

**COMPARATIVE ANALYSIS OF SOME SERUM PROTEINS AND IMMUNOGLOBULIN G CONCENTRATION IN THE BLOOD OF YUGOSLAV TROTTER MARES AND NEWBORN FOALS**

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*The comparison of some serum protein concentrations was performed on 12 Yugoslav Trotter mares and their newborn foals. The mares included in the evaluation were divided into two groups of 6 each. The mares in the first group were vaccinated against equine herpes virus 1 and 4, in the 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> month of pregnancy, while mares in the second group were not vaccinated at all. Pregnant mares were clinically observed during the last stage of pregnancy and blood for biochemical evaluations was sampled immediately after foaling. Foals were clinically observed for seven days after birth and blood samples were collected immediately after foaling (before nursing), and 24, 48, 72 and 168 hours after birth. Foals included in the evaluation were divided into two groups according to the group allocation of the respective mares.*

*All mares gave birth to normal foals in expected terms. Biochemical examination revealed slightly lower total gammaglobulin and IgG values in tested mares compared to the values obtained in other horse breeds. The antibody titres against equine herpes virus-1 reached the level that provides sufficient protection in vaccinated mares. Gammaglobulin and traces of IgG were present in the blood serum of foals tested immediately after birth and before nursing. A significant increase of IgG and gammaglobulin concentration was revealed in all foals after the first 24 hours of life. The observed first day increase of concentration was followed by stagnation of gammaglobulin and IgG levels in all foals. Total protein values showed a significant increase 24 hours after the first intake of colostrum in all foals.*

*Immunoglobulin G concentration established by semi-quantitative test was considered low positive in 16.67% and in 33.34% of foals from vaccinated and unvaccinated mares, respectively. Turbidimetric analyses of the same samples revealed sufficient Ig transfer, i.e. Ig concentration over 8 g/L. Comparison of the results obtained by the two methods indicates that semi-quantitative field test results were clinically valid. There were no antibodies against EHV 1 in foals immediately after birth and before the first colostrum intake, and a*

*highly significant increase of serum antibody level was recorded 24 hours after the onset of nursing in foals born from vaccinated mothers.*

*Key words: colostrum, newborn foal, immunoglobulin, gammaglobulin, Equine herpes virus-1, Yugoslav Trotter*

## INTRODUCTION

It is well known that the crucial source of antibodies, necessary for survival of foals during the first several months of life, is colostrum. Intake and absorption of adequate amount of colostral immunoglobulins is the most important precondition for the establishment of passive immunity in neonatal foals (Le Blanc *et al.*, 1992; Jeffcott, 1974; McGuire and Crawford, 1973; Steven and Samuel, 1975; Perryman *et al.*, 1980). Passive transfer of colostral antibodies is the most important protective factor in foal infections since neonatal sepsis and EHV-1 infections are the most frequent causes of death in newborn foals. Mares can be unapparent EHV-1 carriers for a long period during which virus replication leading to onset of the clinical disease in mares and in foals is possible (O'Callaghan, 2008; Brown *et al.*, 2007, Slater, 2007). It was revealed that abortion outbreaks and neonatal foal deaths occurring in Thoroughbred stud farms in Serbia before 1990 were characterised by isolation of EHV-1 and latent infection of mares complicated by insufficient colostral intake (Trailović *et al.*, 1992).

Insufficient transfer of colostral immunoglobulins is the main cause of disease in all newborns especially in foals. Many diseases from septicaemia to intestinal and respiratory diseases and infections of navel cord and joints, as well as others may be consequential to inadequate colostrum intake. Although the largest risk of these infections exists in the first week of foal life; the inadequate immunity transfer is also responsible for the appearance of diseases all the way to the 3rd or the 4th month of life (Wohlfender, 2009). Studying the causative factors of insufficient transfer of colostral immunity and signs of early recognition of high-risk foals enable reduction of losses caused by infections of newborns. Vaccination of mares during the last third of pregnancy against EHV 1 virus increases the content of specific antibodies in the colostrum (Burrows *et al.*, 1984).

Level of passive immunity provided through colostrum primary depends on: the amount of immunoglobulin in the colostrum, colostral intake and the colostral immunoglobulin absorption efficiency (LeBlanc, 1988, 1992; Giguere and Polkes, 2005). Although colostrum intake is considered as the primary mechanism of IgG transfer, thus providing appropriate antibody titre in the foal's serum, it was proven that the effect of colostrum on neonatal foals is more complex. Today, more attention is focused on colostrum importance in providing an efficient immune barrier to bacteria in the intestinal lumen, so that penetration of microorganisms from the environment into the newborn is prevented. Colostrum is not only a significant IgG source, but also contains other bioactive proteins i.e. immunomodulators and inflammation mediators. All immune system functions

underdeveloped in newborn foals are compensated via colostrum intake (Allen *et al.*, 1993; Bernoco, 1994; Demmers *et al.*, 2001).

Estimation of immunoglobulin status in foals can be of significant preventive importance and therefore, sampling for Ig testing in newborn foals, is done usually 12-72 hours after foaling, although the results of examination of foals performed 6-12 hours after birth are preferred and clinically more appropriate, especially in the case of endangered foal.

#### MATERIAL AND METHODS

The investigation was performed on a total of 12 Yugoslav Trotter mares and their 12 foals. Pregnant mares were clinically observed during the last third of pregnancy and blood samples for biochemical examinations were collected immediately after foaling. Mares were divided into two groups, six each. Mares in the first group were vaccinated against equine herpes viruses 1 and 4 with Duvaxyn® EHV-1, 4 (Fort Dodge Animal Health, The Netherlands) three times during pregnancy, while mares in the second group were not vaccinated. Newborn foals were observed during the first seven days of life and blood samples for biochemical examinations were taken before colostrum intake, and 24, 48, 72 and 168 hours afterwards. Foals were also divided into two groups. The first group of six foals came from vaccinated mares, while the second group of six foals were delivered by unvaccinated mares. Serum samples were examined for the following biochemical parameters: total protein and albumin concentration, serum protein fractions content, IgG and titres of antibodies against EHV-1.

Total protein and albumin concentrations were estimated by colorimetric and spectrophotometric tests on the automatic analyzer Olympus AU400 (Olympus System Reagents, Olympus Diagnostica, Hamburg, Germany). The serum proteins fractions content was established by electrophoresis on cellulose-acetate, and the concentration of immunoglobulin G by immuno-turbidimetry (Olympus AU400, Olympus Diagnostica, Hamburg, Germany). Horse Ig One-Step test (EVL, Woerden, the Netherlands) was used for quick screening, appropriate for the evaluation of colostrum immunoglobulin transfer. This test distinguishes positive (>8 g/L), weak positive (5-8 g/L) and negative (<5 g/L) results. The titre of antibodies to EHV was detected by virus neutralization test. The serology was performed on micro-titre plates with a constant dose of virus and double serial dilutions of tested equine sera (OIE manual 2008, chapter 2.5.9.).

The obtained data was statistically analysed by Tukey test (adapted by Snedecor), Student's t-test and correlation analysis by software program package Statistica 10 and Microsoft Excel 2007.

#### RESULTS

Pregnant mares showed neither signs of health disorders nor deviations of examined haematological and biochemical blood parameters during the last third

of pregnancy (data not included in the paper). Immediately after foaling, total protein and albumin concentrations remained within physiological limits (Table 1). Globulin concentration was  $34.4 \pm 4.3$  g/L in vaccinated mares and  $34.5 \pm 2.9$  g/L in unvaccinated ones; gammaglobulin fraction was 23% of total proteins immediately after foaling in both groups of mares. Immunoglobulin G value in mare serum sample was  $13.1 \pm 1.0$  g/L in the vaccinated group and  $11.8 \pm 2.3$  g/L in the unvaccinated group immediately after foaling, but this difference was not statistically significant ( $p > 0.05$ ). Value of reciprocal EHV-1 antibody titre was 4.5 on average in unvaccinated, and  $85.3 \pm 44$  in vaccinated mares, and the established values are considered as sufficiently protective.

Table 1. Concentration of some serum protein fractions, immunoglobulin G and reciprocal value of EHV-1 antibody titre in vaccinated and unvaccinated mares immediately after foaling ( $\bar{X} \pm SD$ )

Parameter	Vaccinated mares	Unvaccinated mares
Total protein (g/L)	$65.50 \pm 3.8$	$64.2 \pm 2.4$
Albumin (g/L)	$30.90 \pm 2.2$	$29.8 \pm 2.9$
$\alpha$ -1 globulin (g/L)	$2.30 \pm 1.0$	$1.8 \pm 0.6$
$\alpha$ -2 globulin (g/L)	$8.10 \pm 0.6$	$8.4 \pm 1.1$
$\beta$ -globulin (g/L)	$8.90 \pm 1.4$	$9.0 \pm 1.6$
$\gamma$ -globulin (g/L)	$15.30 \pm 1.2$	$15.1 \pm 3.2$
Total globulin (g/L)	$34.60 \pm 4.2$	$34.3 \pm 6.5$
IgG (g/L)	$13.01 \pm 1.0$	$11.8 \pm 2.3$
Antibody titre to EHV-1 (reciprocal values)	$85.30 \pm 44.0$	$4.8 \pm 2.1$

All mares included in the evaluation foaled in expected terms, without complications. The foals were vital and nursed with colostrum without difficulties. Biochemical examinations of blood serum in newborn foals before colostrum intake showed lower total protein value, followed by a significant increase after the first 24 hours of life ( $p < 0.05$ ). The increase of total protein was revealed in all foals, and no significant changes of protein concentration were revealed during the next 6 days of life (Table 2).

Unlike total proteins, albumin concentrations did not follow a similar trend in foals, and only small oscillations were registered in foals after colostrum intake. The  $\alpha$ -2-globulin concentration was a little higher than  $\alpha$ -1-globulin concentration (Table 2). Betaglobulin concentration did not significantly change during the first seven days of foal life either. Gammaglobulin concentration in foals immediately after foaling was low (0.6-1.2 g/L). After 24 hours a statistically significant increase of serum gammaglobulin ( $p < 0.001$ ) was established ( $11.7 \pm 3.3$  g/L and  $9.6 \pm 1.2$  g/L) in the first and second group of foals, respectively. A nonsignificant decrease of gammaglobulin values was revealed in the following six days ( $p > 0.05$ ) in both groups of foals.

Table 2. Concentrations of some serum protein fractions (g/L) and reciprocal values of EHV-1 antibody titres in foals originating from vaccinated (I) and unvaccinated (II) mares

Time of blood collection	Group of foals	Total protein	Albumin	$\alpha$ -1 globulin	$\alpha$ -2 globulin	$\beta$ -globulin	$\gamma$ -globulin	IgG < 1.08	Titre to EHV-1
Before colostrum intake	I	42.8±4.3	26.7±6.6	2.8±2.0	7.2±2.9	5.5±2.5	0.6±0.3	<1.08	<2
	II	44.4±3.2	24.4±1.8	3.9±2.0	7.4±2.1	7.5±1.9	1.2±0.7	<1.08	<2
24 hrs after colostrum intake	I	49.8±5.6	23.6±1.6	3.1±3.0	4.3±0.8	7.0±2.3	11.7±3.3	10.4±2.9	34.7±14.4
	II	48.5±1.9	24.2±1.7	2.9±1.4	5.7±1.5	6.0±0.6	9.6±1.2	9.3±0.9	<4
48 hrs after colostrum intake	I	49.4±5.2	24.0±2.5	2.7±0.7	4.6±1.2	7.0±2.3	11.0±2.6	10.3±3.7	34.7±14.4
	II	48.7±2.4	24.0±1.7	2.8±1.9	6.3±1.6	6.6±0.6	9.0±1.6	9.3±1.0	<4
72 hrs after colostrum intake	I	49.7±4.2	24.2±2.6	3.0±0.5	5.4±1.4	6.7±2.1	10.4±2.1	10.0±3.7	34.7±14.4
	II	48.0±2.5	23.7±1.6	2.9±1.7	6.2±1.6	6.7±0.5	8.5±2.0	8.9±1.2	<4
168 hrs after colostrum intake	I	48.7±5.2	24.4±3.0	3.0±0.6	4.8±0.9	7.2±2.1	9.3±1.0	8.9±3.1	34.7±14.4
	II	48.3±2.2	23.8±1.5	3.2±2.0	6.1±1.4	6.7±0.5	8.4±1.9	8.7±1.4	<4

Immunoglobulin G was present in traces in foal blood, sampled immediately after birth (<1.08 g/L), but 24 hours after onset of nursing a significant increase was revealed ( $10.4 \pm 2.9$  g/L and  $9.3 \pm 0.9$  g/L) in the first and in the second group of foals, respectively.

The antibody titre to EHV-1 was low <1:2 before colostrum intake in both foal groups, but 24 hours after colostrum intake increased significantly in foals delivered by vaccinated mares, i.e. the arithmetic average of reciprocal titre value was  $34.7 \pm 14.4$  ( $p < 0.001$ ).

Semi-quantitative field test (Horse Ig One-step, EVL) showed values which approximately matched the values obtained by different analytic methods (immunoturbidimetry), although values which were obtained by the quick test were lower than the values established by immunoturbidimetry in 16.67% of tested samples.

Table 3. Results of the semiquantitative immunoglobulin detection test in comparison to immunoturbidimetry analysis in foals originating from vaccinated (I) and unvaccinated (II) mares

Group I	SQ	IgG	Group II	SQ	IgG
1	P	11.2	7	P	8.2
2	P	8.2	8	P	13.2
3	WP	9.7	9	WP	9.1
4	P	8.8	10	WP	8.0
5	P	9.0	11	P	8.5
6	P	9.1	12	P	15.5

P – positive (>8 g/L Ig); WP – weak positive (5-8 g/L Ig); N – negative (<5 g/L Ig); SQ – semiquantitative test

## DISCUSSION

The evaluation of colostrum immunoglobulin transfer in Yugoslav Trotter newborn foals confirmed the results obtained by other authors (Bauer and Brooks, 1990; Curardi and Orlandi, 1999; Riond *et al.*, 2009). The content of serum proteins fractions in mares immediately after foaling is in accordance with data from the literature. The  $\gamma$ -globulin concentration established in all pregnant mares before foaling was 23% of total proteins and correlated to the finding of Curardi *et al.* (2007) who revealed almost identical values in Thoroughbreds, while Riond *et al.* (2009) established significantly lower values. Higher gammaglobulin and total protein values immediately after parturition were established by Curardi *et al.* (2007), while Scotoni *et al.* (1992) and Kohn *et al.* (1989) described even higher IgG values in pregnant mares. However, the results obtained by other authors clearly underline the correlation between gammaglobulin and IgG concentration in mare serum (the correlation  $r=0.8724$  was noted in our study). Antibody titre in vaccinated mares in our investigation was  $85.3 \pm 44$  and corresponded to the data

published by Burrows *et al.*, (1984). Although the newborn foals are considered to be gamma-globulin free, the traces of gammaglobulins were found in the blood of all tested foals before taking colostrum. Gammaglobulin traces in foal serum at birth can be attributed to intrauterine production. Foals are immunocompetent at birth, but unless placental leakage of maternal immunoglobulins occurs, they are born without circulatory gammaglobulins. This can theoretically be accepted under assumption that the foetus is located in a completely sterile environment during intrauterine development, without any contact with microbial antigens, a fact that is not realistic in biological systems. Traces of immunoglobulins in newborn foal blood before colostrum intake have been documented by different authors (Curadi *et al.*, 2007; Kohn, 1991; Massey *et al.*, 1992; Lee *et al.*, 1992).

Colostrum immunoglobulin absorption is mostly efficient during the first 12 to 24 hours (Curadi *et al.*, 2007) after birth. Absorption of intact colostrum immunoglobulin molecules is possible both due to the low gastrointestinal activity of proteolytic enzymes immediately after birth and to the presence of trypsin inhibitors in the colostrum. The IgG concentration reaches maximal levels after 24 hours, causing a significant increase ( $p < 0.05$ ) of total protein serum values in both foal groups. The values established in foals delivered by vaccinated mares were higher, but the obtained difference was non-significant.

The gammaglobulin and IgG concentrations in seven days old Yugoslav Trotter foals were lower than in foals of other breeds (Curadi *et al.*, 2007), but the difference cannot be considered clinically important, especially because the tested foals did not have any health problems which could be related to hypogammaglobulinaemia. It is difficult to define which amount of immunoglobulin in the foal serum would provide adequate protection, because there are many other factors that influence the onset of diseases. The side factors influencing foal health include welfare, feeding and nursing conditions. The IgG concentration of 4 g/L should provide sufficient protection from septicaemia, and concentration of 8 g/L should reduce the risk of most infective diseases (Giguere and Polkes, 2005). The values obtained in our study were higher than those reported in the literature, which also confirms the conclusion that the transfer of colostrum immunoglobulins was satisfactory in tested foals.

It is well documented that feeding calves and piglets with clinoptilolite (Stojić *et al.*, 1995; 1998) and mannan oligosaccharides (Lazarevic *et al.*, 2010) during the first 24 hours of life can enhance IgG absorption leading to better protection. However, this phenomenon has yet to be confirmed in other mammalian species.

Antibody titres in both foal groups showed that foals do not have specific serum antibodies to EHV-1 at birth. These results can also confirm the fact that the epithelio-horial placenta of mares does not allow the transfer of antibodies to the foetus. After 24 hours the values significantly increase in foals delivered by vaccinated mares as the average reciprocal titre value was  $34.7 \pm 14.4$ . These results clarify that vaccination of pregnant mares allows efficient transfer of specific antibodies against EHV-1 to the foal via colostrum, thus providing specific antibody titre to remain within the protective range (Burrows *et al.*, 1984). On the other hand the work of Foote *et al.*, (2003, 2004, and 2006) revealed the

appearance of infection in foals occurring during the postnatal weeks regardless of the implemented vaccination of pregnant mares. These studies showed that the presence of maternal antibodies alone cannot absolutely guarantee protection of foals from EHV-1.

Early discovery of newborn foals with insufficient transfer of colostral immunoglobulins represent the key moment for efficient therapy and prevention of life threatening complications, such as septicaemia. The data concerning the reliability of particular diagnostic parameters are therefore very important for the estimation of immunoglobulin status, especially when the determination test(s) can be applied in field conditions. Several authors favoured different methods for the determination of gamma globulin and immunoglobulin G concentrations in the blood including fast semi-quantitative tests underlying the clinical applicability of the results due to the high coefficient of correlation between the gammaglobulin and immunoglobulin G concentrations, and at the same time clarifying that the correlation between either gammaglobulin or IgG concentration and total protein concentration is low (Sugiura, 1997). Analyses like electrophoresis, radioimmunological methods, radiodiffusion test (RID), even indirect estimation of immunoglobulin concentration based on spectrophotometric examination of total protein and albumin concentration used to measure the presence of immunoglobulins in the blood are often unpractical and non-performable in the field and are feasible only under laboratory conditions. RID test gives results after 4 hours and this period is too long to enable efficient treatment i.e. feeding colostrum from other mares in cases of failure of passive transfer (FPT). Therefore, semi-quantitative fast tests are suitable for field diagnostics because they enable a rough estimation of immunoglobulin status in just ten minutes, as well as the test of serum dimming by zinc phosphate also applied on the field by other authors (Giguere and Polkes, 2005; Watson, 2009; Sedlinska *et al.*, 2005). Our evaluation confirmed the diagnostic value and applicability of semi-quantitative testing in field conditions.

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**UPOREDNA ISPITIVANJE KONCENTRACIJE POJEDINIHI FRAKCIJA SERUMSKIH  
PROTEINA I IMUNOGLOBULINA KLASI G U KRVNOM SERUMU KOBILA I  
NOVOROĐENE ŽDREBADI JUGOSLOVENSKOG KASAČA**

LAUŠ S, TRAILOVIĆ RUŽICA, ĐOKOVIĆ S, LAZAREVIĆ M I TRAILOVIĆ D

SADRŽAJ

Ispitivanja su sprovedena na 12 kobila rase jugoslovenski kasač i njihovih 12 ždrebadi. Prva grupa od šest kobila je trokratno vakcinisana protiv EHV-1/4 tokom graviditeta, dok je druga grupa bila nevakcinisana. Od svih kobila su neposredno posle ždrebljenja uzeti uzorci krvi za biohemijski pregled. Novorođena ždrebada su klinički opservirana tokom prvih 7 dana života, uz uzimanje uzoraka krvi pre uzimanja kolostruma, zatim nakon 24, 48, 72 i 168 časova.

Kod svih kobila su utvrđene nešto niže vrednosti gama globulina i IgG od vrednosti koje su zabeležene kod drugih rasa, pri čemu su vrednosti titra antitela na EHV-1 kod vakcinisanih kobila bile na nivou koji pruža zadovoljavajuću zaštitu, za razliku od nevakcinisanih kobila koje su bile ili seronegativne ili imale minimalan titar anti EHV-1 antitela. Kod većine ždrebadi je neposredno posle rođenja a pre prvog napoja u krvnom serumu ustanovljeno prisustvo gama globulina i IgG u tragovima, pri čemu je statistički značajno povećanje i gama globulina i IgG ustanovljeno posle prvih 24 časa. Primenom semikvantitativnog testa za određivanje IgG kod 25% ždrebadi utvrđene su niže vrednosti IgG (slabo pozitivne - što ukazuje na vrednosti od 5-8 g/L), iako je koncentracija IgG utvrđena imunoturbidimetrijom i kod njih bila veća od 8 g/L. Pre prvog unosa kolostruma u serumu ždrebadi nema antitela protiv EHV-1.