0	1
22	Maha Sarakham	5	17
23	Mukdahan	9	13
24	Nakhon Nayok	0	1
25	Nakhon Pathom	0	12
26	Nakhon Phanom	1	8
27	Nakhon Ratchasima	47	102
28	Nakhon Sawan	0	4
29	Nakhon Si Thammarat	19	38
30	Nan	3	6
31	Narathiwat	0	5
32	Nong Bua Lam Phu	0	1
33	Nong Khai	3	4
34	Nonthaburi	1	4
35	Pathum Thani	16	19
36	Pattani	0	1
37	Phatthalung	6	7
38	Phayao	0	3
39	Phetchabun	0	7
40	Phetchaburi	1	7
41	Phichit	3	10
42	Phitsanulok	0	6
43	Phra Nakhon Si Ayutthaya	9	11
44	Phrae	0	3
45	Prachinburi	18	30
46	Prachuap Khiri Khan	13	26
47	Ranong	3	5
48	Ratchaburi	0	4
49	Rayong	25	33
50	Roi Et	34	59
51	Sakon Nakhon	0	1
52	Samut Prakan	9	9
53	Samut Sakhon	3	7
54	Samut Songkhram	5	5
55	Saraburi	5	8
56	Satun	26	37
57	Sing Buri	0	1

No.	Provinces	Positive cases	Total submitted cases
58	Sisaket	29	35
59	Songkhla	50	73
60	Sukhothai	0	3
61	Suphan Buri	3	19
62	Surat Thani	5	11
63	Surin	69	107
64	Tak	2	6
65	Trang	16	22
66	Trat	0	2
67	Ubon Ratchathani	10	16
68	Udon Thani	0	9
69	Uthai Thani	0	1
70	Uttaradit	0	4
71	Yasothon	41	50