The purpose of Veterinary Immunogenics Ltd is to promote all the benefits of immunoprophylaxis and immunotherapy and to be established as the premier producer of the finest animal plasma in the world.

It succeeds in this by adopting and implementing current best practice, using state of the art equipment, in the production of blood products in total compliance with the EU regulations for the manufacture, storage and supply of Veterinary Medicinal Products to the Certificated standard of Good Manufacturing Practice (GMP). It supports specialist meetings for equine veterinary surgeons in both the UK and Europe and has formed links with fellow professionals in many countries in Europe and elsewhere in the world. Above all, it continues to embrace the highest standards of customer service in product supply and technical advice in product use.
**A Review of Plasma in Equine Practice**

The original primary use of equine plasma was to transfer immunity from adult horses to foals with Failure of Passive Transfer which became accepted practice following Jeffcott’s work, originally published in 1974. While this is probably still the main use of plasma in foals, there are a number of additional indications for plasma to be considered as part of a preventive or treatment strategy in horses of different ages:

1. Treatment of FPT
2. Use in septic foals
3. Transfer of specific antibody
4. Miscellaneous: colic
   - Colitis
   - intraoperative lavage
   - reproduction.

**TREATMENT of FAILURE OF PASSIVE TRANSFER - HYPERMUNE**

**Introduction**

All foals are born with a deficiency of humoral antibody (1). They rely on the adequate intake of good quality colostrum within a few hours of birth to supply significant amounts of IgG and IgG(T) and lesser amounts of IgM and other immunoglobulin classes to provide significant transient protection against infectious agents (2,3,4,6). Unfortunately, this transfer of antibodies from dam to offspring does not always occur successfully due to various factors such as low quantity or poor quality in maiden mares, aged mares losing the ability to concentrate IgG in colostrum or running colostrum before parturition, aggressive mothering and interference with absorption in the foal. This results in “failure of passive transfer” (FPT), as depicted by foal serum gammaglobulin levels (usually specifically IgG) of less than 4g/l. If this deficiency is confirmed before the gut’s ability to absorb antibodies ceases (from 12 hours of age), oral supplementation of good quality colostrum or plasma may well rectify the situation. If, on the other hand, the foal is over 12 hours old when this deficiency is diagnosed, the foal has to be considered at risk to infection, especially in a “high challenge” environment. In such circumstances transfusion of plasma should be considered, the benefits of which are well documented (7,8).

**The Practical Use of HYPERMUNE**

The main use for equine plasma is as a source of equine IgG when used prophylactically in foals with failure of passive transfer or partial failure of passive transfer. The IgG molecule is a Y shaped structure of linked polypeptide chains. Each of the two branches of the Y is the Fab fragment because it has the ability to bind antigen, while the “body” of the Y is called the Fc fragment, which has the ability to bind to receptors on the surface of innate immune cells. This part is crucial in recruiting phagocytes to engulf and kill pathogens, and activating the
complement cascade, leading to disruption of cell membranes and destruction of invading microorganisms. It is from this background that Hypermune is harvested and stored to ensure these desirable properties of IgG are preserved. Although there are a number of beneficial proteins in equine plasma, IgG has always been the focus of attention because not only is it of major immune importance but it is also relatively easy to measure semi-quantitatively in foal blood due to the availability of commercial test kits such as Gamma-Check-E or quantitatively using a validated test in an approved laboratory.

The veterinary application of equine plasma for the treatment of FPT in foals is not new, having been recommended for over 20 years in text books (12,13,14), conference proceedings (15), satellite article (16), practice tip (13), and a manual of equine neonatal medicine (17). In the United States, where commercial equine plasma usage has been well established for much longer than in the United Kingdom, the whole matter was reviewed in 1994 (18), with the conclusion that the process of plasmapheresis has made the use of equine plasma safe and efficacious. The review states that research with equine plasma has shown that treatment of disease or protection against disease is in proportion to the amount of IgG in the plasma. It also summarises one of the earliest studies on equine plasma in 1984, which saw 119 transfusions being made in a variety of equine disease states including FPT and septicaemia.

In the United Kingdom, two publications by Durham (16) and Stoneham (11), advocate that the administration of plasma to a foal can be a life saving procedure and that plasma administration has important applications in the therapeutic and prophylactic management of conditions in equine practice.

Indications for Plasma Transfusion

Equine plasma transfusion is indicated where a foal of 12-18 hours old or more has been diagnosed as having inadequate circulating gammaglobulin levels. The definition of “inadequate” is open to some degree of interpretation and dependent on several factors (8,9). Generally, veterinary medical standards (and often some insurance company requirements) now dictate that foals be checked for IgG within the first day or so of life and those showing a deficiency given some form of supplementary antibodies.

In the treatment of Failure of Passive Transfer, the administration of plasma should be based on the accurate IgG level of the foal. It is, therefore, extremely important to employ a test which provides a meaningful result. On receipt of the result, the following considerations should be borne in mind:

- IgG< 2g/l Strong predisposition to development of infection
- IgG<2-4g/l Increased risk of developing infection
- IgG 4-8g/l Moderate transfer, but risk of developing infection remains especially in a high challenge environment.
- IgG > 8g/l  Good to excellent transfer, no action needed

IgG is the immunoglobulin isotype found in the highest concentration in blood and plays a major role in antibody mediated defence mechanisms. Because of its size, it can escape from blood vessels more easily than other immunoglobulin molecules thus readily participating in the defence of tissue spaces and body surfaces. IgG can opsonise, agglutinate and precipitate antigen but it can activate the complement cascade only if sufficient molecules have accumulated in a correct configuration on the antigen surface.
A 50kg foal has a plasma volume of approximately four litres and therefore any transfused antibodies will be immediately distributed within this volume. The administration of one litre of plasma containing 24g IgG, Hypermune’s minimum level, will initially raise the recipient’s blood level by 6g/l (24 divided by 4). However, within 24 hours following transfusion there is some movement out of the circulation and only about 50% remains in the vascular system after 24 hours. It is important to wait approximately 24 hours before measuring the IgG level after transfusion. Following transfusion of one litre of plasma containing 24g IgG, the foal’s circulating level will be increased, therefore, by about 3g/l (10). By accurately knowing the initial level and the desired final level the amount of plasma to be administered can be calculated. This may be provided by infusing up to four litres of plasma from a normal resting horse but in a foal this would cause serious volume overload problems. The design of Hypermune equine plasma is to overcome this problem by providing a greater concentration of IgG per litre using hyperimmunised horses.

The dose of Hypermune is **20ml/kg body weight**. Following plasma transfusion, often a second sample is tested for IgG status. Interpretation of the result depends on a number of factors, such as:

1. **How soon the sample is taken following transfusion?**
2. **Has the foal an active septic process in which IgG is consumed?**
3. **The dilution factor effect on the transfused IgG in the foals circulating blood volume.**
4. **The speed which IgG leaves the vascular system.**

Failure of Passive Transfer may, therefore, also require more than one administration of Hypermune to sustain satisfactory levels of foal serum IgG. The results of a 2003 study (Figure 1) suggest that FPT foals may benefit from a second litre at 3-4 weeks old to close the window of susceptibility to septic challenge, concurring with the literature that foal serum IgG increases naturally only after 8-10 weeks of age (19).

**Figure 1:** IgG levels of 4 foals pre- and post- Hypermune transfusion; 2003 Field Study.
Table 1: Customer feedback

<table>
<thead>
<tr>
<th>Foal Identity</th>
<th>Before Plasma</th>
<th>After Plasma</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age in Hours</td>
<td>IgG level</td>
<td>Age in hours</td>
</tr>
<tr>
<td>13/6-5</td>
<td>24</td>
<td>4-8</td>
<td>72</td>
</tr>
<tr>
<td>6/6</td>
<td>24</td>
<td>&lt;4</td>
<td>72</td>
</tr>
<tr>
<td>4/6-2</td>
<td>24</td>
<td>4</td>
<td>96</td>
</tr>
<tr>
<td>31/5-5</td>
<td>24</td>
<td>&lt;4</td>
<td>48</td>
</tr>
<tr>
<td>21/5-8</td>
<td>24</td>
<td>&lt;4</td>
<td>72</td>
</tr>
<tr>
<td>3/5-3</td>
<td>24</td>
<td>4-8</td>
<td>48</td>
</tr>
<tr>
<td>8/3-6</td>
<td>24</td>
<td>&lt;4</td>
<td>120</td>
</tr>
<tr>
<td>12/2</td>
<td>24-48</td>
<td>4-8</td>
<td>96</td>
</tr>
</tbody>
</table>

References
In the presence of sepsis, immunoglobulins are rapidly consumed whether derived from colostral transfer or plasma, resulting in a shortened half-life. Plasma proteins provided by transfusion normally have a half-life similar to autologous proteins, which in the case of immunoglobulins is about 21 days. In the presence of infection, the half-life might be as low as a few hours and a plasma transfusion might not appear to give the expected increase in IgG when given to a sick foal. It needs to be emphasised that the sick foal would have had a lower IgG if plasma had not been given. To keep IgG levels sustained in the face of rapid consumption, severely compromised foals can require a number of litres over a few days to prevent volume overload.

Scientific evidence in recent years supports the use of equine plasma in the treatment of septic foals (2, 5, 6). The opsonic ability of foal serum has been found a limiting factor for the phagocytosis of pathogens in foals up to the age of 3 – 4 weeks (2, 3, 4). Phagocytic activity has been found to increase when mixed with adult plasma (1, 2). This was associated with greater opsonic factors provided by adult plasma, with fibronectin and complement being suggested as possible opsonic contributors. Improved oxidative burst activity of neutrophils has also been recorded in septic foals post transfusion of plasma (5). This suggests that specific and non-specific factors in plasma in some way promote white cell activity.

A retrospective study, covering 65 septicaemia cases, found the administration of plasma was significantly associated with foal survival (7). On this evidence, it is recommended that plasma is administered as
soon as septicaemia is suspected or confirmed in the neonatal foal. At the Foal Care Course in Newmarket in January 2008, John Madigan emphasised that in his clinic, all septic foals are given a litre of plasma at the outset, irrespective of IgG status. Hypermune should therefore be a prime consideration as part of the treatment protocols when faced with septicaemia in the foal.

References


SPECIFIC Antibody Transfer

**Rhodococcus equi** Pneumonia and Hyperimmune-RE

*R. equi* infection represents a significant cause of disease in foals between 1 and 6 months of age producing chronic bronchopneumonia with extensive abscessation, which is often fatal. Much research over the past two decades has focused on immunity to this pathogen (both in the immune adult and susceptible foal), and developing preventive strategies, such as Hyperimmune plasma, to provide passive transfer of specific antibodies. Some researchers have reported a reduction in *R. equi* related morbidity and mortality as a consequence of plasma administration (1, 2, 3, 4, 5). Currently, development of a protective vaccine has been unsuccessful.

Attendance at the 4th Havemeyer Workshop on *R. equi* in Edinburgh in July 2008, enhanced Veterinary Immunogenics Ltd’s current knowledge on organismal biology, pathogenesis, immunology, clinical aspects and epidemiology of this disease. A recurrent debate between scientists in the field concerning the age at which young foals are infected with *R. equi* was apparent. The old, yet accepted paradigm suggests that foals can become infected at anytime throughout the first 1 – 6 months of age, and is often linked to the wane of maternal antibodies. It was argued that this age range is when foals develop clinical signs, not when most infections are initiated. Some researchers have provided evidence that most foals are actually infected within the first few days of life (6), which may mean it will be very difficult to develop a primary vaccination strategy to protect foals within the first week of life from *R. equi* disease. It was accepted however that some foals can become infected at a more advanced age, as do some adults, which is related to the level of environmental challenge in paddocks, and more likely in the stables and horse walkers of breeding farms in temperate climates such as Ireland and the UK (7, 8, 9, 10).

There was general concern at the meeting on the use of antibiotics, such as Azithromycin and Erythromycin, as a chemoprophylactic tool, for fear of developing multi-drug resistant virulent strains of this pathogen. The use of hyperimmune plasma as an immunoprophylactic tool was still generally supported. Data collected over 4 years from an endemic farm, and presented as a poster at the workshop, clearly showed a reduced incidence of disease in foals that received Hyperimmune-RE (average 10% with disease), compared to control foals (average 50% with disease) (11).

A further observation made at the workshop was the timing of transfusing a second litre of hyperimmune plasma. It is current practise to transfuse a second dose approximately 21 days after the first, which is administered at birth. Data, also presented by the previous referenced author, revealed a sharp drop in antibody levels in foals between 21 and 28 days of age (11). A large field study conducted on an endemic farm by Veterinary Immunogenics Ltd in 2006 found foals seroconverted at 21 days of age (Figure 2), which was thought to indicate environmental challenge. It was evident that
control foals seroconverted at a faster rate than either of the treatments groups, but an endogenous production of antibodies does not necessarily correlate with clinical infection or indeed protection (22). It was therefore discussed that it may be beneficial to reduce the interval between the first and second transfusion of Hypermune-RE to prevent a sharp drop in antibody and other immune components in the face of environmental challenge.

![Graph](image)

**Figure 2:** Mean anti-\(R.\ equi\) antibody levels in foals used in Hypermune-RE efficacy study 2006, \(n = 90\). Plasma transfused at 1 and 21 days of age (12).

**Endotoxaemia and Hypermune**

Endotoxaemia is a life-threatening condition that is associated with many gastrointestinal diseases in adult horses and septicaemia in foals. Successful treatment of endotoxaemia relies on preventing movement of gram-negative endotoxins into general circulation, neutralising endotoxins before they react with inflammatory cells, and preventing synthesis and release of inflammatory mediators (13). The neutralisation of endotoxins through the use of anti-endotoxin (lipopolysaccharide, LPS) antibodies in equine plasma has been found successful, relating to an increased survival rate and reduction in time to recovery (14, 15, 16, 17). Since circulating endotoxins and infectious agents are an important risk factor for the development of laminitis, the use of hyperimmune plasma for treatment of endotoxaemia and septicaemia is advised to help prevent laminitis in horses during hospitalisation (18).

Veterinary Immunogenics Ltd produced a plasma product called Hypermune-J from donors hyperimmunised with a gram-negative core antigen vaccine for treatment of endotoxaemia. A specific ELISA was used to assess the level of gram negative endotoxin antibodies in Hypermune products and the general adult UK horse population.

Clinical signs of endotoxaemia are related to the levels of circulating endotoxin. Low levels (0.03ug/kg) produce symptoms such as mild depression with clinical "silence" but changes in the blood, medium levels (10 ug/kg) are clinically "noisy" and high levels (125ug/kg) will cause death.
Figure 3 demonstrates the comparable level of anti-endotoxin antibodies in all Hypermune products, which is significantly greater than levels found within the general horse population (P=<0.001). Recent publications have reported that ordinary equine plasma provides a measurable benefit in terms of prolonged opsonisation and improved neutrophil function when given to septic foals (19, 20). In addition, this was endorsed at Rossdales Foal Care Course in January 2008 when it was stated that all septic foals are given 1 litre of plasma irrespective of IgG status (21). On the basis of evidence supporting the use of existing licensed Hypermune products and the difficulties in complying with current regulatory demands to license additional Hypermune products as “new” Veterinary Medicinal Products, reluctantly the production of Hypermune-J was ceased. Veterinary Immunogenics Ltd now advocates the use of Hypermune or Hypermune-RE for endotoxaemia as part of an overall treatment strategy.

![Figure 3: Comparison of anti-endotoxin antibodies in Hypermune products and the general UK adult horse population at 1/300 plasma dilution.](image)

### References


Intraoperative Lavage

Hyperimmune plasma has been reported to reduce the adhesion of bacteria to bone surfaces, compared to Plasmalyte™ (6). Hypermune could therefore be useful as an Intraoperative lavage solution to decrease post-op infections.

MISCELLANEOUS Plasma Use

Colic

In addition to immunoglobulins, plasma provides a source of many other non-specific proteins, such as coagulation factors, anti-thrombin III and albumin, to maintain oncotic pressure (1), which is important in cases of hypoproteinaemia and hypovolaemia. Hyperimmune plasma has been reported to help reduce abdominal inflammation and minimise the effects of endotoxaemia associated with gastrointestinal adhesions (2). Hypermune is frequently used in large equine hospitals in the UK for post-operative management of colic.

Colitis and Diarrhoea

Colitis, whether associated with gram-negative bacteria in the large intestine (Salmonella spp) or gram positive bacteria (Clostridium spp), or antibiotic use, is frequently coupled with diarrhoea and subsequent hypoproteinaemia and hypovolaemia. Plasma provides many benefits, but is primarily used intravenously for colloidal support. Plasma is also the treatment of choice if protein loss is accompanied by coagulation disorders (3). Although intravenous transfusion of plasma is more commonly used, there is evidence, especially in the human literature, that oral administration of plasma can effectively minimise causative agents of colitis and diarrhoea, such as Clostridium spp, Candida spp, E. Coli and Rotavirus (4). Hyperimmune plasma has also been reported to decrease the time to resolution of diarrhoea (5).
Reproduction

Bacterial infections in the uterus are recognised as a major cause of reproductive failure in mares (7). The use of plasma transfusion therapy to improve fertility has been studied and discussed (13). Intrauterine administration of hyperimmune plasma was found to increase pregnancy rates in susceptible mares by reducing uterine infections caused by common bacterial strains, such as *Streptococcus zooepidemicus* (8,9,10). The mechanism by which this occurred was associated with plasma enhancing the phagocytic activity of uterine neutrophils (9,14). An earlier study also showed considerable promise of intrauterine infusions of plasma through successfully breeding many barren mares (11), though some authors have suggested that plasma may only be beneficial in mares that do not possess mechanical clearance problems (14,15). Some authors debated whether the efficacy of plasma exceeded the efforts required to collect, store and administer plasma (8), but the availability of commercial Hypermune now provides reliable access to high quality and safe plasma. The use of Hypermune for the management and treatment of endometritis could be considered to try to improve reproductive rates and possibly prevent the development of bacterial resistance associated with repeated antibiotic administration.

A further use for Hypermune in equine breeding is for the prevention of Neonatal Isoerythrolysis. If a mare is at risk of transferring anti-red cell antibodies via colostrum to the foal, hyperimmune plasma should be considered as an immunological passive transfer replacement (12). This method could allow breeders to continue using mares associated with Neonatal Isoerythrolysis.

References


Safety and Quality of Hypermune Products

Hypermune equine plasma is designed to be:

- Red cell group compatible with recipient horses
- Produced by a consistently repeatable process
- Free from endotoxins
- Free from external contaminants
- Free from important named viruses
- Free from known pathogens
- Consistent with normal fresh equine plasma
- More consistent in its immunoglobulin and total protein content than plasma from the foal’s dam or a random donor, specifically IgG ≥ 24g/l
- More value in its IgG content due to VIL’s vaccine protocols than plasma from the foal’s dam or a random donor
- Immediately available
- Free from excess citrate

In addition Hypermune-RE contains high levels of specific antibodies to European strains of pathogenic *R. equi*.

Avoiding Problems with Frozen Hypermune Products

Plasma Considerations:
Adoption of instructions on product label and package insert

Receiving, CAREFULLY handling and placing in dedicated frozen storage at -25°C (± 5°C).

Selecting, removing from storage and DILIGENTLY thawing at not greater than 40°C.

Slowly warming thoroughly to body temperature.

Using the correct blood giving set with large filter.

Foal Considerations:

Between birth and 1 month the sympathetic nervous system matures dramatically (Table 3). Until then, the immature baro receptor reflexes mean that there is an inherent degree of haemodynamic instability and neonates do not tolerate sudden or large changes in blood pressure or volume very well. It is therefore essential to evaluate carefully the physiological state of the foal, in each and every situation, prior to administration of plasma. Consultation of breeding records together with a thorough clinical examination is good veterinary practice.
All the safety studies carried out on Hypermune products relate to the administration to normal full term foals. Extra care should be taken when plasma is to be administered to foals out with this category. In particular it should be assessed:

- If the foal is premature <320 days or
- Dysmature >320 days with signs of immaturity
- Or normal and full term, and that a full term foal is not a miniature adult

Table 2: Physiological Parameters of the Developing Neonatal Foal

<table>
<thead>
<tr>
<th>Age</th>
<th>Heart rate (beats/min)</th>
<th>Respiratory Rate (breaths/min)</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>60-80</td>
<td>Gasping</td>
<td>37-39</td>
</tr>
<tr>
<td>0-2 hours</td>
<td>120 - 150</td>
<td>40 - 60</td>
<td>37-39</td>
</tr>
<tr>
<td>12 hours</td>
<td>80 - 120</td>
<td>30 - 40</td>
<td>37-39</td>
</tr>
<tr>
<td>24 hours</td>
<td>80 - 100</td>
<td>30-35</td>
<td>37-39</td>
</tr>
</tbody>
</table>

RERAINT and the SEDATION of FOALS

Foals up to 1 month of age can usually be restrained manually by a single person. Veterinary Immunogenics Ltd adopts the advice of leading equine veterinary surgeons and advises that chemical restraint should be considered only in extreme circumstances and then only using drugs with minimal side effects on cardiovascular and respiratory systems.

Chemical Restraint

Alpha 2 adrenoreceptor agonists (Xylazine, Detomidine, Romifidine)

It should be borne in mind that foals have inherent dynamic instability and their cardiac output is heart rate dependent. Since these agents cause marked changes in haemodynamic variables, including bradycardia and reduced cardiac output, they must be used with caution especially in neonatal, hypovolaemic or sick foals.

Benzodiazepines (Diazepam - Vallium)

These drugs are particularly useful because of their minimal side effects on the cardiovascular and respiratory systems.
CONTACT DETAILS

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For further information, updated regularly throughout the foaling season, please visit:

www.veterinaryimmunogenics.com

Hypermune Products are shipped Monday to Thursday for overnight delivery in most cases, but national holidays, weather and traffic problems may interfere with this prompt and efficient service. It is wise, therefore, to hold some plasma in the practice freezer especially during the anticipated period of use. (Hypermune products have a proven frozen shelf life of two years from the date of production.)

Orders may be placed by fax using the Faxback order form which can be downloaded from the website or by telephone or by email.

Please note that in order to comply with Good Manufacturing Practice, Veterinary Immunogenics Ltd has to adopt a NON RETURNS POLICY, and thus will NOT accept return of any unused product under any circumstances.