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Title: Eveluation of *Leptospira* infection in blood samples of horses in a number of horse farms in Mazandaran province by PCR method

Summary:
Leptospirosis is a zoonotic disease that caused by *Leptospira interrogans*, is a gram-negative and aerobic bacterium. The disease can cause abortion, infertility, decrease in milk production, swelling of the testicles, decrease in sperm production, and subsequently imposed high treatment costs. This study was conducted to evaluate the *Leptospira* contamination in some farms of Mazandaran province using PCR methods. So, one hundred blood and thirty urine samples were collected from seemingly healthy and unvaccinated horses. The samples were stored at -70°C until molecular assay. Genomic DNA of all samples were extracted by a commercial DNA extraction kit (Favorgen, Taiwan). Then, the genomic DNA were stored at -20°C. Molecular detection of *Leptospira* was performed by amplification of *rrs2*, *LipL32* and *SecY* genes in PCR. For initial identification of bacteria *16s rRNA* gene was amplified by using a set of specific primers in Nested-PCR. Finally, sequencing of all PCR products was performed by Pishgam, Iran. Results showed that out of 130 samples, 15 (11.5%) were infected with *Leptospira*, seven (7%) and eight (26.6%) of blood and urine samples respectively. The information obtained from the samples was evaluated in SPSS software version 24 and chi square test was used to analyze the data.
Also, the results of phylogeny and gene blast analysis showed that all positive samples were 99-100% similar to the reference in the gene bank.. Results of sequencing data confirmed that all positive samples were *Leptospira interrogans* serovars. It's should be noted that PCR can be used for leptospirosis identification, because of its specificity and sensitivity. As well as, due to the importance of leptospirosis in horse and as a zoonotic disease, fast diagnosis and early treatment are necessary.

Keywords: Blood, Horse, Leptospirosis, PCR method, Urine