

surveillance and continued monitoring should be needed to provide early warning of transcontinental avian influenza threats by migratory waterfowls.

#### References

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### Tick Distribution at Horse Riding Course, Horse Ranch, and Horse Stables Site in the Republic of Korea, 2016-2017

Keun Ho Kim<sup>1</sup>, Hyun-Ji Seo<sup>1</sup>, Heung-Chul Kim<sup>2</sup>, Mi-Sun Yoo<sup>1</sup>, Kyu-Won Kwak<sup>1</sup>, Bang Hun Hyun<sup>1</sup>, and Yun Sang Cho<sup>1\*</sup>

Parasitic and Insect Disease Laboratory, Bacterial Disease Division, Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon-si, Gyeongsangbuk-do, 39660 Republic of Korea, <sup>2</sup>5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205-5247, Yongsan, Seoul, Republic of Korea

**Introduction:** Tick is an important vector that transmits diseases to both humans and animals and nowadays increases pathogenic potential due to climate change, livestock population growth, increase of livestock import, especially equid species, such as donkeys, horses, and so on. Most of rickettsiae and protozoa, including *Ehrlichia* spp., *Anaplasma* spp., *Bartonella* spp., *Rickettsia* spp., and *Theileria/Babesia* spp., are transmitted by tick. Climate change, resulting in a habitat more favorable for vectors, may have raised the risk of potential introduction of exotic tick-borne diseases into the Republic of Korea. Horse population has been increasing in Republic of Korea (ROK), and then tick-borne diseases in horse could be increased in ROK. However, there is no information on the tick on horse raising environment. In this study, we selected the tick collected site in the area around horse stable. To examine the pathogenic potential of tick-borne diseases in horse, a monitoring project for horse tick has been started. Therefore, we monitored horse tick and identified the species level of ticks in the Republic of Korea from April 2016 to June 2017.

**Materials and Methods:** BioQuip's tick drag was used for monitoring ticks at 34 sites consisting of 8 *Korea Racing Authority*, 8 personal ranch, 15 horse riding course, and other 2 sites. Tick collection was operated from 2016 to 2017. Tick were removed from the traps, and transported to laboratory, where the collected ticks were stored at -70°C until identified at species level by microscopic morphological identification on a cold table using standard keys (Yamaguti *et al.*, 1971). Ticks were placed, up to 30 specimens by species.

**Results:** A total of 9,791 ticks were collected from horse riding course, ranch, and horse stables from 2016 to 2017. The collected ticks ( $n = 9,791$ ) were identified 2 species of ticks. Interestingly, 99.96% of collected ticks was *Haemaphysalis longicornis* ( $n = 9,787$ ), which was the predominant tick at horse raising environment in Korea.

**Conclusions:** Our study has showed the tick species in *Korea Racing Authority*, personal ranch, and horse riding course in ROK. *Haemaphysalis longicornis* was the dominant species in our selected site. Four of *I. nipponensis* were collected. Despite small number of collection, *I. nipponensis* might be important role in the transmission of tick-borne pathogens to humans, because human tick bites have been reported more frequently for this species among patients at medical clinics in Korea. This study is ongoing and the collected tick samples are to be tested for the presence of tick-borne disease such as anaplasmosis, ehrlichiosis, babesiosis, theileriosis, and rickettsiosis.

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### Quantitative Duplex Real-Time PCR for *Babesia caballi* and *Theileria equi*

Hyun-Ji Seo, Keun Ho Kim, Mi-Sun Yoo, Kyu-Won Kwak, Bang Hun Hyun, and Yun Sang Cho\*

Parasitic and Insect Disease Laboratory, Bacterial and Parasitic Disease Division, Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon-si, Gyeongsangbuk-do, 39660 Republic of Korea

**Introduction:** *Babesia caballi* and *Theileria equi* are two pathogens of equine piroplasmiasis which caused large economic losses in global equine industry. It is one of OIE list diseases and is a tick-borne disease. The detection methods are monoplex PCR as molecular diagnosis and complement fixation test, ELISA, and IFA as serological diagnosis. But the previous detection methods have low sensitivity and are time-consuming. Therefore, we developed a highly sensitive specific and rapid diagnostic method based *B. caballi*- and *T. equi*-specific gene segments to detect simultaneously and quantitatively.

**Materials and Methods:** Oligonucleotide primers and probes were used to amplify the genes encoding the 16S rRNA. The duplex quantitative PCR (qPCR) was used to detect each control plasmid DNA using specific primer, and probe. The duplex qPCR were tested using IQ super mix (Bio-rad) kit according to the manufacturer's instructions. The reaction contained 5  $\mu$ l DNA templates, 5  $\mu$ l primer-probe mix (Final concentration of 3.2  $\mu$ M for each primer and 200 mM for each probe). All reactions were performed on an Bio-Rad CFX96 with the following cycling: 95 °C for 5 min, and then 45 cycles of 95 °C for 10 sec, 60 °C for 30 sec. The sensitivity of duplex qPCR was evaluated 10-fold dilution of control DNA. The duplex qPCR for