Immunoglobulin isotypes in sera and nasal mucosal secretions and their neonatal transfer and distribution in horses

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Objective—To determine concentrations of IgA and IgG subclasses in serum, colostrum, milk, and nasal wash samples of adult horses and foals.

Animals—Seven 2-year-old Welsh ponies, 27 adult mixed-breed horses, and 5 Quarter Horse mares and their foals.

Procedure—Serum was obtained from ponies and adult horses. Colostrum and milk were obtained from mares and serum and nasal wash samples from their foals immediately after parturition and on days 1, 7, 14, 28, 42, and 63. Nasal wash samples were also obtained from 23 adult horses. Concentrations of immunoglobulins were determined by use of inhibition ELISA. To determine transfer of maternal isotypes to foals, concentrations in colostrum and milk were compared with those in foal serum. Serum half-lives of isotypes in foals were also determined.

Results—IgGb was the most abundant isotype in serum and colostrum from adult horses, whereas IgA was the predominant isotype in milk. The major isotype in nasal secretions of adult horses and foals ≥ 28 days old was IgA, but IgGa and IgGb were the major isotypes in nasal secretions of foals ≤ 14 days old. Serum half lives of IgGa, IgGb, IgGa(T), and IgA in foals were 17.6, 32, 21, and 3.4 days, respectively.

Conclusions and Clinical Relevance—The early immunoglobulin repertoire of neonatal foals comprised IgGa, IgGa(T), and IgA; endogenous synthesis of IgGb could not be detected until 63 days after birth. The restricted repertoire of immunoglobulins in foals may influence humoral immune responses to vaccination. (Am J Vet Res 2000;61:1099–1105)
foals were clinically normal during the entire study. Serum, colostrum, and milk samples were stored at –20 °C for later determination of immunoglobulin isotype concentrations by use of inhibition ELISA.

Nasal mucosal secretions were collected from 3 of the 5 foals before suckling (day 0) and on days 1 (24 hours after suckling for the first time), 7, 14, 28, 42, and 63 after birth and from 23 mixed-breed adult horses as described elsewhere. Briefly, 50 ml of sterile PBS solution (PH 7.2) was instilled into the nares, and the fluid that drained out was collected, centrifuged at 500 × g for 30 minutes to remove particulate material, and saturated to 45% with ammonium sulfate (PH 7.6). Precipitated proteins were separated by centrifugation (30,000 × g for 30 minutes), redissolved in 1 ml of PBSS, and stored at –70 °C for later determination of immunoglobulin isotype concentrations by use of inhibition ELISA.

Purification of IgA and IgG subclasses for use in inhibition ELISA—Specific mAb against equine IgG subisotypes (CVS48, IgGa-specific; CVS39, IgGb-specific; CVS52, IgGc-specific; and CVS38, IgG[T]-specific) and equine IgA (BVS2) were used. Each mAb was coupled to synthetic hydrophilic polymer beads as described. Horse serum (approx 5 ml) from a mixed-breed horse was passed through each column. Bound immunoglobulin isotypes were eluted with 50 mM glycine hydrochloride (pH 2.5) and neutralized immediately with 3 M Tris buffer. After concentration and equilibration by dialysis against PBSS, concentrations of purified IgA and IgG subclasses were determined by use of a bicinchoninic acid protein assay reagent. Known concentrations of an equine IgG standard were used to produce a standard curve. Purity of IgA and the IgG subclasses was verified by use of an indirect ELISA. Briefly, separate wells of 96-well ELISA plates were coated with the purified IgA and each IgG subclass at a concentration of 0.1 µg/well, and the isotypes were tested for their ability to bind each isotype-specific mAb. Purified isotypes were stored at –20 °C until used in the inhibition ELISA.

Inhibition ELISA—Concentrations of IgA and IgG subclasses in serum, colostrum, milk, and nasal wash samples were measured by use of a modification of the inhibition ELISA described elsewhere. Briefly, wells of separate flexible 96-well flat bottom polystyrene microtiter ELISA plates were coated with 100 µl of an optimal concentration of purified IgA, IgGa, IgGb, IgGc, and IgG(T) diluted in 0.1 M carbonate-bicarbonate buffer (pH 9.2). Optimal coating concentrations as determined by use of a checkerboard ELISA were as follows: IgGa, 0.3 µg/ml; IgGb, 0.3 µg/ml; IgGc(T), 0.3 µg/ml; IgGc, 0.15 µg/ml; and IgA, 0.15 µg/ml. Coated plates were incubated overnight at 4 °C and washed 3 times with PBSS containing 0.05% Tween 20 (PBSS-T). The uncoated sites of the wells were blocked by addition of 100 µl of blocking solution (2% nonfat dry milk powder in PBSS-T) to each well for 1 hour at 37 °C. After decanting the blocking solution and washing the plates 3 times with PBSS-T, 100 µl of purified IgGa (20 µg/ml), IgGb (20 µg/ml), IgGc (25 µg/ml), IgGc(T) (22 µg/ml), and IgA (44 µg/ml) were added to duplicate wells in the first column of plates coated with the respective isotype and then serially diluted 2-fold in PBSS-T across the rows, leaving a final volume of 50 µl/well. One hundred microliters of each serum, colostrum, or milk sample diluted 1:100 in PBSS-T or nasal wash sample diluted 1:10 in PBSS-T was added to duplicate wells in the first column of each of the 5 plates coated separately with IgGa, IgGb, IgGc, IgGc(T), and IgA and serially diluted across rows. Fifty microliters of the appropriate mAb at the optimum dilution was quickly added to each well. Optimum dilution of a mAb was defined as that dilution that resulted in an absorbance at the mid-point of the slope of the titration curve and were as follows: mAb CVS48 (anti-IgGa), 1:1.25; mAb CVS39 (anti-IgGb), 1:6.25; mAb CVS52 (anti-IgGc), 1:6.25; mAb CVS38 (anti-IgG[T]), 1:6.25; and mAb BVS2 (anti-IgA), 1:500.

After addition of mAb, plates were incubated for 1 hour at 37 °C and washed 3 times with PBSS-T. One hundred microliters of peroxidase-conjugated goat antimouse IgG diluted 1:1,000 in PBSS-T was added to each well, and plates were incubated for 1 hr at 37 °C. After a final wash, 100 µl of substrate consisting of 0.07% orthophenylene diamine and 0.05% hydrogen peroxide in citric acid-phosphate buffer (pH 5.0) was added to each well. The reaction was stopped after 5 (all isotypes except IgGc) or 10 (IgGc) minutes by addition of 50 µl of 3 M sulfuric acid to each well. Absorbance at 490 nm was read in an automated microplate reader. The optical density (OD) values of wells containing known amounts of a particular isotype were used to generate a standard curve for that isotype. Concentrations (mg/ml) of IgA and IgG subclasses in each sample were determined by choosing wells for which OD values were within the linear region of standard curves and calculated from the graphs of standard curves by multiplying the observed values in chosen wells by the appropriate dilution factor. The configuration of the ELISA allowed 3 samples to be assayed per plate together with the isotype required to generate a standard curve. Plate to plate variations were controlled by measuring only 1 isotype per plate and plotting a standard curve for the isotype on that plate.

Determination of half-lives of immunoglobulins in foal serum—To calculate half-lives of immunoglobulin isotypes, mean concentrations of immunoglobulins in foal serum were analyzed by use of a commercially available software program. This program determines half-life by linear regression of the log-linear portion of the serum immunoglobulin isotype concentration versus time curve.

Statistical analyses—Concentrations of immunoglobulin isotypes in serum samples from horses and ponies and in nasal wash samples of horses were compared by use of a Friedman 2-way ANOVA by ranks. When results of the Friedman test resulted in rejection of the null hypothesis, all possible differences among isotypes were compared by use of a multiple comparison procedure used with the Friedman test to control the experiment-wide error to α = 0.05. Concentrations of immunoglobulin isotypes in colostrum, milk, foal serum, and nasal wash samples were compared on days 0, 1, 7, 14, 28, and 63 by analyzing the blocked data for individual horses by use of a 2-way ANOVA. This procedure was also used to compare concentrations of immunoglobulin isotypes in nasal wash samples between foals and horses. Concentrations of immunoglobulin isotypes in foal serum samples were compared across days.

To stabilize variability, a natural logarithmic transformation was used for data compared by use of parametric tests. Values of P < 0.05 were considered significant.

Table 1—Mean (± SD) serum concentrations (mg/ml) of immunoglobulin isotypes in seven 2-year-old Welsh Ponies and 27 adult mixed-breed horses

<table>
<thead>
<tr>
<th>Group</th>
<th>IgGa</th>
<th>IgGb</th>
<th>IgGc</th>
<th>IgG[T]</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pony</td>
<td>2.3 ± 0.8*</td>
<td>19.2 ± 5.2*</td>
<td>0.17 ± 0.07*</td>
<td>3.3 ± 1.5*</td>
<td>ND</td>
</tr>
<tr>
<td>Horse</td>
<td>3.4 ± 2.0</td>
<td>19.6 ± 6.5</td>
<td>0.2 ± 0.1</td>
<td>4.0 ± 2.5</td>
<td>0.4 ± 0.3*</td>
</tr>
</tbody>
</table>

*Within a row, values with different superscripts are significantly (P < 0.05) different.

ND = Not determined.
Table 2—Mean (± SD) concentrations (mg/ml) of immunoglobulin isotypes in colostrum and milk of 5 Quarter Horse mares after parturition

<table>
<thead>
<tr>
<th>Day after parturition</th>
<th>IgGa</th>
<th>IgGb</th>
<th>IgGc</th>
<th>IgG(T)</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>82.0 ± 44.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>183.0 ± 38.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3 ± 0.10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>44.0 ± 25.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.0 ± 3.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>1.6 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.0 ± 1.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.6 ± 0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8.6 ± 0.62&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.0 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>0.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>0.3 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.7 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>0.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5 ± 0.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>0.2 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.7 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>28</td>
<td>0.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>0.1 ± 0.08&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.7 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>42</td>
<td>0.1 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>0.08 ± 0.08&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>63</td>
<td>0.08 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1 ± 0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>0.06 ± 0.04&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.6 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Colostrum collected immediately after parturition and before the foal had suckled.
<sup>ND</sup> = Not detectable.
<sup>*</sup>Within a row, values with different superscripts are significantly (P < 0.05) different.
<sup>c</sup>Within a column, values with different superscripts are significantly (P < 0.05) different.

Results

Immunoglobulin isotype concentrations in serum of adult ponies and horses—Immunoglobulin Gb was the predominant, and IgGc the least abundant, IgG subclass in all serum samples (Table 1). The concentration of IgGb was significantly higher than the concentrations of all other isotypes. Concentrations of IgGa and IgG(T) were also significantly higher than those of IgGc and IgA. However, concentrations of IgGa and IgG(T) in both ponies and horses and IgGc and IgA in horses were not significantly different. Concentrations of IgA were not measured in serum samples from ponies.

Immunoglobulin isotype concentrations in colostrum and milk—Concentrations of all immunoglobulin isotypes in colostrum collected on day 0 differed significantly (Table 2). Mean (± SD) concentrations of IgGa, IgGb, IgG(T), and IgA in day-0 colostrum were several times higher than concentrations in serum obtained 45 days before parturition (IgGa, 5.4 ± 2.0 mg/ml; IgGb, 23.5 ± 5.4 mg/ml; IgGc, 0.05 ± 0.01 mg/ml; IgG(T), 7.4 ± 2.4 mg/ml; IgA, 0.8 ± 0.6 mg ml). This difference was also suggested by low mean isotype serum-to-colostral concentration ratios (IgGa, 0.06; IgGb, 0.12; IgGc, 0.16; IgG(T), 0.17; IgA, 0.18). By day 1, IgGc was detected in the colostrum of only 3 of 5 mares; IgGc was not detected in colostrum collected at later days from any mare. Concentrations of each isotype on day 1 differed significantly from concentrations of all other isotypes. Immunoglobulin Gb was the predominant isotype in colostrum samples from all mares. The concentration of IgGb in milk did not differ significantly from that of IgGa on days 7, 14, 28, 42, and 63, and from that of IgA on days 7 and 14. The difference between concentrations of IgA and other isotypes (excluding IgGc, which was not detectable) was not significant on day 7 but was significant on day 28. On and after day 28, IgA became the predominant isotype in milk.

Concentrations of IgGa, IgGb, and IgG(T) in colostrum decreased by 43- to 75-, 40- to 91-, and 42- to 107-fold, respectively, from day 0 to 1. Decrease in concentrations of these subclasses in colostrum and

Table 3—Mean (± SD) serum concentrations (mg/ml) of immunoglobulin isotypes in 5 Quarter Horse foals

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>IgGa</th>
<th>IgGb</th>
<th>IgGc</th>
<th>IgG(T)</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.03 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>10.0 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.0 ± 3.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>4.8 ± 2.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>7.3 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.0 ± 6.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>3.8 ± 2.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>6.5 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.2 ± 6.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>2.8 ± 1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.06 ± 0.04&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>28</td>
<td>3.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.5 ± 4.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>2.0 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.06 ± 0.004&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>42</td>
<td>4.94 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.0 ± 4.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>1.4 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08 ± 0.004&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>63</td>
<td>14.3 ± 5.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.5 ± 3.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>3.8 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Serum obtained immediately after birth and before the foal had suckled.
<sup>ND</sup> = Not detectable.
<sup>*</sup>Within a row, values with different superscripts are significantly (P < 0.05) different.
<sup>c</sup>Within a column, values with different superscripts are significantly (P < 0.05) different.
Immunoglobulin Gc was not detected in serum samples from any foal at any time. We did not detect IgGc in serum samples from foals. Immunoglobulin G(T) was detected at low concentrations in samples from foals until day 14 after birth, and IgGc was not detected at any time; IgG(T) and IgGc were not detected in samples from horses.

Concentrations of IgG subclasses in nasal wash samples from 1-day-old foals were significantly higher than concentrations in samples from horses. Concentration of IgGb in nasal wash samples from 7-day-old foals also differed significantly from that in samples from horses. However, IgGa and IgG(T) concentrations in nasal wash samples from 7-day-old foals did not differ significantly from concentrations in horses. Also, IgGa and IgG(T) concentrations on days 14, 42, and 63 in foals did not differ significantly from concentrations in horses. In foals, IgGb and IgGa were the predominant isotypes in nasal wash samples until 14 days after birth. Immunoglobulin A could not be detected in nasal wash samples for the first 14 days, but on day 28, IgA was the most abundant isotype. The concentration of IgA in nasal wash samples from 28-day-old foals was significantly lower than that in samples from horses, but concentrations in foal samples on days 42 and 63 did not differ from those in horse samples.

Serum half-lives of immunoglobulins in foals—Serum half-lives of IgGa, IgGb, IgG(T), and IgA in foals were 17.6, 32.0, 21.0, and 3.4 days, respectively. The half-life of IgGc could not be calculated, because we did not detect IgGc in serum samples from foals.

### Discussion

The mAb-based ELISA that we used in the present study to measure immunoglobulin isotype concentrations was highly specific and theoretically more sensitive than radial immunodiffusion (RID) assays used previously. We found that IgGb constitutes > 60% and IgGc < 1% of total serum IgG concentrations in adult horses and ponies. The low serum concentrations of

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**Table 4—Mean (± SD) concentrations (mg/ml) of immunoglobulin isotypes in nasal wash samples from 3 Quarter Horse foals and 23 adult mixed-breed horses**

<table>
<thead>
<tr>
<th>Group</th>
<th>Isotype concentration (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foals (age in d)</td>
<td>IgGa</td>
</tr>
<tr>
<td>0*</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>(0.00–0.05)</td>
</tr>
<tr>
<td>7</td>
<td>(0.09–0.10)</td>
</tr>
<tr>
<td>14</td>
<td>(0.00–0.20)</td>
</tr>
<tr>
<td>28</td>
<td>ND</td>
</tr>
<tr>
<td>42</td>
<td>(0.00–0.04)</td>
</tr>
<tr>
<td>63</td>
<td>(0.02–1.0)</td>
</tr>
<tr>
<td>Horses</td>
<td>(0.00–0.02)</td>
</tr>
</tbody>
</table>

*Values within a row with different superscripts are significantly (P < 0.05) different.

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milk was more gradual after day 1. In contrast, concentrations of IgA decreased 23- to 38-fold from day 0 to day 1 and remained constant after day 7. In mare serum obtained 45 days before parturition, the concentration of IgG(T) was higher than that of IgGa, but concentrations of IgG(T) in colostrum and milk were always lower than those of IgGa (Table 2). Serum concentration of IgGa was less than a fourth that of IgGb. In contrast, IgGa concentrations in colostrum collected on days 0 and 1 were half those of IgGb.

Concentrations of immunoglobulin isotypes in foal serum—IgGa was the only immunoglobulin isotype detected in sera of 3 foals immediately after birth (Table 3). Concentrations of IgG subclasses and IgA peaked on day 1 and decreased thereafter. Comparison of immunoglobulin isotype concentrations measured on the same day revealed significant differences, except for the concentration of IgGb on day 42: at that time, its concentration did not differ from that of IgGa. Serum concentrations of IgGa decreased from day 1 until day 28 but then increased significantly on days 42 and 63. On the other hand, IgGb concentrations continued to decrease from day 1 until day 63, and IgG(T) concentration decreased until day 42 but then increased significantly on day 63. Serum IgA concentration decreased until day 28, increased slightly but not significantly on day 42, and increased significantly from day 42 to 63. Immunoglobulin Gc was not detected in serum samples from any foal at any time.

**Immunoglobulin isotype concentrations in nasal wash samples**—Comparison of the IgG subclasses measured on the same day in nasal wash samples from foals indicated that concentrations of IgGa and IgGb were significantly higher than concentrations of IgG(T); concentration of IgGa was never significantly different from that of IgGb (Table 4). Concentration of IgGa in nasal wash samples from horses also never differed significantly from that of IgGb. Immunoglobulin G(T) was detected at low concentrations in samples from foals until day 14 after birth, and IgGc was not detected at any time; IgG(T) and IgGc were not detected in samples from horses.

Concentrations of IgG subclasses in nasal wash samples from 28-day-old foals was more gradual after day 1. In contrast, concentrations of IgA decreased 23- to 38-fold from day 0 to day 1 and remained constant after day 7. In mare serum obtained 45 days before parturition, the concentration of IgG(T) was higher than that of IgGa, but concentrations of IgG(T) in colostrum and milk were always lower than those of IgGa (Table 2). Serum concentration of IgGa was less than a fourth that of IgGb. In contrast, IgGa concentrations in colostrum collected on days 0 and 1 were half those of IgGb.
IgGc that we detected, together with the low concentrations of this IgG subclass in response to infection with Streptococcus equi and equine influenza virus, and the absence of complement binding suggest that IgGc is the least important IgG subclass in protective humoral immune responses. Immunoglobulin Gb is shown to be the dominant isotype in foal serum with the longest half-life. This, together with its known importance in protective immune responses, suggests that IgGb is the most important IgG subclass in equine protective humoral immune responses. The ratio of serum IgGa to serum IgG(T) concentration varied greatly among ponies and horses. In some, IgGa was present at higher concentrations than IgG(T), whereas in others, the converse was true. Our results indicate that IgGb and IgA are the dominant isotypes in colostrum and milk, respectively. As expected, we also found that IgA was the major isotype in nasal secretions of adult horses and foals ≥28 days old. However, IgGa and IgGb were the major isotypes in nasal secretions of foals until 14 days after birth. We did not determine IgM concentrations.

Because equine IgGa, IgGb, and IgGc are classified on the basis of increasing anodal mobility during electrophoresis, the corresponding mAbs used in the present study were designated accordingly. However, commercial RID kits, used for quantitating IgGa and IgGb, classify equine IgG subclasses on the basis of serum concentration. Screening of commercial affinity-purified peroxidase-conjugated antisera specific for the heavy chains of IgGa, IgGb, IgGc, and IgG(T) with our mAb affinity-purified IgGa, IgGb, IgGc, and IgG(T) revealed that IgG(T) measured by use of the RID kit is actually IgGc.

It must be emphasized that breed, age, sex, and herd management may influence serum immunoglobulin isotype concentration. Moreover, the values may also be affected by the different assay methods used to determine isotype concentrations. The serum concentrations of IgG(T) that we determined for ponies in the present study (2.1 to 6.4 mg/ml) were similar to values published by Kent and Blackmore, Paton et al., and Rouse but lower than values reported by McGuire and Crawford. Serum IgG(T) concentrations in horses in the present study (1.0 to 9.6 mg/ml) were higher than values reported for Thoroughbreds (0.7 to 4 mg/ml) reported by Rouse but similar to values in Thoroughbreds reported by Kent and Blackmore and in Quarter Horses reported by McGuire and Crawford. However, Kent and Blackmore also reported high concentrations of serum IgG(T) (23.3 mg/ml) for 1 group of horses of unspecified breed. Serum IgG(T) concentration has been shown to increase in response to infection with the nematode Strongylus vulgaris. The combined serum IgGa, IgGb, and IgGc concentrations for horses (13 to 53 mg/ml) and ponies (18 to 35 mg/ml) in the present study were higher than total IgG concentrations reported earlier for Thoroughbreds and Quarter Horses (7 to 20 mg/ml), whereas IgA concentrations (0.1 to 1.3 mg/ml) were lower than values reported by others (0.6 to 3.5 mg/ml, 1.5 to 4.8 mg/ml, and 0.8 to 3 mg/ml).

It has been suggested that breed, age of the dam, body weight, lactation number, yield of colostrum, and herd management may influence colostral immunoglobulin isotypes; the method used to quantitate isotypes may also affect values measured. Colostral IgA concentrations determined in the present study agree with a previous value of 9 mg/ml in an unspecified breed but not with the range reported for Quarter Horses (1.5 to 3 mg/ml). However, in the latter study, concentrations of colostral immunoglobulin isotypes may have been inaccurate, because colostral IgGa, IgGb, IgGc (6.6 to 10.8 mg/ml), and IgG(T) (1.9 to 3.5 mg/ml) at the time of parturition were less than serum concentrations. Total colostral IgG concentrations in the present study (205 to 398 mg/ml) were higher than values reported for Arabian horses (108 mg/ml), heavy draft breeds (64 mg/ml), Shetland Ponies (40 to 85 mg/ml), Thoroughbreds (23 mg/ml and 46 mg/ml), and Quarter Horses (8.5 to 14.2 mg/ml).

Receptors on mammary epithelial cells of ruminants may be involved in uptake of serum IgG1, and it has been suggested that these receptors are specific for the Fc portion of the molecule. Immunoglobulin G1 is the principal isotype in ruminant milk, and serum is considered the chief source, although IgG1 can be synthesized in vitro by cultured mammary gland tissue. Receptor-mediated uptake of immunoglobulin isotypes by mammary gland cells of swine has not been studied, but it has been shown that almost all colostral IgG and as much as 40% of colostral IgA in swine are of serum origin. Immunoglobulin A is found at higher concentrations than IgG in swine milk and is believed to be of local origin. We found that concentration of IgA increased and that of IgG decreased in mare's milk during the first 28 days after parturition, suggesting that lacteal IgA is locally produced, and colostral IgG is derived from serum. This finding confirms results of previous studies that indicate IgA is the dominant immunoglobulin isotype in equine milk.

As expected, and in agreement with results of earlier studies, concentrations of all immunoglobulin isotypes (except IgGc) in equine colostrum were several times higher than serum concentrations. However, these results are in contrast to results of a previous study, which indicated that concentrations of colostral IgGa, IgGb, IgGc, and IgG(T) in Quarter Horses on day 0 (immediately after parturition) are less than serum concentrations measured at the same time. In the present study, concentrations of IgGa, IgGb, and IgG(T) in colostrum decreased more than 40-fold from day 0 to day 1. In contrast, IgG in mammary secretions of swine and Holstein cattle decreased by only 7- and 4-fold, respectively, from day 0 to day 1. This suggests that mares may be unique in that transfer of immunoglobulins from serum to mammary secretions ceases at or possibly before parturition. A finding by Rouse and Ingram also supports this hypothesis; mammary secretions of nursing mares changed from colostrum to milk in <12 hours. Rouse and Ingram
also noticed a 20-fold decrease in IgG concentrations in mammary secretions of Shetland Ponies between 0 to 3 hours and 9 to 24 hours after parturition.

In the present study, concentration of IgA in mare serum was 4 times less than that of IgGb, whereas in day-0 (immediately after parturition) colostrum, IgGa concentration was only 2 times less than that of IgGb. This suggests that IgGa is selectively transferred from serum to colostrum. However, this selective transfer is not as great as that observed for IgG1 in cows.35 In serum to colostrum. However, this selective transfer is not as great as that observed for IgG1 in cows.35 In cows, IgG1 and IgG2 are present at nearly equal concentrations in serum, but in colostrum, the concentration of IgG1 is 16-fold greater than that of IgG2. The selective transfer of IgGa in mares was also supported by the observation that IgGa concentration, although approximately 1.5 times less than IgG(T) concentration in serum, was approximately 2 times greater than IgG(T) concentration in day-0 colostrum.

During the first few weeks of life, we detected high concentrations of IgGa and IgGb in nasal secretions of foals; however, we did not detect IgA in nasal secretions until day 28. This finding suggests that during the first few weeks of life, maternally derived IgG subclasses protect the nasal mucosal surfaces of foals. Concentrations of IgA in nasal secretions of 42- and 63-day-old foals did not differ significantly from those in adult horses, suggesting that foals ≥ 42 days old, similar to adult horses, are able to synthesize and secrete IgA into nasal secretions.

Unlike serum IgG, we detected little or no transport of IgA from serum to nasal mucosa of foals, because serum concentration of IgA was low. The ratios of IgGa, IgGb, IgG(T), and IgA concentration in foal serum at day 1 to concentrations in colostrum at day 0 were 0.12, 0.16, 0.11, and 0.04, respectively. This suggests that intestinal uptake of IgA into sera of foals following ingestion of day-0 colostrum may be minimal, presumably because the monomeric form of IgA, which lacks secretory component, is preferentially absorbed in contrast to the polymeric forms, which accumulate in the intestinal crypts as described in 1-week-old piglets.9 Previous results indicating that radiolabeled IgA from milk is absorbed and translocated into the nasopharyngeal mucosa within 2 hours of ingestion may be explained by contamination of the IgA preparation with radiolabeled IgG.40 Absorption of colostral IgGc could not be determined in the present study, because colostral concentrations were extremely low.

Immunoglobulin isotype responses of equine fetuses have not been studied in detail, but the finding of restricted immunoglobulin isotype repertoires during the first weeks of life in this study supports previous indications of prenatal immunoglobulin synthesis.41-43 The significant increase in serum IgGa concentration detected on day 42, as well as the increase of IgG(T) and IgA on day 63, compared with day-0 values, suggests that the immune system of foals responds to immunogens in the first 42 days after birth, and this response does not involve IgGb, an important protective immunoglobulin. Interestingly, high concentrations of IgGa, another protective immunoglobulin, probably compensate for the lack of IgGb. The presence of IgGa in serum samples obtained from 3 of 5 foals before they had nursed suggests that the fetus also produces this isotype. Low concentrations of IgG (possibly IgGa) and IgM have been detected in newborn foals.44

The restricted immunoglobulin isotype repertoire of foals suggests either that isotype expression is under developmental control based on the proximity of the heavy chain C region gene segment to the heavy chain variable region gene segments, or the immunoglobulin response may be biased in 1 direction (eg, T-helper 1 vs T-helper 2 response). In a previous study,4 IgG(T) synthesis could not be detected before 1.2 weeks of age in Thoroughbred foals and 7 weeks of age in pony foals. Moreover, IgG synthesis did not increase until 11 and 20 weeks of age in pony and Thoroughbred foals, respectively. Results of the present study indicate either that IgGb synthesis is delayed in foals until after 63 days of birth, or synthesis is insufficient to offset the slow catabolism of maternal IgGb. The persistence of maternally derived IgGb in foal serum suggests that this IgG subclass is of greatest clinical importance in interfering with immune responses to vaccination during this time.45

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