Functionality of Innate Defense Mechanisms Influenced by Raising Technologies in Horses

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Abstract. This study investigated the functionality of horse innate defense mechanisms under the influence of raising technologies. This evaluation was conducted on three experimental groups: group I (horses from a semi intensive farm), while group II and III included horses raised in extensive conditions and cohabitating with bovine and bovine and pigs, respectively. From each animal, blood was collected by jugular vein puncture and the serum samples were processed for the evaluation of total immunoglobulins and immune circulating complexes. Two nephelometric tests were carried out: zinc sulphate turbidity assay for the total protein concentration (Serb method) and the 4.2% polyethylene glycol (PEG) precipitation technique in order to estimate the level of the circulating immune complexes (CIC). The monitoring of the results obtained for the total immune globulins indicated lower values for the animals raised in shelters along with others species when compared to the animals grown under semi-intensive conditions. There was no statistical significance of the differences for the obtained results. The CIC were reduced for the intensively raised horses when compared to groups II and III, while the differences between groups II and III were statistically non significant. The lowest value of CIC, recorded for the horses belonging to group III, indicated the influence exerted by the raising conditions, the overcrowding induced stress and the housing and microclimate conditions on this parameter.

Key words: horses, immune globulins, circulating immune complexes, technology

INTRODUCTION

The active life of a horse, either economic or sportive, implies a permanent contact with physical, chemical or biological stressing factors that induce changes, often clinically unapparent, to one of the most sensitive radars of the organism, the immune system. Monitoring these modifications may indicate the animal health status and may provide useful information for the breeder, owner, user or sportsman, therefore being able to take the optimum measures for the animal welfare (Drăghici C., 2002). Modern animal breeding pleads for modern technologies, with small, medium and big heard, taking into consideration all the relationships and by this, accomplishes an acceptable compromise between biology, physiology, behaviour of horses depending on their breed, age category, production and economical, technical, hygienic, sanitary and ecological objectives (Man et al., 2002).
MATERIALS AND METHODS

The research was carried out on three experimental groups:

**Group I**, composed by twenty sport horses, with different sex and age (ranging in age from 2 to 16 years), from Transylvanian Equestrian Club - Sibiu, raised in semi-intensive system. All animals were healthy and annual anthrax vaccinated, and the hygienic and microclimate conditions were satisfactory.

**Group II**, horses raised in extensive system, housed along with bovine.

**Group III**, that included horses housed in the same stables with bovine and pigs.

From each animal, a 5 mL volume of blood was collected by jugular venipuncture using sterile procoagulant Vacutainers. The serum samples needed for the evaluation of several humoral immune parameters (total immune globulins, immune circulating complexes) were obtained after the centrifugation of the clotted blood. Sera were kept at –20°C until tested.

Two nephelometric tests were carried out: zinc sulphate turbidity assay for the total immune globulin concentration (Serb method) and the 4.2% polyethylene glycol (PEG) precipitation technique in order to estimate the level of the circulating immune complexes (CIC).

**Immunoglobulin measurement.** Total immunoglobulin, known as opsonins, play an important role in the ‘first line of defense’, that is innate immunity, against aggressors. The serum level of the immunoglobulins represents an indicator of the humoral immune reactivity, while its modifications may suggest either the initiation of an immune response or the presence of some pathological state associated with an increased immunoglobulins synthesis, or a diminished reactivity determined by the action of external or/ and internal factors (Roitt and Delves, 2001). At a pH·7.4, the electric charge and colloidal stability of gamma globulins are lower than those of serum albumins. Thus, concentrations as low as 24·mg·l–1 of metal salts precipitate the immunoglobulin. A volume of 6.6·µl of serum was mixed with 193.4·µl of a 0.024% barbital buffer zinc sulphate solution and allowed to precipitate for 30·min at room temperature (22–23°C). Optical density (ODU) then was read spectrophotometrically (l=475 nm, d=0.5 cm).

**Circulating immune complex (CIC) measurements.** Circulating immune complexes result from specific antibodies binding with the correspondent antigens as a common physiological mechanism for eliminating different pathogens from the organism (Roitt et al., 2001; Andries et al., 1992; Răpunteanu, 1997). If the microbial aggression is massive and the eliminating capacity is exceeded, these immune complexes may accumulate in the circulatory system, some time dependent by the antigen type, or may be deposited in several organs, but mainly in the basal membranes determining immune mediated lesions. Measurement of the level of circulating immune complexes (CIC) allows the evaluation of the molecular clearance capacity at a particular moment. A 4.2% polyethylene glycol (PEG) solution in borate buffer was used as the precipitating agent, while buffer-treated samples served as controls for borate-induced precipitation. The reaction was performed in a 96-well-plate to enhance spectrophotometrical readings. Volumes of 196.7·µl of borate buffer and PEG solution, respectively, were mixed with 3.3·µl samples of the serum, for each sample, in parallel wells. The samples were allowed to precipitate at room temperature (22–23°C) for 60-min, then read spectrophotometrically at a wavelength of 450-nm in the test plate (d=0.5-cm) (multichannel spectrophotometer SUMAL PE2). CIC concentrations, expressed in optical density units (ODU) were calculated by subtracting the value of the control (serum + buffer) from that of the PEG precipitate.
The data are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSIONS

During their phylogenic evolution, organisms developed and continually selected defense systems, reaching to the highly improved system of the inducible immunity that functions using specific immune effectors synthesis, maintaining the homeostasis and providing the antimicrobial protection. Determination of the total circulating immunoglobulins concentration may complete the global image of the antimicrobial unspecific defense capacity, as well as for the immune removal and elimination of antigens (Bellido C. et al., 1981).

Considering the results obtained for the evaluation of the serum total immunoglobulins (Table 1) (Vernes degrees), we can observe superior mean values for the horses raised at the Equestrian Club (22.03 ± 6.382 Vernes degrees) compared to those of horses housed along with bovine (14.95 ± 5.798 Vernes degrees) and bovine and pigs (14.02 ± 2.151 Vernes degrees), respectively.

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
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<tr>
<td>Mean values</td>
<td>22.03</td>
<td>14.95</td>
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<tr>
<td>Standard deviation (SD)</td>
<td>6.382</td>
<td>5.798</td>
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As proven before, an important role in the macroorganism’s defense first line is accomplished by the circulating immunoglobulins. Their concentration, as determined by zinc sulphate turbidity assay, has little value on its own, but correlated with other specific serological assays may suggest the functional level of the antimicrobial potential (Roitt et al., 2001; Andrieş et al., 1992).

Analyzing these results it can be noticed that the circulating gammaglobulins level was diminished for both experimental groups raised by extensive conditions, when compared with the semi intensively raised horses. Similarly, all values obtained for this immune parameter were lower than the normal values in horses (0.4 ODU or 40 Vernes degrees) (Ghergariu et al., 2000). This aspect can be the direct consequence of stress factors’ influence, mainly due to the housing conditions, and implicitly of the corticosteroids secretion triggered in the general adaptation syndrome.

In case of the extensively raised horses, an inhibition of the immune globulins’ synthesis was noticed, the mean values being closely related and with one third reduced when compared to the semi intensively raised horses.

The housing conditions and the raising system type seem to have an impact on the immunoglobulins’ levels in horses. Thus, it can be observed that for the extensive system, where the horses are housed along with other species (bovine and bovine and pigs, respectively), with poor microclimate conditions (light, temperature and humidity), the immunoglobulins’ synthesis was reduced when compared to those in healthy animals housed under optimal conditions at the Sibiu Equestrian Club.
However, for the sport horses, the stress due to the active life, training, competitions abroad or within the country, as well as the transportation stress should be also taken into consideration.

The differences between the mean values recorded for the extensive raised horses, group II and group III, can not be considered statistically significant, as the values are very similar.

As for the horses from the Sibiu Equestrian Club, the values obtained for the circulating immune complexes (Table 2) vary between large limits, from one unit (U) to 19 units. The mean value of CIC in this group was established for 6.4 ± 5.113 U, a value closely related to that obtained for group II (6.5 ± 5.61 U). In case of horses included in group III (horses housed along with bovine and pigs) the mean values were the lowest (4.2 ± 2.38 U).

### Table 2

| Mean values for the circulating immune complexes in the three experimental groups |
|----------------------|----------------------|----------------------|
| Mean values          | Group I              | Group II             | Group III            |
| Standard deviation (SD) | 6.4                  | 6.5                  | 4.2                  |
|                      | 5.113                | 5.61                 | 2.38                 |

There are not few diseases for that the estimation of the circulating immune complexes levels indicates the microorganism reactivity potential, as well as the severity of the disease. For the bacterial infections, excessive formation of the circulating immune complexes may conduct to their accumulation and fixation in several organs and tissues, also may trigger the activation for the cell-killing membrane attack complex (complement system) and the consecutive destruction of the noble, normal, tissue, also the releasing of new antigens and the synthesis of specific antibodies against the modified self (Zahao, 2006).

Using the 4.2% polyethylene glycol (PEG) precipitation technique, the presence of circulating immune complexes is pointed out. As an alternative to this method, it is mentioned in literature the indirect detection of the immune complexes for the circulatory system by determining the complement level, this one being activated by the immune complexes’ formation (Algers et al., 1987).

Therefore, it can be considered that the circulating immune complexes levels indicated the level of the organism’s reactivity under normal and stressful circumstances, determined by several factors, such as: different species cohabitation, crowded shelters. The microorganism’s presence in the body of the animals, as non self, determines an excessive formation of the CIC (Andriesc et al., 1992).

During the experiment, we evaluated if the raising conditions and system, and also the cohabitation with other species, may have a negative influence on this immune parameter in horses raised under different circumstances.

Analyzing the CIC values obtained for the three experimental groups, we remarked similar mean values for groups I and II, while the lowest value was recorded for the horses belonging to the group III, leading us to the conclusion that this immune parameter is influenced by the raising conditions, the crowded environment-induced stress and the housing and microclimate conditions. All these factors, increasing the elimination of CIC, determined a small decrease of the serum level of CIC in group III of horses.

The mean values for all three experimental groups were considered between normal values (Ghergariu et al., 2000).
CONCLUSIONS

The results obtained for the total immunoglobulins indicated lower values for the animals raised in shelters along with others species, when compared to the animals from the intensive system.

The differences observed between the three experimental groups could not be considered statistically significant, as the values were almost similar.

The circulating immune complexes were reduced for the intensively raised horses when compared to groups II and III, while the differences between groups II and III were statistically non-significant.

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