

EXPERIENCE OF USING SEROLOGICAL AND MOLECULAR TESTS TO DETECT EQUINE INFECTIOUS ANEMIA VIRUS IN HORSE

N.N. GERASIMOVA, O.L. KOLBASOVA, S.ZH. TSYBANOV, A.V. LUNITSIN,
D.V. KOLBASOV

All-Russian Institute of Veterinary Virology and Microbiology, Russian Academy of Agricultural Sciences, Pokrov, Petushinskii Region, Vladimir Province, 601120 Russia, e-mail gerasimova-nadya-88@yandex.ru, vniivim@niiv.petush.elcom.ru
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Abstract

Equine infectious anemia in horses is caused by equine infectious anemia virus (EIAV, *Lentivirus*, *Retroviridae*), affecting hematopoietic organs. The symptoms of the disease are relapsing or continued fever, anemia and a disturbance of cardiovascular functions. Duly virus detection is the only effective way to control infection. Serological methods used to indicate EIAV have some limitations. For instance, they did not allow identifying infected animals prior to seroconversion. Also an immunodeficiency can really occur when the content of virus specific antibodies is too low to be indicated. Here we report the results of comparative study of different serological and molecular techniques for diagnosis of equine infectious anemia in experimentally inoculated susceptible 9 month old foal. In the experiment, we used the kits for agar gel immunodiffusion assay (Russia) and ELISA (France) as serological tests, and our own developed test-systems for viral RNA detection by nested reverse transcription polymerase chain reaction (RT-PCR) with electrophoretic control and by real-time RT-PCR. Developed PCR-tests is based on amplification of EIAV *gag*-gene fragment using specific oligonucleotide primers. In blood, the viral RNA was detected by both test-systems from day 2 after animal inoculation until the end of observation at day 35. Specific antibodies were detected in diffuse precipitation reaction from day 30 and in ELISA from day 21 after animal inoculation. Thus, PCR-analysis could be used as an express-test for detecting EIAV genome prior to seroconversion, and then other diagnostic methods should be further applied to verify the RCR data. The developed Russian test-systems and investigation techniques confirm the benefits of the PCR diagnosis in the early stages of the EIA.

Keywords: equine infectious anemia, experimental infection, PCR, serodiagnostics.

Equine infectious anemia in horses affects hematopoietic organs with relapsing or continued fever, anemia and a disturbance of cardiovascular functions (1-4). Horses of all ages, ponies, asses, mules can be naturally infected (5, 6). Diseased growing horses often die (3, 7). Because of the consequences of outbreaks the national programs are implemented to eradicate equine infectious anaemia (8, 9). The etiological agent of the disease is RNA containing equine infectious anemia virus (EIAV) from *Lentivirus* genus of *Retroviridae* family (3, 4, 10).

At EIAV infection the nonsterile immunity is developed, meaning the immunity is exists only in the presence of the infectious agent in the organism. In horses attacked by EIAV the antibodies to its different antigenic determinants appear in blood serum due to immune response to viral infection (3).

The EIAV diagnostics is currently based on a detection of specific antibodies. Special kits are developed and commercially distributed for a diffuse precipitation test (DPT) and ELISA test. Of serological methods, the diffuse precipitation test (DPT, or the Koggin's test, is considered the golden standard (7, 11).

Nevertheless, some disadvantages of the these techniques should also be noted. Of them the first is inability to detect sick animals prior to seroconversion. Moreover, the immune deficient status is described resulting in low virus specific antibodies production, so that their small quantity prevents detec-

tion. In these cases the obtained negative immunological results must be considered false (12).

Additionally to indirect serological indication the EIAV genome can be detected directly. PCR diagnostics of EIA disease is most common prior to seroconversion occurs. Abroad different protocols based on RT-PCR are developed and successfully applied (2, 4, 13). A number of researchers and official experts consider the EIAV molecular detection essential at early diagnostics of equine infectious anemia (12, 14), and the molecular methods are also recommended by the World Organization for Animal Health (Paris) (14).

Importantly, the duly virus detection is the only effective way to control infection, as to date there are no preparations for its specific treatment and prevention (1, 3).

Here we report the results of comparative study of different serological and molecular techniques for diagnosis of experimentally developed equine infectious anemia.

Technique. A susceptible 9 month old foal was intravenously injected with 10 ml of 10 % suspension of spleen tissue from a seropositive horse slaughtered at EIA outbreak in Nizhegorodsakay Province in 2011. Then the animal was kept in the vivarium under daily clinical control including thermometry.

Blood was sampled daily from day 1 to day 4 after infection and then each 2 days until the end of experiment on day 35. Blood serum was sampled on days 2, 7, 14, 21, 28, 30 and 35.

EIAV RNA was detected by nested RT-PCR with electrophoretic separation of amplified fragments and by real time RT-PCR assay (15, 16). To perform nested RT-PCR a Palm Cycler (Corbett Research, Australia) was used, and the real time RT-PCR amplification was carried out on a RotorGene-6000 (Corbett Research, Australia) according to recommended protocols (15, 16).

For serological diagnostics a commercial Kit for diagnostics of equine infectious anemia by diffuse precipitation (Russia) and a Test system for detection of antibodies against EIAV in blood serum by indirect ELISA (IDvet, France) were used according to the manufacturers' recommendations.

Results. Prior to inoculation the animal was EIAV-seronegative as confirmed by a diffuse precipitation test.

During our experiment the characteristic symptoms of equine infectious anemia were observed such as fever, hyperthermia, strong thirst, depression and weigh loss. From day 10 a hyperthermia was observed, with the body temperature reached 40.9 °C on day 15 and then decreased to a physiological norm.

Developed PCR-tests is based on amplification of EIAV *gag*-gene fragment using specific oligonucleotide primers (10, 11).

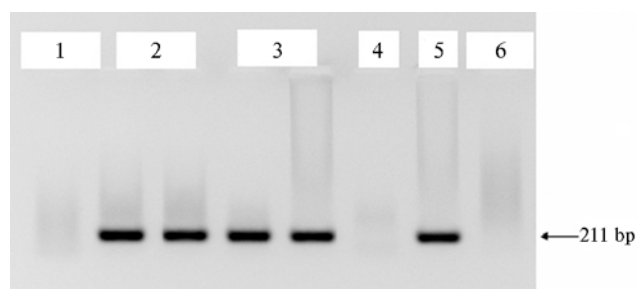


Fig. 1. Amplification of specific genome fragment of equine infectious anemia virus from blood of 9 month old experimentally infected foal in nested RT-PCR: 1 — day 1 after inoculation, 2 — day 2 after inoculation, 3 — day 35 after inoculation, 4 — negative control of the reaction, 5 — positive control

of the reaction, 6 — negative control of amplification.

In blood the viral RNA was found from day 2 after animal inoculation by both nested RT-PCR with electrophoretic detection (Fig. 1) and by real time RT-PCR (Fig. 2). Further, EIAV RNA was detected by both test systems until

the end of observation at day 35.

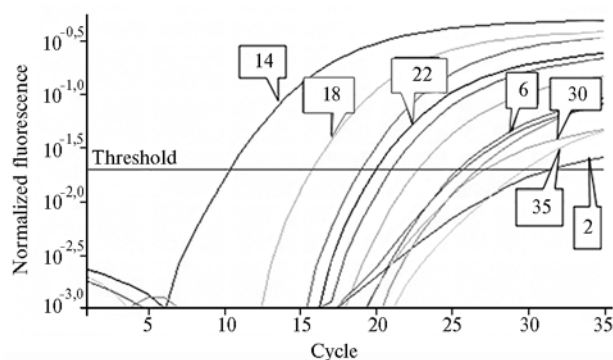


Fig. 2. Amplification of specific genome fragment of equine infectious anemia virus from blood of 9 month old experimentally infected foal by real time RT-PCR (the curves for day 2, 6, 14, 18, 22, 30, and 35 of the experiment shown).

Specific antibodies against EIAV were identified by diffuse precipitation test from day 30 after inoculation and by indirect ELISA test from day 21 after inoculation until the end of our observation on day 35. These data are in line with the reported findings that the concentrations of specific antibodies in blood serum enough to be serologically indicated cannot be reached prior to day 14 to 35 period of infection (3).

Thus, PCR-analysis could be used as an express test for detecting EIAV genome prior to seroconversion, and other diagnostic methods should be further applied to verify the RCR data. The developed Russian test-systems and investigation techniques confirm the benefits of the PCR diagnosis in the early stages of the EIA. Particularly, EIAV can be effectively detected by PCR analysis from day 2 of infection.

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