Emergence of zoonotic filariasis, *Dirofilaria repens*, in eastern Thailand 2019-2020: The impact of climate change

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Research Article

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Abstract

Dirofilaria repens is a zoonotic vector borne parasite in dogs and cats. It is not commonly found in every part of Thailand excepts Southern part. The aims of this study to investigate the prevalence and correlation of climate parameters in eastern Thailand during 2019–2020. A total of 15,552 blood samples were collected from private veterinary clinics and animal hospitals in eastern Thailand. Blood parasites were examined by using buffy coat thin blood smear with Wright-Giemsa stained. *D. repens* was morphologically identified and confirmed with acid phosphatase activity technique. Molecular analysis from randomly selected *D. repens* positive samples revealed they were grouped in *D. repens* group. The first emerging was found in March 2019. The prevalence of *D. repens* during March 2019 and January 2020 was 0.50 % (38/7,595) and increase rapidly to 1.76 % (140/7957) during February 2020 to October 2020. The correlation of climate parameters, for example rainfall, humidity and average temperature were tested with Pearson's correlation. The results showed that the prevalence of *D. repens* related with rainfall with $p \leq 0.05$. The Pearson's coefficients of rainfall, humidity and average temperature were 0.80, 0.58 and − 0.36, respectively. The rainfall seems to affect the natural habitats for mosquitos as well as the rubber orchard and agricultural area which served as suitable habitat for *Armigeres* spp. like in Southern part of Thailand which plenty of rubber orchard. In this study, dog is considered as a reservoir host due to only one cat was positive. In conclusion, the prevalent data of *D. repens* in eastern Thailand should be informed as a zoonotic vector borne diseases. Strategic plan to control zoonotic transmission, preventive program should be emphasized and encouraged to pet owner and veterinarian.

1. Introduction

Several filarial nematodes were found worldwide especially in tropical country including Thailand which have suitable climate for mosquitoes. In Thailand, filariasis has been reported; *Dirafilaria immitis*, *Brugia malayi*, *B. pahangi*, and *Acanthochelionema* (*Dipetalonema*) *reconditum* (Kamyingkird et al., 2017; Kanjanopas et al., 2001; Satjawongvanit et al., 2019; Wongkamchai et al., 2014). *D. repens* is one of neglected canine filariasis but also found in cats and human (Yilmaz et al., 2016). The epidemiology of *D. repens* was firstly studied Southern Europe and expanded to Northern Europe (Deksne et al., 2020; Otranto et al., 2013; Pupic-Bakrac et al., 2020). Recently, some study suggested that *D. repens* in Asia had higher diversity than in European country which purposed name Candidatus *D. hongkongensis* from Hong Kong and *D. repens* "Thailand II" found in Narathiwat province, Thailand (To et al., 2012; Yilmaz et al., 2016; Yilmaz et al., 2019). In other part of Thailand also found *D. repens* including Bangkok but the prevalence was very low (Taweethavonsawat and Chungpivat, 2020).

Human is considering as an accidental host for Dirofilariasis. Human infected with *D. repens* commonly presented in two clinical forms, subcutaneous and ocular (Pupic-Bakrac et al., 2020). Nodules in subcutaneous tissues usually about 1 cm. in size and sometime may present clinical signs like cutaneous larval migration including irritation and itching (Capelli et al., 2018). Ocular form mainly found in subconjunctival region. In chronic infection which cannot diagnose timely manner, parasites can migrate in the peri-, intra- or retro-ocular space causing complication with damaged vision, floaters,

glaucoma, retinal detachment, vitreous opacity, loss of visual acuity and blindness (Ilyasov et al., 2013). In Thailand, ocular dirofilariasis by *D. repens* infection was reported causing cystic mass in the eyelid of woman living in Phangnga Province (Jariya and Sucharit, 1983). Recently, the first woman case of subconjuntival dirofilariasis has been reported in Bangkok, Thailand (Sukudom et al., 2018)

The transmission of *D. repens* is similar to *D. immitis*. Mosquitoes in genera *Anopheles*, *Aedes*, and *Culex* were identified as a potential vectors for *Dirofilaria* spp. (Labarthe et al., 1998; Ledesma and Harrington, 2011). *D. immitis* infection in dog caused cardiopulmonary diseases but *D. repens* caused milder diseases in subcutaneous tissues (Demirci et al., 2020). Dirofilariasis causing by *D. repens* was considered as non-pathogenic disease. But recently, some studied suggested that subcutaneous dirofilariasis is related with dermatological symptoms together with concomitant pruritus, neoplastic processes, inflammation and blindness in dogs and humans. The blood parameters in dogs were changed during infection along with deceasing of white blood cell, red blood cell and platelet and increasing of alkaline phosphatase and creatinine activity (Wysmolek et al., 2020).

In Thailand, most of studied focused on Southern part of Thailand which is an endemic area of filariasis due to the climate conditions were suitable for vector. However, the epidemiological data of canine subcutaneous dirofilariasis in eastern Thailand which has the climate-like the Southern was limited. Then, this study aims to investigate the prevalence and correlation of climate parameters in eastern Thailand during 2019–2020.

2. Materials And Methods

2.1. Study sites and blood collection

The retrospective study was done between March 2019 and October 2020. The records of *D. repens* were given by Vet Central lab, Rayong branch which collected canine and feline blood samples from veterinary private clinic and animal hospital in 7 provinces in eastern Thailand including Chonburi, Rayong, Chanthaburi, Trat, Chachoengsao, Prachin Buri and Sa Kaeo (Fig. 1.) except Pattaya city, a municipal area. Climate parameters including rainfall, relative humidity, and average temperature were obtained from information service of The Thai Meteorological Department, Ministry of Digital Economy and Society.

2.2. Blood collection and parasite identification

The total 15,552 EDTA-blood samples were collected from owned dogs, and submitted to Vet Central lab, Rayong branch. Buffy coat thin blood smears were performed and stained with Wright-Giemsa stain. Stained smears were examined the presence of filarial nematode using light microscope. Unsheathed microfilaria with 2 nuclei at cephalic space was confirmed together with acid phosphatase activity to identified *D. repens*.

2.3. Genetic characterization of *D. repens*

Five positive samples were randomly confirmed microfilaria species with PCR targeting cytochrome C oxidase subunit I (COI) using DR COI -F1 (5’AGTGTTGATGGTCAAC- CTGAATTA’3) and DR COI -R (5’GCCAAAACAGGAACAGATAAAACT’3) (Rishniw et al., 2006). PCR reaction comprised of 12.5 µl of 2x ViRed Taq Master Mix (Vivantis, Malaysia), 10 µM of each primer and 1 µl of DNA. Cycle condition was set for 35 cycles of 94°c 30s, 58°c 30s, 72°c 30 sec and 94°c 2 min for pre-denaturation and 72°c 7 min for final extension. PCR products were run with gel electrophoresis, and all samples were submitted to sequencing. The phylogenetic tree was conducted using MEGA X software.

2.4. Statistical analysis

The prevalence of *D. repens* was demonstrated using descriptive statistics and 95% confident interval (95% CI). The relationship between *D. repens* infection rate and climate parameters were analyzed using Pearson’s correlation.

3. Results

3.1. Prevalence of *D. repens* in eastern Thailand during March 2019 to January 2020

During March 2019 and January 2020, a total of 7,595 blood samples were examined and 0.50 % (38/7,595) (0.35–0.69) samples were *D. repens* positive. The prevalence from each month is shown in Fig. 4. Microfilaria of *D. repens* has been identify using buffy coat smear with Wright-Giemsa stained and acid phosphatase activity test (Fig. 2A and 2B, respectively). The private veterinary clinics and animal hospitals in Rayong and Chanthaburi provinces showed in Fig. 3. All positive samples were dogs except for one sample of a cat. However, co-infection with *Brugia pahangi*, *Hepatoon canis*, *Babesia canis*, *Ehrlichia canis* and *D. immitis* were observed (Table 1.)

Table 1
Coinfection of *D. repens* with other vector borne pathogens

Coinfection	**Prevalence (n)**	**95% CI**
Single infection of *D. repens*	0.34 (26)	0.22–0.50
D. repens with *D. immitis*	0.03 (2)	0.00–0.10
D. repens with *B. pahangi*	0.07 (5)	0.02–0.15
D. repens with *Babesia canis*.	0.01 (1)	0.00–0.07
D. repens with *B. pahangi* and *H. canis*	0.04 (3)	0.01–0.12
D. repens with *B. pahangi*, and *E canis*	0.01 (1)	0.00–0.07

3.2 Prevalence of *D. repens* in eastern Thailand during February to October 2020 and association with climate change.

From February to October 2020, a total of 7,957 blood samples were examined and 1.76 % (140/7957) (95% CI: 1.48–2.07) samples were positive microfilaria of *D. repens*. The prevalence of *D. repens* was rapidly increasing from 0.50–1.76%. The trend of prevalence from March 2019 to October 2020 was demonstrated in Fig. 4. The highest prevalence was 2.54% in June, 2020.

The climate parameters including rainfall, humidity and average temperature were tested with Pearson's correlation to identify the correlated with prevalence of *D. repens* from February to October 2020. The results revealed that rainfall was correlated with prevalence of *D. repens* ($p < 0.05$) (Table 2). The prevalence of *D. repens* from February to October 2020 and bar chart revealed rainfall, average temperature and humidity shown in Fig. 5A-C.

Table 2
The Pearson's correlation results from climate parameters and prevalence of *D. repens*

Parameters	Pearson's correlation coefficient	95% CI	P value
Rainfall	0.80	0.28–0.96	0.01*
Humidity	0.58	-0.14–0.90	0.10
Average temperature	-0.36	-0.83–0.40	0.34
*$p < 0.05$			

3.3. Phylogenetic tree analysis of *D. repens* emerging in eastern Thailand

A total of 5 positive samples were submitted to sequencing, and the phylogenetic tree was created using the Maximum Likelihood method and Tamura-Nei model. The phylogenetic tree revealed that all samples collected from eastern Thailand were grouped with *D. repens* isolated from human in India (KT588609.1) and distinct from *D. hongkongensis* clade (MN564742.1, KY750548.1, JX187591.1)

4. Discussions

Since February 2020, prevalence of *D. repens* in eastern Thailand increased rapidly. Due to the climate condition of Southern, the filariasis endemic area and Eastern part seem to be similar (Fig. 6). The Pearson's correlation revealed that rainfall, humidity, and average temperature in Eastern were correlate with in Southern with coefficient 0.58, 0.89 and 0.94, respectively.

In this period, rainfall in Eastern was higher than Southern especially in June and September which rainfall reached the peak. The Thai Meteorological Department reported tropical cyclone, Nuri and tropical depression Noul on June and September, respectively. Tropical depression Noul moved from South China Sea and then to Vietnam, Laos and Northeastern of Thailand while Tropical cyclone Nuri occurred in North South China Sea and moved to Guangdong Province, China. Two tropical storms occurred in the

South China Sea and may result in increasing rainfall in Eastern but had less effect in Southern part which had effect when tropical storm from Thai gulf or Andaman Sea occurred. The rain made humid habitat more abundant in the area. The female mosquitoes did not need to fly in long distance for eggs laying area. The male mosquitoes also easily found nectar which abundant in orchard for feeding (Elbers et al., 2015). So the hypothesis that the influents of two tropical storms tend to affect Eastern part's rainfall and increase the prevalence of *D. repens* in this period are considered. However, there are evidence suggested that climate conditions can increase the vector activity and also accelerate larval development in vectors resulting in increasing diseases transmission (Simon et al., 2012).

Since *D. repens* has a high genetic diversity in Asia (Yilmaz et al., 2016; Yilmaz et al., 2019), mitochondrial genome should be analyzed to differentiated sub-species of *D. repens* including Candidatus *D. hongkongensis* from Hong Kong and *D. repens* "Thailand II" in Thailand. Dog is a definitive host and also considered as a reservoir host. In this study, only one cat has *D. repens* infection. In a total of 38 *D. repens* cases in 2019, 3 dogs had been recorded clinical sign such as lethargy, loss of appetite and epistaxis. The hypothesis that infected dogs might not show any clinical signs had been made. In order to control zoonotic transmission, preventive program should be emphasized to owner and veterinarian. Apart for aforementioned potential vector, *Armigeres* spp. was recognized as potential vector in some regions (Dissanaike et al., 1997; Lee et al., 2007). The tropical fruit and rubber orchards were abundant in Eastern part agricultural area which served as suitable habitat for *Armigeres* spp. same as in Southern part which rich of rubber orchard as well. The clinical signs in *D. repens* infected human and animals are mild and might be neglected especially in orchard laborers or orchard guarding dogs due to socioeconomic problems.

The eastern Thailand shares some climate conditions with Southern, the filarial nematode endemic area. In order to prevent the transmission of canine zoonotic vector borne diseases to human, the eastern Thailand will be an interested study site to investigate the prevalence of other canine vector borne diseases and its vectors.

5. Conclusion

D. repens was emergence in eastern Thailand in March 2019. The impact of two tropical cyclones were considered as a cause of high occurrence in July and September 2020 by increasing rainfall. The phylogenetic tree analysis from randomly selected *D. repens* positive samples revealed they were grouped in *D. repens* group. However, since *D. hongkongensis* and *D. repens* "Thailand II" were reported, further mitochondrial genome analysis should be conducted.

Declarations

Author statement

Piyanan Taweethavonsawat: Conceptualization, Project administration, Resources, Supervision, Writing Original draft preparation. Wanarit Jitsamai: Methodology, Validation, Visualization, Writing Original draft preparation. Patchana Kamkong: Validation, Methodology. Sariya Asawakarn: Project administration, Writing-Review & Editing.

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Declaration of Competing Interest

The authors declare they have no conflicts of interest.

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Figures

Figure 1

Location of 7 provinces in eastern Thailand. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

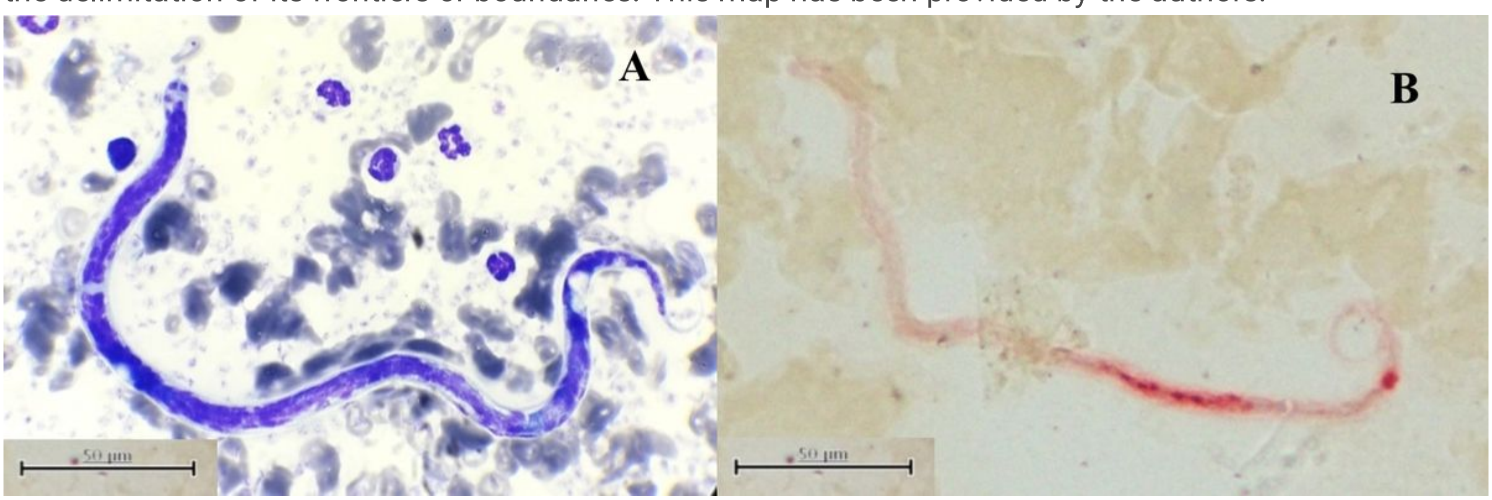

Figure 2

Microfilaria of D. repens from buffy coat smear Giemsa stained (A) and acid phosphatase activity test (B)

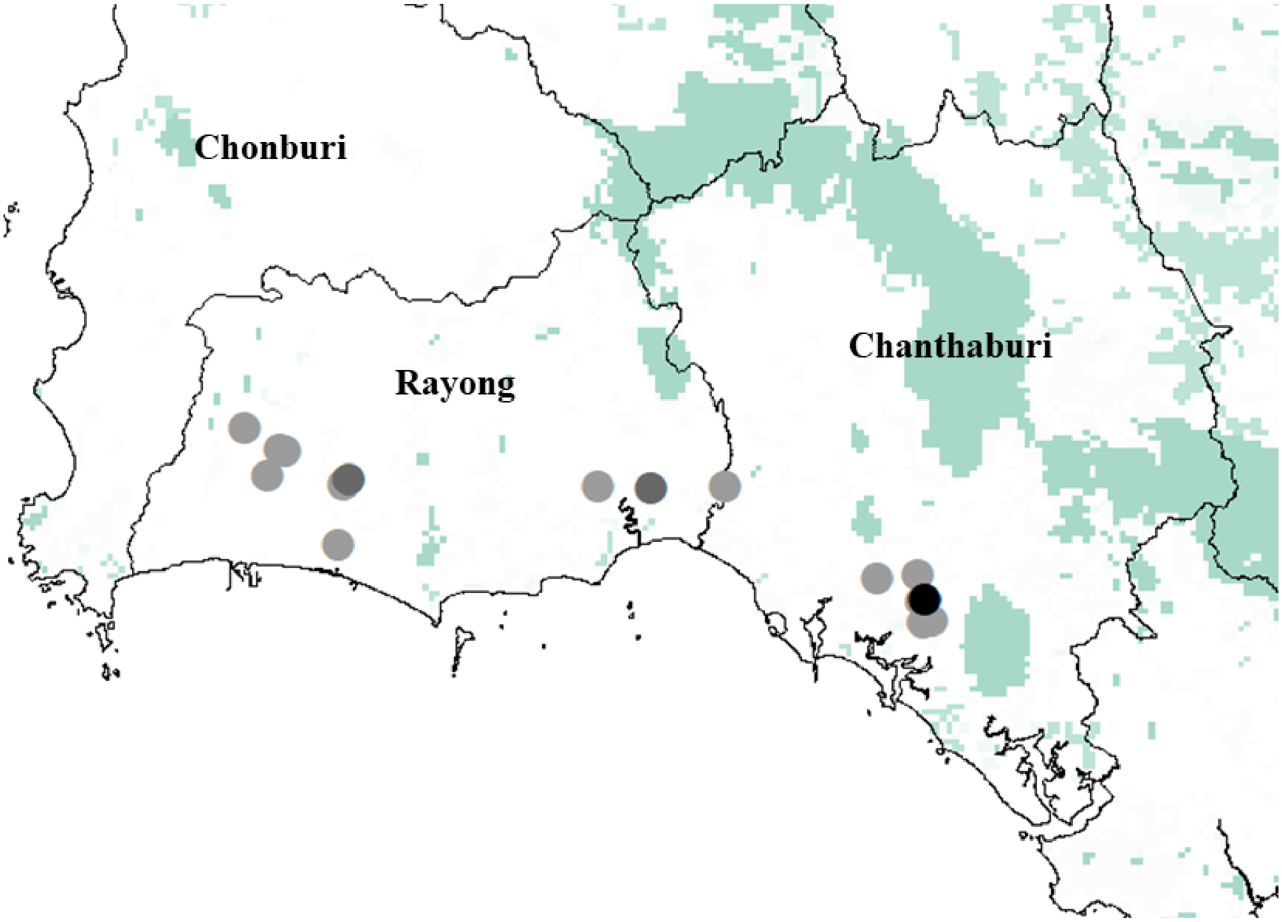

Figure 3

Location of private veterinary clinics and animal hospitals demonstrated by dot (grey scale < 4cases, dark grey 4 – 8 cases and black >8 cases) during March 2019 to January 2020. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

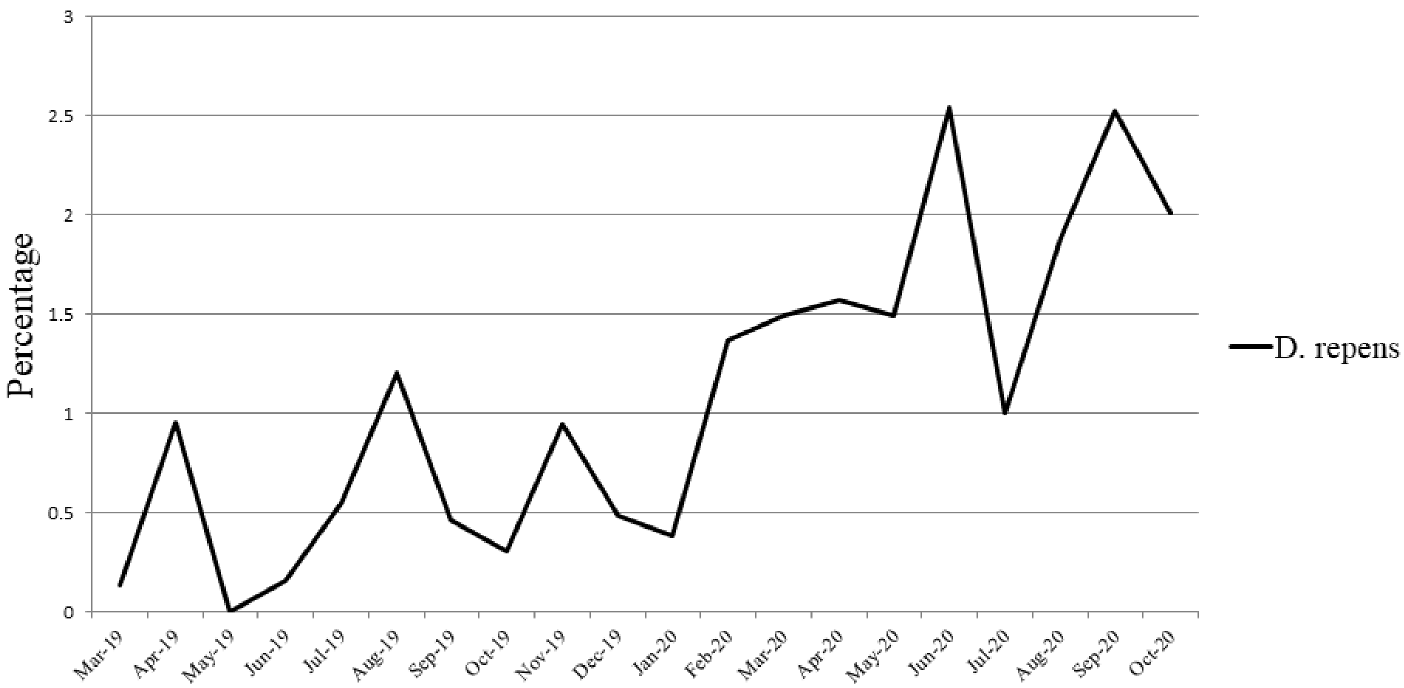

Figure 4

The prevalence of D. repens during March 2019 to October 2020

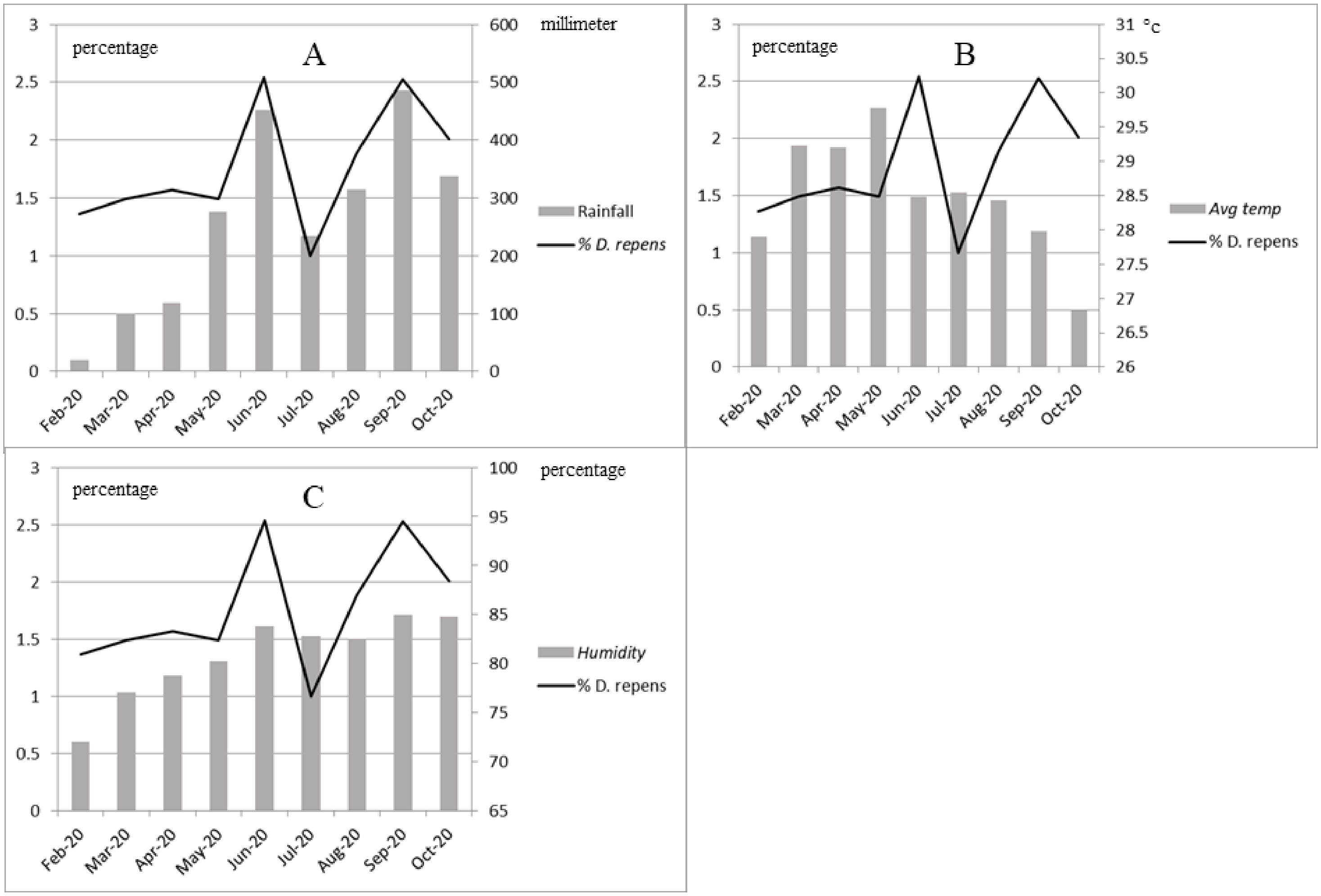

Figure 5

Line chart demonstrated prevalence of D. repens from February to October 2020 and bar chart revealed rainfall (A), average temperature (B) and humidity (C)

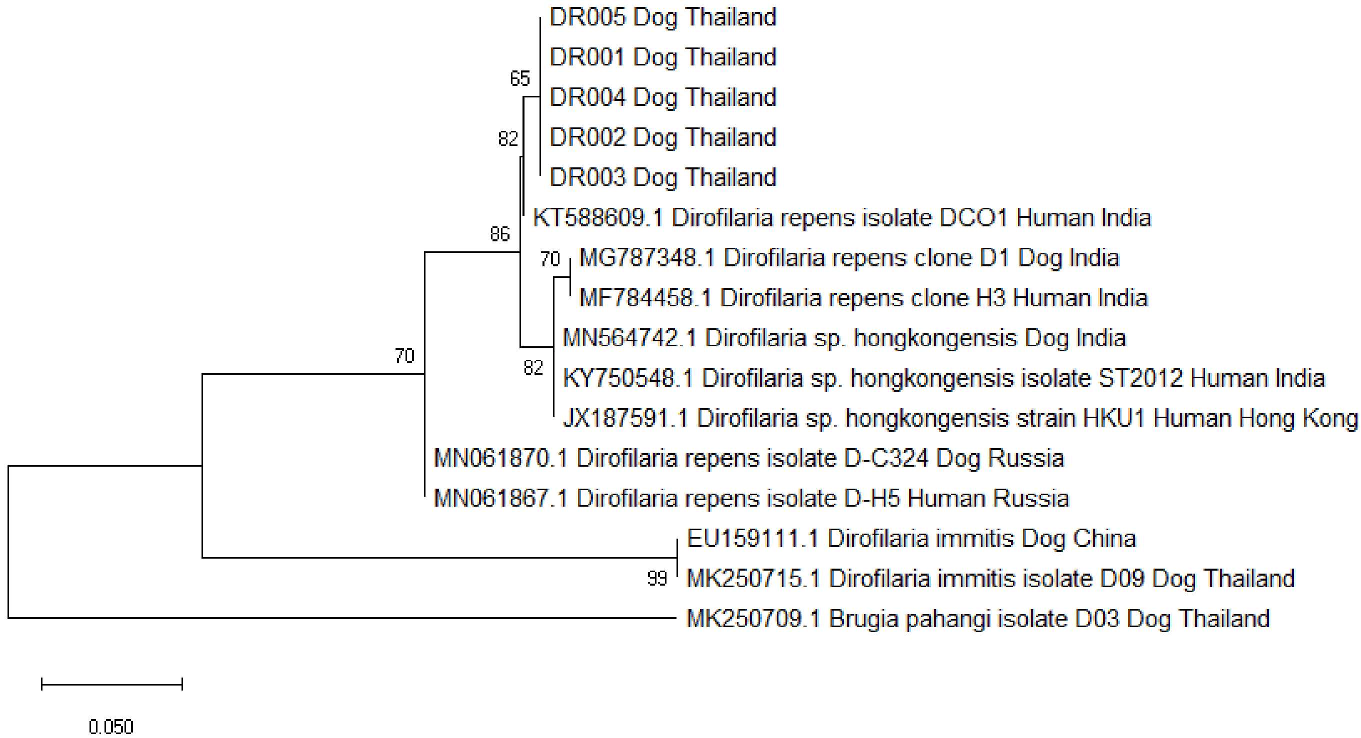

Figure 6

Phylogenetic tree analysis of D. repens in eastern Thailand

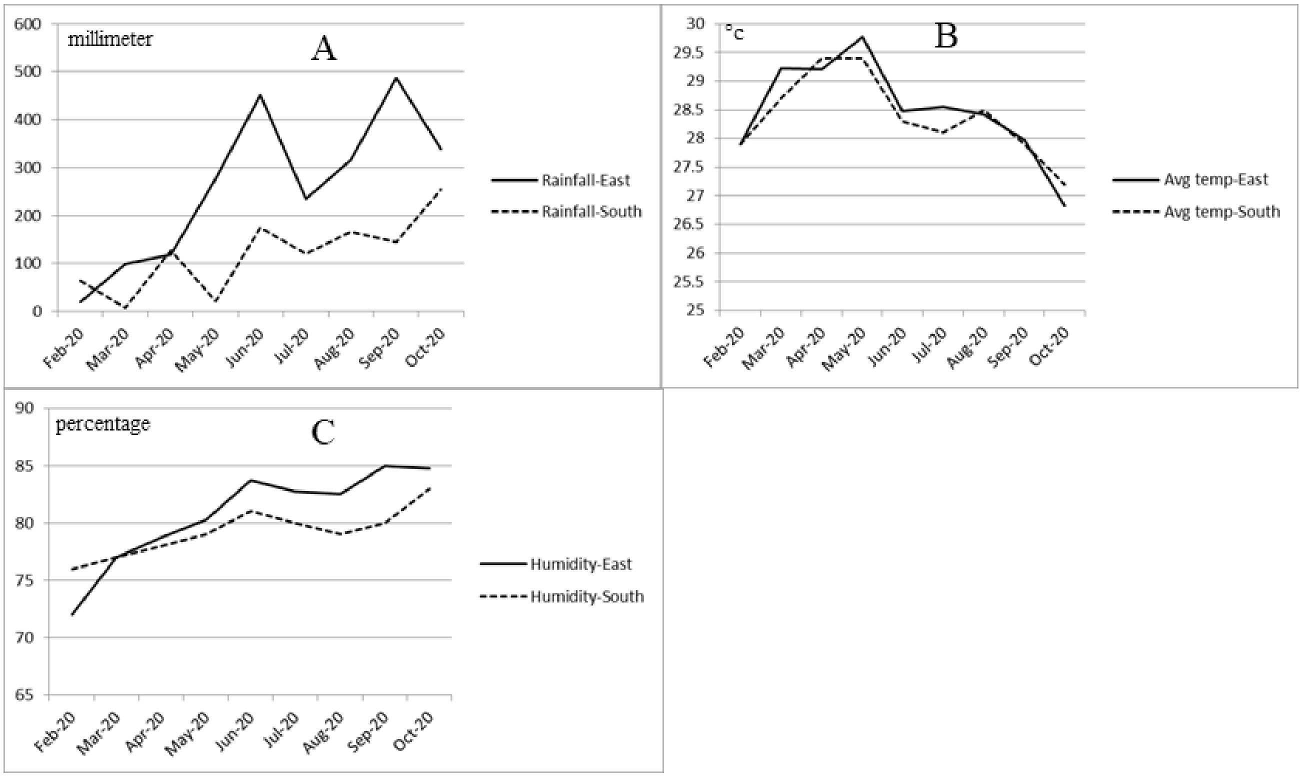

Figure 7

Comparison of climate condition including rainfall (A), average temperature (B) and humidity (C) form Southern and Eastern part during February and October 2020.