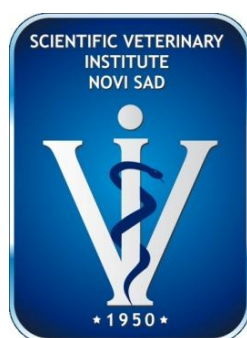


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INSTITUTE OF VETERINARY MEDICINE OF SERBIA

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THE HEALTH STATUS OF BREEDING STALLIONS FOR NATURAL BREEDING AND ARTIFICIAL INSEMINATION: REGULATORY COMPLIANCE IN EUROPEAN UNION AND WEST BALKAN

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Abstract

Diagnostic and health condition control procedures of breeding stallions in Serbia are in accordance with Regulation on the establishment of animal health care measures for 2014. Diagnostic tests for breeding stallions are performed on each breeding animal one time in a year, and those include only tests for Equine infectious anaemia. Nevertheless, the majority of horse owners and keepers tend to follow their previous routines and preventive measures. When it comes to stallions, tests which are conducted on horse-farm prior to breeding season include serum neutralization test for Equine Herpes Virus -1 (EHV-1) and Equine viral arteritis.

What is the looming problem nowadays is that there is no existing system control whether would that involve certain institutions or associations (sport or breeding animals). Examination of equine sperm for arteritis is not a common procedure - however, in justified cases of doubt solely it is. On the other hand, as an example of regulations in South America, we shall mention compulsory measures in Argentina about the obligation of examination of Equine Viral Arteritis on stallions. These are following: Every breeding association must submit a certification of negativity (serum neutralization test) for Equine Viral Arteritis of all active stallions before the annual breeding registration or AI. Frozen semen samples imported from foreign countries must be checked by state laboratories for detection of viral presence by PCR before commercialization and insemination. On balance, it can be emphasized, that in relation to further development of veterinary profession and horse industry in Serbia as well, it is not recommended to "a priori" implement every EU model currently, and completely, due to the difference in horse breeding between our system and similar in EU countries. Nevertheless, in relation to mentioned legal framework and de facto situation in horse breeding in Serbia, we would recommend thoroughly planned control and primarily to make evidence of stallions used for breeding of mares in sport and rural areas.

Keywords: equine, semen, legislative, Serbia, EU

Introduction

The state of horse breeding and reproduction in Serbia

Horse breeding as an animal husbandry branch has a long and significant tradition in Serbia through the use of horses in work, sport and nowadays increasingly more as a hobby. According to the records from the Bureau for statistics of Serbia from 2014 here are 16.000 horses, which include 8.000 of mares and fillies. However, when it comes to the number of stallions there are no official records. Although, those are the official records we consider them as not viable enough and they are

taken with caution owing to the fact that those do not reflect the actual state especially regarding the breeding animals.

Moreover, the additional records available to us are containing the information about the population size on the Province Vojvodina territory (Trivunovic, 2014). This report, from main breeding organisation in the animal husbandry at the Faculty of Agriculture of Novi Sad from 2014, includes only stallions that are registered, and are under the regular productivity control. The active population counts 184 animals. Thus, there is an assumption that for breeding is used a larger number of the breeding animals, however the breeders are not completely adjusted to breeding programmes from the unknown reason whether they are not keeping records or they do not hand in breeding reports to basic breeding organisations. In addition to those mentioned facts there have not been a proper number of scientific publications when it comes to fertility and problems in horse reproduction due to infectious disease in Serbia. In the continuation of this work we are going to describe what are the laws that should be obeyed regarding the health conditions of stallions, if they are compared with the legislation in Serbia (West Balkan) and EU.

Legislation about breeding stallions in Serbia

Diagnostic and health condition control procedures of breeding stallions in Serbia are in accordance with Regulation on the establishment of animal health care measures for 2014 (Anonymous, 2014A). Diagnostic tests for breeding stallions are performed on each breeding animal one time in a year, and those include only tests for Equine infectious anaemia (EIA). Nevertheless, the majority of horse owners and keepers tend to follow their previous routines and preventive measures. When it comes to stallions, tests which are conducted on horse-farm prior to breeding season include serum neutralization test in blood sample for Equine Herpes Virus-1 (EHV-1) and Equine viral arteritis (EVA).

Nevertheless, the present Program of measures states that in order to do establishment, monitoring and prevention of Leptospirosis specific diagnostic examination (microscopic agglutination test-MAT), is biannually conducted in stallions for natural mating and for semen production for artificial insemination. Furthermore, there are no precise data about the number of breeding mares and stallions that are under regular serological control for Leptospirosis in the herds in Serbia. Owing to that, we cannot accurately determine the nature of epizootic situation for this contagious disease.

What is the looming problem nowadays is that there is no existing system control whether would that involve certain institutions or associations (sport or breeding animals). Examination of equine sperm for EVA is not a common procedure - however, in justified cases of doubt solely it is.

In any case, in our country no systematic control of breeding animals for CEM (Contagious equine metritis) is done which is hardening the insight on the field. However, there is existing risk of CEM transmission during the import of breeding stallions and mares from the neighbour countries and EU, as well as the stallions provided and borrowed for breeding.

Compulsory blood examination for EIA with the Coggins test has to be conducted in the following cases: once in a year in the breeding horse-farms, horse-farms owned by Serbian Military Force, sports horse-farms, stud sections, forests and other places of work, as well as in every herd containing more than 10 horses; 90 days prior their arrival to hippodromes, fairs, exhibitions, reviews, sporting competitions etc. If the owner of an animal sells the animal or alienates it in any other way, the certificate about the examination of EIA must not be older than 30 days. Diagnostic examination can also be done with ELISA test, with confirmation Coggins test in addition.

Equine viral arteritis

All newly purchased horses should be kept in quarantine and subjected to serological testing

(serum neutralisation test), necessarily paired blood samples.

In relation to the regulations on the health status of breeding stallions in Serbia rules about semen quality ought to be mentioned (Anonymous, 2014B).

It is necessary to obtain certificate of the official veterinary organization claiming that the samples are taken from studs with high in quality with well-familiar health status and that the standard measures are conducted which are in accordance with program of measures of animal health care; Certificate of the official veterinary organization claiming that standard clinical examination is conducted and that no symptoms are established responsible for causing diseases which can be transmitted by the semen of the donor animal.

Such records are kept for each production run of conserved semen, and in a manner when it is possible at any time to determine the origin of semen and it is kept for at least five years from the date of placing on the market of the last batch produced.

The semen meets the requirements in terms of quality if: it is produced in a centre for reproduction and artificial insemination; it meets the health and hygiene conditions in accordance with recognized international standards; it is taken from an animal that has been clinically examined and at which no symptoms of diseases which can be transmitted by semen of an animal which the semen is taken from; it is taken from the high in quality breeding animals with known health status over which are implemented the measures prescribed by a special regulation governing the program of measures of animal health care;

European Union legislative

An interesting example of legislation is in Croatia, where the Regulations on traffic equine semen are in fact the provisions of Directive 92/65/ EEC (Anonymous, 1992) on health requirements for trade and imports into the European Union (EU) of animals, semen, ova and embryos. Regardless of its issuing in 2009, the mentioned Regulations came into force on the day of Croatia entering the EU in 2013.

As in the whole EU veterinary certificate for trade of semen of the equine animal is issued or authorized by the official veterinarian and above all he specifies the type, race and identity of donor semen, as well as the time of collection of semen. The official veterinarian confirms that the Centre for semen collection in which that semen was collected, processed and stored for transport, meets the following requirements: that is situated on the territory or in the region of the Member State on the day of collection of semen until the date of dispatch of semen or until the expiry of a period of 30 days mandatory storage of frozen semen that is free of African horse sickness (AHS) in accordance with special regulations; that during the period commencing 30 days prior to the date of semen collection until the date of dispatch of fresh / chilled semen or until the expiry of a period of 30 days mandatory storage of frozen semen fulfil the requirements of the Ordinance on the veterinary conditions for the transfer of the equine animal and imports from third countries. This Ordinance applies to most contagious animal diseases, in accordance with Council Directive EC. In the first place, the definition of "third country" means States that are not members of the EU. But in the narrow sense of "third country" in relation to the status of AHS, means a Member State of the EU free from AHS, or most precisely where no clinical, serological (in unvaccinated equidae) or epidemiological evidence of AHS have not been established in the past two years and in which in the last 12 months has not implemented a vaccination against this disease. The term "official veterinarian" means that the veterinarian is appointed by the Minister in accordance with the provisions of the Veterinary Act.

The official veterinarian must during the inspection and on the basis of declarations by the owner or breeder to establish the absence of any reason to suspect that the equidae have been in contact with animals suffering from infectious or contagious diseases during the 15 days immediately prior to the

examination. This applies to a negative status of the animal from a disease which should be reported: Dourine, Glanders, Equine encephalomyelitis (all types including Venezuelan encephalomyelitis), EIA, rabies, anthrax, AHS, Vesicular stomatitis.

The certificate of health in the case of a registered equine must be issued within 48 hours before loading or no later than the last working day prior to loading, in the language of the country of export of semen and at least one of the official languages of the EU. The health certificate is valid for 10 days and consists of a single sheet. Interestingly, the experts of the European Commission, in cooperation with the Ministry of Agriculture (as the competent body of the exporting country), may do the examination on the spot, to the extent that is necessary to ensure the implementation of this Regulation. The competent body shall provide all necessary assistance to the experts referred to in paragraph in carrying out their work. If it is about the import of equidae from the third countries to Croatia it is only allowed if those countries are officially enlisted by the European Commission. This approval relates to the whole territory of a third country or to only a part of its area. We should not neglect the legislation of the third country which applies in relation to animal health and welfare.

The conditions to be met by the Centre for artificial insemination in EU are: That during the period commencing 30 days prior to the date of semen collection until the date of dispatch of fresh / chilled semen or until the expiry of a period of 30 days of mandatory storage of frozen semen possessed only equidae which were free of clinical signs of CEM and EVA. That the above described semen comes from donor stallions, which: on the day of collection of semen have not shown clinical signs of an infectious or contagious disease; during at least 30 days prior to collection of semen have not been used for natural mating; during at least 30 days prior to collection of semen have been kept on farms where no equine animal showed clinical signs of EVA; during at least 60 days prior to collection of semen have been kept on farms where no equine animal showed clinical signs of CEM; according to the information / check the official veterinarian of the 15 days immediately prior to collection of semen have not been in contact with equidae suffering from an infectious or contagious disease; that they are subjected to the following tests in a laboratory approved by the competent authority in accordance with a test program: Coggins test for EIA with negative result, serum neutralisation test with serum dilution of 1 in 4, or with virus isolation test on an aliquot of the entire semen of the donor stallion, with the negative result; a test for CEM carried out on two occasions with an interval of seven days by isolation of *Taylorella equigenitalis* from pre-ejaculatory fluid or a semen sample and from genital swabs taken at least from the penile sheath, urethra and urethral fossa with negative result in each case.

That the stallions donors undergo one of the following testing programs: that at least 30 days prior to and during the collection of semen continuously resident in the collection centre of semen and in that period no equine animal in the centre has not come into direct contact with equidae of lower health status than the one in the centre. Certainly, the exact date is stated when they carried out indicated tests on samples of semen or blood.

During the completion of this certificate, parts that are not related to the shipment must be crossed out. In order to prevent forgery of documents, the colour of the seal and signature of veterinarian should differ from the colour of the printed text.

Discussion

Unfortunately, for now, in Serbia some kind of diagnostics includes only sport horses or other categories of horses that are exposed to diagnosis because of bringing them to the event, fairs or export.

The impression is that the most widely implemented serological diagnosis is the one of EIA. Although the Ordinance on eradication EIA specifies the compulsory serological diagnostic measures in horses once in five years it is highly dubious that this is fully carried out. This is indicated by the occasional discovery of seropositive animals in the past 5 years. In contrast, most sports horses are regularly controlled, sometimes several times a year, and it is partly regulated by law, and partly by sporting regulations.

Regardless of whether it is possible titre of antibodies against influenza, herpes virus infection, or EVA result of vaccination or infection it raises the question of what this finding means when we are aware that a large number of horses are positive to all these diseases, without evidence of vaccination.

There are some interesting experiences and research on the occurrence of EVA in Croatia in the period from 2009 to 2012 (Kolaković, 2013). According to the legislation in Croatia, EVA is mandatory controlled with serological examinations of all stallions before the breeding season. Serologically positive animals then are subjected to an overview of at least two semen samples for the presence of the virus, with the PCR method. Stallions with a positive test for EVA in the sperm must be excluded from breeding. The author concludes that this reduction in the number of stallions serologically examined presents a significant risk of uncontrolled spread of EVA in the horse population in Croatia. It underlines the need to careful approach when it comes to the implementation of vaccination against this disease, because the mentioned measure cannot eliminate the cause of the permanently infected stallions. Therefore, it is recommended to provide vaccination of only seronegative animals under strictly controlled conditions with the required stay in quarantine after the vaccination. In addition, vaccination, particularly in countries with unreliable labelling and record-keeping of horses (i.e. Croatia and Serbia), completely loses the ability to control the disease with serological examination, primarily because of the problems in differentiating infectious and vaccination titre. Moreover, epizootic situation in Serbia, together with the manner of keeping, manipulating and breeding animals, is not suitable to disease control implementing vaccination against EVA.

As an example of regulations in South America (Argentina), we would mention compulsory measures (National Animal Sanitary Service-SENASA- 434/01) in Argentina about the obligation of examination of EVA on stallions. These are following: Every breeding association must submit a certification of negativity (serum neutralization test) for EVA of all active stallions before the annual breeding registration or artificial insemination. Frozen semen samples imported from foreign countries must be checked by state laboratories for detection of viral presence by PCR before commercialization and insemination.

In relation to the occurrence of Dourine, interesting is the situation in Italy (Calistri et al. , 2013), where the disease was diagnosed in the regions Sicilia and Campagna in 2011. In Italy, the program of measures requires examination of about 4000 breeding stallions annually to this parasitic infection. Having established five outbreaks of Dourine in spring 2011, competent institutions have made a plan to eradicate and above all to provide the determination of the prevalence of this disease in Italy. Consequently were found two, but later seven outbreaks. It has been discovered that the infection is created directly from infected individual coitus. Although Italy is considered the country with developed horse breeding, this prevention of Dourine has been used to detect a number of deficiencies in recording and registration of horses in the Central Database in relation to the situation on the field. All this made it difficult to implement measures on eradication Dourine and identify the source of infection. This refers to an uncontrolled mating mares (without proper records), non-implementation of the prescribed veterinary measures and on the impossibility of monitoring the movement of animals. This example confirms that effective control of infectious diseases that occur occasionally involves primarily the establishment of effective communication between veterinarians, authorized diagnostic institutes and laboratories, veterinary inspection,

government and international institutions which are responsible for recording the occurrence of certain diseases and regular reporting when that is the case.

Conclusion

It can be concluded that the aforementioned regulations differ significantly only in the control of CEM and EVA, which are the most actual epizootiological problem in Serbian horse population, because we have no real information about their presence in horse studs in Serbia.

Actually it is understood the free status of centres for artificial insemination and horse studs of especially dangerous infectious diseases, such as AHS. We believe that more important would be consistent implementation of regulations related to the diagnosis of infectious diseases in stallions.

On balance, it can be emphasized, that in relation to further development of veterinary profession and horse industry in Serbia as well, it is not recommended to “a priori” implement every EU model currently, and completely, due to the difference in horse breeding between our system and similar in EU countries. Nevertheless, in relation to mentioned legal framework and de facto situation in horse breeding in Serbia, we would recommend thoroughly planned control and primarily to make evidence of stallions used for breeding of mares in sport and rural areas.

Thus, based on these data, we propose appropriate measures. First of all, audits (as amended) regulations and their alignment with the current EU directives, bearing in mind the epizootiological situation in the country and the region. As a prerequisite, it should be the training of certified laboratories for fast and effective diagnosis of infectious diseases of horses, those that are regularly or occasionally occurs in our country, as well as diseases that can potentially introduce horses or otherwise from other countries. This mainly refers to identifying EVA in the sperm (PCR), as well as with evaluation of genital swabs for CEM. However, as far as it is possible we would not recommend vaccination against EVA. The reason for this is that the disease situation in Serbia, the way of keeping animals, the use and breeding horses, including the records and marking of animals, are not appropriate and in accordance with control of the disease by introducing just these preventive measures.

To ensure effective diagnosis, it is necessary to review and offer laboratory analysis of all veterinary institutes related to infectious diseases of horses. Constant communication and exchange of information and experience would ensure effective coordination of all involved in the health care of horses, and certainly with international organizations such as OIE and the relevant EU bodies. Because of the introduction of legal solutions for immunoprophylaxis should enable simpler procedure of import of vaccines for infectious diseases of horses. And last but not least, a crucial measure for combating infectious diseases, breeding a horse and raising awareness of the importance of this issue, both for the owner or holder of the animals themselves and veterinarians who care about their health condition.

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