Evaluation of Stress in Riding Horses, During the Horse-Assisted Therapy Activities in the Case of Children with Autism Spectrum Disorders

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RESEARCH ARTICLE

Abstract
Animal stress is a current topic intensely addressed. The general adaptation syndrome, following short exposure to stressors with low or medium intensity, has a positive effect, inducing eustress and improving adaptive capacity. Exposure to strong stressors or over a long period of time has the effect of inducing distress. The aim was to evaluate the stressful effect they have on horses, the specific activities of equine-assisted therapy, in the case of children with autism spectrum disorders. We used 10 riding horses (8 females and 2 males), of purebred English breed, divided into 2 groups, aged between 4-8 years. The animals had the same maintenance conditions and lifestyle. Samples were taken from the jugular vein in 5 ml heparinized tubes. The parameters were determined using a ProCyte Dx Hematology Analyzer. The data obtained were statistically analyzed, calculating the Mean and Standard Deviation (Microsoft Excel). The results show that the specific activities of equine-assisted therapy do not have a stressful effect on the pathways used. These activities were less stressful than regular recreational riding activities in a riding school, subjecting the animal to less intense physical exertion than daily activities.

Keywords: Equine; stress; autism.

INTRODUCTION
Animal stress is an intense topic addressed by researchers in the field. The general adaptation syndrome, produced after short exposure to stressors with low or medium intensity, has a positive effect, inducing stress and improving the body's adaptive capacity (Asselin, et al., 2012; Basset, et al., 2009; Koolhaas, et al.2011; Popescu, et al., 2014; Selye, 1956). Exposure of animals to strong stressors or over a long period of time has the effect of inducing distress. It has negative implications that can lead to a pathological evolution (Campbell and Sandoe, 2015; Peeters, et al., 2011; Popescu, et al., 2019). Researchers have sought to identify techniques for assessing stress in various species of animals in general and in horses in particular. In this sense, methods were tested such as dosing of cortisol in plasma, saliva or feces, clinical evaluations that focus on the effect of stress on heart function or blood pressure, etc. Recent studies have highlighted the effects of stress on hematological parameters. This increased the range available to assess the intensity of stress. In this context, it can be emphasized that stress induces changes such as an increase in the number of red blood cells, hematocrit and hemoglobin (Corley, 2008; Despopoulos and Silbernagl, 2003;
Hausberger, et al., 2020; Paulescu, 1999-2000). Stress also induces changes in the leukogram, such as an increase in the number of leukocytes, neutrophils and a decrease in the number of lymphocytes (Basset, et al., 2009; Cunningham, 2002; Popescu and Diugan, 2017; Lesimple, 2020). Here the specific effect of stress on the Neutrophils/Lymphocytes ratio stands out, which increases with stress. Thus, the evaluation of the Neutrophils/Lymphocytes ratio has become an effective method in the evaluation of stress in mammals (Popescu, et al., 2014).

The issue of stress has a great relevance for horses. Species characterized by a special temperament, which makes it more sensitive to the action of stressors (Asselin, et al., 2012; Dantzer and Kelley, 1989). A special case is represented by the horses used in equine-assisted therapy. This type of therapy has begun to be used more and more often, as an additional way of therapeutic intervention in patients with various mental traumas or psycho-behavioral disorders (Hession, et al., 2014; Rigby and Grandjean, 2016). These treatments, according to research, have given positive results that have encouraged the application of these methods on an increasingly large scale. Among the psycho-behavioral disorders that can be treated with the help of equine-assisted therapy are autism spectrum disorders in children.

Our research has focused on assessing the stressful effect they have on horses, the specific activities of equine-assisted therapy in children with autism spectrum disorders. We initiated this approach by evaluating the hematological changes induced by these activities in the body of horses. The experiments were performed on a riding school in Ilfov County, where the therapy of children with autism spectrum disorders has been practiced for several years, using riding horses.

MATERIALS AND METHODS

In our experiment we used 10 riding horses (8 females and 2 neutered males) of purebred English breed. The 10 horses were between 4-8 years old. The sample of animals was uniform in terms of maintenance. The animal feed consisted of: ad libitum hay, oats 6 kg/per animal/day and bran 1.5 kg/per animal/day. These animals were maintained in a riding school in Ilfov County, where in addition to other activities specific to entertainment riding, they were used to treat children with autism spectrum disorders through equine-assisted therapy.

The experiment was done with the written consent of the animal owners, but also of the parents of the children who participated in the experiment.

The experimental procedures did not affect the level of well-being of the horses, the only experimental intervention being the collection of some blood samples. This action was done in compliance with the rules of ensuring animal welfare.

The animals were divided into two groups. Lot 1 (witness), consisting of 5 horses (4 females and one male) and Lot 2 (experimental), consisting of 5 horses (4 females and one male).

During the experimental period, in the intervals between physical activities, the horses were kept in the stables of the riding school. Under these conditions the animals were fed ad libitum. The food consisted of hay and concentrate supplements. The water was provided at will.

The animals from group 1 were used for 3 days for the usual specific activities, from a riding school where the animals are ridden by various clients for recreational purposes. These animals were ridden by teenagers and children without autism. The horses were controlled during the activities carried out by the riding staff. This activity was performed by each horse in group 1 daily, for 3 days, during two hours. The exercises performed consisted in carrying out the following activities: contact with the rider (approach and riding), walking, running on a sitting trot, jumping trot, light gallop, jumped trot and gallop lightly.

The animals from group 2 were used for 3 days only for the specific activities of equine-assisted therapy, applied in the case of children with autism spectrum disorders. The horses were controlled during the activities carried out by the employees of the riding school. In addition to the situation presented in the case of group 1, this activity was attended by a therapist, specialized in assisting children with autism spectrum disorders. This activity was performed by each horse in group 2 daily, for 3 days, during two hours. The exercises performed consisted in carrying out the following activities: contact with the rider (approach and riding), walking, running on a sitting trot, jumping trot and light gallop.

At the beginning of the first day of the experimental period, but also at the end of the third day of the experiment, the animals were subjected to blood samples. At the beginning of the first day of the experimental period, but also at the beginning of the third day of the experiment, the animals were subjected to blood sampling (Table 1).

The collection of blood samples, labor with potential stress, was performed by medical staff who monitor daily the number of animals, staff with which the animals are accustomed, thus contributing to the reduction of stress following the collection of blood samples. At the same time, taking into account the fact that both groups were subjected to the same maneuvers with potential stress, the level of induced stress being similar, we consider that the comparisons between the two groups are valid. Also, although the act of collecting blood samples is stressful, the samples are taken exactly at the time of the work, which does not influence the values of the parameters we follow, parameters that, in order to change, need a certain latency period.
Blood samples were taken from the jugular vein in 5 ml heparinized tubes. These samples were used to evaluate the following parameters: hemoglobin, hematocrit, erythrocyte count, leukocyte count, and neutrophil and lymphocyte weight in the leukocyte formula. The parameters were determined using a ProCyte Dx Hematology Analyzer.

Table 1. The experimental protocol to which the animals in the two lots were subjected

<table>
<thead>
<tr>
<th>Blood sampling no.</th>
<th>Lot 1 (Witness) (n=5)</th>
<th>Lot 2 (Experimental) (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>At the beginning of the experiment</td>
<td>At the beginning of the experiment</td>
</tr>
<tr>
<td>2</td>
<td>At the end of the experiment</td>
<td>At the end of the experiment</td>
</tr>
</tbody>
</table>

The data obtained were statistically analyzed, calculating the Media and Standard Deviation (SD), using the Microsoft Excel application. At the same time, the statistical significance of the differences between the batches was calculated using the t test (Student), using the Microsoft Excel application. Of course, the data were tested for normality and homogeneity of variations.

RESULTS AND DISCUSSIONS

In the case of group 1, the level of red blood cell count was higher by 1.9% at the second collection, compared to the one recorded in the card of the first determination, the differences being statistically significant (P <0.05) (Table 2).

For the 2 group, the level of the number of red blood cells in the blood was higher by 0.1% at the second collection, compared to the one registered in the card of the first determination, the differences being statistically insignificant (P> 0.05).

Table 2. Hemogram values in the case of the two experimental groups (Media ±SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Average number of red blood cells (M/mL)</th>
<th>The average hematocrit (%)</th>
<th>Medium hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 1</td>
</tr>
<tr>
<td>1 Witness</td>
<td>9,2±0.45</td>
<td>9,4±0.38</td>
<td>36,4±4.81</td>
</tr>
<tr>
<td>2 Experimental</td>
<td>9,8±0.40</td>
<td>9,83±0.32</td>
<td>35,5±3.62</td>
</tr>
</tbody>
</table>

*P <0.05 (DF=8)

These results indicate an effect with a more intense stress potential of current activities, specific to recreational riding, compared to those following equine-assisted therapy. The differences could be induced by the specifics of the exercises performed by the two groups of animals. In the case of group 2, the one used in the equine assisted therapy, the exercises performed required a lower physical effort compared to the exercises performed by the animals in group 1. These differences cannot be attributed to dehydration and changes in body fluids induced by exercise, as blood samples were taken each morning before physical activity began.

These results are consistent with those published by Rigby and Bass, who noted similar effects in the general syndrome of adaptation in riding horses.

Results obtained by us in the evaluation of hematocrit and hemoglobin, in the conditions of our experiment, were in line with those obtained by us in evaluating the number of red blood cells. Thus, we recorded evolutions of these parameters that closely followed the fluctuations in the number of red blood cells. These results highlight the fact that there is an effect with a more intense stress potential of current activities, specific to recreational riding, compared to those following equine-assisted therapy.

Results for group 1, the level of leukocytes in the blood was higher by 19.44% at the second collection, compared to the one recorded in the card of the first determination, the differences being statistically significant (P<0.05) (Table 3). In the case of group 2, the level of leukocytes in the blood was higher by 2.4% at the second collection, compared to the one recorded in the card of the first determination, the differences being statistically insignificant (P> 0.05) (Table 3). The share of neutrophils in the leukocyte formula, for group 1, was higher by 3.26% at the second harvest, compared to that recorded in the card of the first determination, the differences being statistically significant (P<0.05). The share of neutrophils in the leukocyte formula, for the 2 group, was higher by 0.35% at the second harvest, compared to that recorded in the card of the first determination, the differences being statistically insignificant (P>0.05). The share of lymphocytes in the leukocyte formula, for group 1, was lower by 9.09% at the second harvest, compared to that recorded in the card of the first determination, the differences being statistically significant (P<0.05). The share of lymphocytes in the leukocyte formula, in the case of group 2, was lower by 4.12%
at the second harvest, compared to that recorded in the card of the first determination, the differences being statistically insignificant (P>0.05).

Table 3. Leukogram values in the case of the two experimental groups (Media±SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Leukocytes (K/mL)</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 1</td>
</tr>
<tr>
<td>1 Witness</td>
<td>7.2±0.50</td>
<td>8.6±0.22</td>
<td>58.2±2.15</td>
</tr>
<tr>
<td>2 Experimental</td>
<td>8.2±0.42</td>
<td>8.4±0.12</td>
<td>56.2±2.15</td>
</tr>
</tbody>
</table>

*P<0.05 (DF=8)

These differences cannot be attributed to dehydration and changes in body fluids induced by exercise, as blood samples were taken each morning before physical activity began.

These results indicate a more intense stressful effect of current activities compared to those following equine-assisted therapy.

In group 1, these results are induced by an increase in blood levels of glucocorticoid hormones (Basset, et al., 2009; Tsigos, et al., 2004). Indeed, these hormones induce the centralization of neutrophils, respectively the inhibition of their adhesion to the vascular endothelium and the delay of diapedesis (Campbell and Sandoe, 2015; Despopoulos and Silbernagl, 2003; Glaser and Kiecolt-Glaser, 2015), hence the increase in the total number of leukocytes in the blood fluid. Also, the increase in blood levels of glucocorticoid hormones, leads to tissue sequestration of lymphocytes (Basset, et al., 2009; Koolhaas, et al., 2011; Lesimple, 2020) and decrease their weight in the leukocyte formula.

Our results are consistent with those published by Asselin et al. (2012), who noted similar effects in the use of horses for therapeutic purposes. Our results show that the specific activities of equine-assisted therapy do not have a stressful effect on the pathways used. These activities were obviously less stressful than the usual recreational riding activities in a riding school, induced by the specific activities of equine-assisted therapy, activities that subject the animal to less intense physical effort than those following daily activity from riding.

CONCLUSIONS

The results showed that the specific activities of equine-assisted therapy in the case of children with autism spectrum disorders, have a weak effect of stress on the animals used.

This effect is placed below the level of the stressful effect found during the current activities in a riding school, which proved to be much more stressful.

It can thus be emphasized that this type of therapy does not have the consequence of increasing the level of stress to which they are exposed to the animals used.

These results encourage the further application of these therapeutic methods, which have no negative effect on the welfare of the animals used.

Research confirms that evaluating the number of leukocytes and the share of neutrophils and leukocytes in the leukocyte formula is an effective way to assess stress in horses, being easy to apply and providing relevant results.

Author Contributions: A.M.Z.(M) designed the study, wrote the working protocol and performed the statistical analysis. G.Z. wrote the draft of the manuscript and completed the manuscript. G.C. and G.G. collected and processed the samples. M.G. and A.M.I. managed the searches in the specialized literature and organized the material. A.D. and D.C.C. managed and interpreted the analysis of the study. All authors read and approved the final manuscript.

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REFERENCES