INTRODUCTION

As part of fulfilling its purpose of promoting all the benefits of immunoprophylaxis and immunotherapy Veterinary Immunogenics Ltd (VIL) is pleased to produce this second edition of HYPERMUNE-REview. The lead article is by Professor Michelle Henry Barton, DVM, PhD, Diplomate ACVIM, and is reproduced by her kind permission and by Carey Ross, American Association of Equine Practitioners (AAEP) 4075 Iron Works Parkway, Lexington, KY 40511 who first published it in AAEP Proceedings Focus Meeting in 2008.

VIL is always pleased to receive any comments or feedback on this publication and any queries regarding its plasma products HYPERMUNE and HYPERMUNE-RE.
What’s Got Them Covered? Innate Immunity and Passive Transfer of IgG

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Introduction

A key player in the ultimate battle between microbial colonization and establishment of infection is the innate immune system. Unlike adaptive immunity, which provides targeted defense against specific antigens that is magnified with each subsequent exposure, the innate immune system is comprised of components that provide unconditional defense, and therefore relies on immediate and less specific recognition of invading microbes. Neonatal foals are capable of initiating directed and specific responses against microbes, however, it is the lag in the adaptive response that makes foals particularly susceptible to infection and more dependent on the innate immune system, compared to adult horses. The innate immune system entwines several redundant levels of protection including the macroenvironment of physiologic barriers, the microscopic protection furnished by phagocytes, as well as molecular defense. A key player in molecular defense is immunoglobulin. Immunoglobulins traditionally are a component of the adaptive immune system, and thus a specifically targeted immunoglobulin response by lymphocytes and plasma cells takes days to weeks to successfully launch. Thus along with the innate immune system, the foal is dependent on the nonspecific molecular defense provided by immunoglobulin that is passively transferred via ingestion of colostrum during the immediate postnatal period. The main purposes of this session are to discuss the principal components of the innate immune system that are operative in the neonatal period and the importance of passive transfer of colostral immunoglobulin.

Components of the Innate Immune System

Physical Barriers

The skin deters bacterial invasion by providing a physical barrier between the environment and deeper host tissue, in the form of host cells and normal flora. Other important components of resistance to colonization of the skin are an acidic pH, secretion of fatty acids, and the ability to “shed” cells. Like the skin, the mucosal surfaces of the gastrointestinal, respiratory, and genitourinary tracts provide a physical cell barrier that can desquamate, the presence of normal flora, and abundant mucus that prevents colonization. The respiratory tract contains numerous
“filters” that can trap bacteria that is inhaled, such as the turbinates, the long trachea, and ciliated respiratory tract lining cells. Mucosal epithelial cells in the gastrointestinal, respiratory, and genitourinary tracts secrete molecules, such as defensins and collectins that cause bacteriolysis and increase opsonization by phagocytes. Additional chemical defenses in the gastrointestinal tract include peroxidases, the acidic pH of the stomach, proteolytic enzymes, and lysozyme, all substances that can challenge bacterial cell wall integrity. Finally, physiologic activities such as sneezing, coughing, salivation, and peristalsis can also serve to remove potential pathogens.

Although innate immunity tactics are present within the gastrointestinal tract of the neonatal foal, the fact that the single most commonly reported pathogen identified in equine neonatal septicemia is Escherichia coli has lead to the speculation that the gastrointestinal tract is the primary route of microbial invasion of the neonatal foal. Indeed, although other species that absorb colostral immunoglobulin G do so via specific molecular receptors on their enterocytes, the process of immunoglobulin absorption in foals in much less specific and occurs by pinocytosis. Again, considering the incidence of E. coli septicemia in foals, it also has been speculated that microbes may concomitantly gain access internally during pinocytosis of immunoglobulins.

**Phagocytic Cells**

Phagocytes, principally neutrophils, monocytes, and tissue-fixed macrophages, play a key role in innate immunity. In addition to engulfing and destroying bacteria via various oxidative, acidifying, and enzymatic mechanisms, neutrophils and monocytes secrete numerous substances, such as cytokines, growth factors, lactoferrin, and interferon, that can induce chemotaxis, enhance phagocytosis, cause bacteriolysis, or inhibit microbial replication. Although it has been shown that equine neutrophils are functional at birth, compared to neutrophils from adult horses, phagocytosis, oxidative burst activity, and killing are reduced in the first 1 to 2 weeks of life. These deficiencies are further exacerbated by failure of passive transfer, as IgG and complement are needed for optimal phagocytosis of bacteria. Clearly these deficiencies in phagocytic activity in the newborn foal increase the susceptibility to microbial invasion in the perinatal period.

**Molecular Defense**

Lastly, the innate immune system is comprised of an extensive array of soluble molecules in the circulation, including pathogen recognition receptors, cytokines, chemokines, immunoglobulins, acute phase proteins, and other pro-inflammatory and anti-inflammatory mediators. Many of these molecular components are also present on cell surfaces, intracellularly, and on mucosal surfaces. Their overlapping presence in more than one tissue, the dynamic discovery of new molecules and rediscovery of new roles for previously identified molecules confuses their classification. A main feature of the innate immune system that enables immediate discrimination is pattern-recognition receptors (PRRs) that are capable of detecting a variety of microbial ligands, referred to as pathogen-associated molecular patterns or PAMPs. These ligands are evolutionally conserved molecules that are unique to microbes, are often shared by a broad range of organisms, and are usually essential for microbial survival or
virulence. PRRs may be present on host cell membranes, in the circulation, or on mucosal surfaces and they may be constitutively expressed and/or are released or secreted during the acute response to microbial invasion. Examples of PAMPs include bacterial cell wall extracts, such as endotoxin, peptidoglycan and lipoteichoic acid, and prokaryotic DNA. There is a complex and overlapping arsenal of PRRs, examples including the family of toll like receptors that recognize bacterial endotoxin, cationic antimicrobial peptides, such as defensins; and some of the acute phase proteins. Ultimately, the interaction of a PRR with its PAMP can result in direct neutralization of the PAMP or microbe, or it may activate other components of the host immune system to deploy further defense mechanisms, initiate an inflammatory response, or commence tissue repair.\textsuperscript{1,2}

**Passive Transfer of Immunoglobulin G**

Although immunoglobulins are classically linked to adaptive immunity and anamnestic defense, they are a critical component for opsonization and the immediate defense against bacteria. The unique placental attachment in horses prevents placental transfer of immunoglobulins during gestation, thus foals are born agammaglobulinemic.\textsuperscript{7} Colostrum provides an immediate source of immunoglobulins and failure of acquisition or absorption of colostral antibodies or failure of passive transfer (FPT) in the first day of life has been well recognized as a major risk for infection.\textsuperscript{8-10} The fact that some foals with adequate serum IgG still acquire infection and that some foals with inadequate serum IgG do not acquire infection in the perinatal period\textsuperscript{11} supports the notion that the likelihood of infection is a combination between the risks of exposure and colonization, counterbalanced by the ability to defend from invasion. Regardless of inadequate serum IgG concentration, if exposure to microbes is minimized, then intuitively, infection is less likely to occur. Furthermore, IgG is not the only factor available to the foal for immediate defense against microbes, thus despite failure of passive transfer, other components of the immune system may provide sufficient protection.

The mare mobilizes antibodies to the mammary gland in the last month of pregnancy. The concentration of immunoglobulin varies considerably between mares but good quality colostrum typically has an IgG concentration of at least 3000 mg/dl (specific gravity of $>1.060$ on an equine colostrometer, $>20\%$ on a Brix refractometer).\textsuperscript{12} Foals should consume 1 to 2 liters of colostrum within the first 12 hours of life. Because absorption rapidly falls to less than 25\% efficiency by 3 hours of age, foals should be encouraged to nurse as soon after birth as possible.\textsuperscript{7}

It has been stated that the incidence of failure of passive transfer ranges from 3 to 40\%.\textsuperscript{13} Many risk factors including fescue toxicity, premature lactation, and illness during pregnancy result in low colostral antibody production. Separation of the mare and foal at parturition, weakness, other illness, or musculoskeletal problems that result in failure of the foal to stand and/or nurse, or failure to absorb ingested antibodies (i.e. prematurity or dysmaturity) can contribute to the development of failure of passive transfer.

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**GAMMA-CHECK-E:**

Is an inexpensive rapid foal side semi quantitative screening test for the detection of equine gammaglobulin (IgG) in whole blood or serum. There are six tests in a kit. See page 18.
Diagnosis of Failure of Passive Transfer

The time of peak serum concentration of IgG is between 18 to 24 hours of age and thus this is the ideal time frame in which to test foals for failure of passive transfer. Although somewhat arbitrary, the cut off value most frequently suggested for diagnosis of failure of passive transfer is 800 mg IgG/dl. Healthy foals will often have greater than 2,400 mg IgG/dl. There are several methods for determining the serum IgG concentration. Single radial immunodiffusion is considered the most accurate method of quantifying IgG; however, the inherent flaw of this test is 24 hour assay time. Other tests that are used to estimate serum IgG include total protein concentration, zinc sulfate turbidity test, glutaraldehyde coagulation test, and stall side enzyme linked immunosorbent assays. In foals, refractometer measurements of total protein do not correlate well with failure of passive transfer. However, serum biochemical determination of serum total protein concentration can be somewhat reliable in that a serum total protein concentration < 5.0 g/dl has good sensitivity for diagnosis of FPT in that few foals that truly have FPT will be missed. However, serum total protein has very poor specificity and therefore many foals with adequate transfer will be falsely diagnosed with failure of passive transfer. The zinc sulphate test works on the premise that salt will precipitate the IgG and increase sample turbidity. The test has very good sensitivity but low specificity. The gluteraldehyde coagulation test has a similar efficacy as the zinc sulfate test. Stall side quantitative and semiquantitative enzyme linked immunosorbent tests have the advantage of using whole blood, plasma or serum for testing and has the best overall combination of sensitivity and specificity.

Treatment of Failure of Passive Transfer

When failure of passive transfer is identified, the next plan of action will depend on the health of the foal and the risk for sepsis, weight against the cost and risks of treatment. The rapid decline in absorption of antibodies in the first few hours of life often precludes the oral treatment route by the time failure of passive transfer is identified diagnostically. However, if agalactia is the problem or the foal is orphaned at birth, equine colostrum can be used, when available. A total volume of 1 to 2 liters of colostrum divided into hourly feedings of 200-400 ml should suffice if the colostrum is of good quality and it can be given within the first 6 to 12 hours of life. If equine colostrum is not
available, bovine colostrum can be used, though the circulating half life of bovine IgG is only 9 days in the foal (versus 26 days for equine IgG) and the specificity of bovine IgG against equine pathogens is limiting.\textsuperscript{17} Concentrated equine IgG is available as an oral product and when given to foals according to the label recommendation (two 150 ml doses given 2 hours apart within the first 4 hours of life), the mean serum IgG concentration was considerably lower than 800 mg/dl.\textsuperscript{18}

Any steps which the equine veterinary surgeon can take to improve the welfare of the equine neonate by decreasing the susceptibility to painful, debilitating and potentially fatal infectious disease such as septicaemia, joint-ill, navel-ill, diarrhoea and pneumonia must be given serious consideration.

Equine plasma provides that facility for the foal which has Failure of Passive Transfer of colostral immunity. The intravenous administration of equine plasma produced and marketed in compliance with national regulations as a Veterinary Medicinal Product is accepted and utilised in the United Kingdom and Europe.

For foals older than 12 hours, the most reliable method by which to increase plasma concentration of IgG is to give an equine plasma transfusion. There are several USDA licensed equine plasma products in the United States\textsuperscript{b,f,g} with guaranteed minimal IgG concentrations from donors tested for absence of anti-red blood cell antibodies. Although recommendations on the amount of plasma to be given have been suggested over the years, there is poor correlation between amount of plasma given and the final plasma IgG concentration in the recipient.\textsuperscript{13} However, in general, between 1-4 liters of plasma is recommended for a 50 kg foal. Frozen plasma should be thawed in warm water and not microwaved, and the plasma should be given through a blood administration filter. The first 50 ml of plasma should be given as a slow drip. If no adverse signs are seen, then the rate of administration may be increased to 20-40 ml/kg/hour. The plasma IgG should be rechecked in 12 to 24 hours to assure adequate IgG concentration. Plasma administration should be stopped if any of the following signs develop: fever, tachycardia, tachypnea, piloerection, urticaria, or colic.

Summary

The immune system is not fully developed at birth and thus the neonatal foal is at greater risk for acquiring infection than is the mature horse. The degree of immunocompetence in both the innate and adaptive immune systems versus the risk of pathogen exposure, colonization, and invasion is difficult to predict in any given foal. Failure of passive transfer of maternal colostral IgG receives much attention as a risk factor for neonatal sepsis and has merit based on the fact that phagocytosis of bacteria is enhanced with increasing serum concentrations of IgG. Early identification of foals with failure of passive transfer, in conjunction with risk assessment must be evaluated in individual foals to ascertain whether further intervention with IgG containing products or antimicrobials is warranted.
Permissions

References and Footnotes
 a. Equi-Z, TM VMRD, Pullman WA.
 b. Gamma-Check-E, TM Plasvacc, Templeton, CA.
 c. DVM Stat, TM VDX Inc. Belgium, WI.
 d. Snap Foal IgG,TM Idexx Laboratories, Westbrook, ME.
 e. Seramune, Sera Inc., Shawnee Mission, KS.
 f. Mg Biologics, Ames, IA.  g. Lake Immunogenics, Inc. Ontario, NY.
FOAL SEPTICAEMIA – EXPOSING THE SUBTLETIES OF ITS CLINICAL SIGNS

**Septicaemia** is a common cause of illness and death in the equine neonate. Unfortunately the preliminary signs of sepsis are vague and often are not easily recognised. Knowledge of normal foal behaviour and physical parameters is essential to identify septicaemia early. Indicators of normal health for foals are summarised in Table 1 and Table 2.

### Table 1

<table>
<thead>
<tr>
<th>From Birth to:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sternal Recumbency</td>
<td>1-2 minutes</td>
</tr>
<tr>
<td>Standing</td>
<td>Within 1-2 hours</td>
</tr>
<tr>
<td>Suck reflex</td>
<td>2-20 minutes</td>
</tr>
</tbody>
</table>
| Suckling                  | Within 2 hours then 4-7 times per hour
                              | Gets up to suckle if woken |
| Meconium                  | Within 4 hours |
| First Urination           | Within 9 hours |

### Table 2

<table>
<thead>
<tr>
<th>Normal Temperature</th>
<th>37.2-38.6°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99-101.5°F</td>
</tr>
<tr>
<td>Heart Rate: At birth</td>
<td>&gt;60 beats per minute</td>
</tr>
<tr>
<td>Heart Rate: From 6-60 minutes</td>
<td>80-130 beats per minute</td>
</tr>
<tr>
<td>Heart Rate: Day 1-5</td>
<td>80-120 beats per minute</td>
</tr>
<tr>
<td>Respiratory Rate after 1 hour old</td>
<td>30-40 per minute</td>
</tr>
</tbody>
</table>


**The Clinical Examination**

The clinical examination should focus on primary potential portals of entry of infection for signs of reaction to infection and indicators of movement of infection into adjacent areas. The primary sites of infection should include the skin, umbilicus, digestive, respiratory and urogenital tracts which should be carefully examined as potential sites of primary bacterial invasion.

**Digestive Tract.** Gram-negative enteric bacteria in general and specifically *Escherichia coli* are involved in the majority of cases of septicaemia in foals with often the gastrointestinal tract as the most common primary site of infection.

Diarrhoea is not normal in the first few days of life and may be the only important clue of colonization and invasion of the gastrointestinal tract. Teeth grinding, disinterest in suckling and signs of abdominal pain may also be signs that point to risk of bacterial movement from the gastrointestinal tract.
**Umbilicus.** External evidence of navel infection may not be grossly apparent from several days to one to two weeks after initial infection. Occasionally, despite significant infection of the umbilical vessels or urachus interior to the body wall, there will be no obvious evidence of disease in the external umbilical stump. Clinical signs consistent with infection of the umbilical remnants include heat, swelling, patency, pain of the umbilical stalk or discharge or moistness from or around the stalk.

**Respiratory System.** Surprisingly, even severe infection of the respiratory tract may not appear as obvious external clinical signs in neonatal foals. Often the only signs of respiratory-tract disease are the presence of unexplained rapid breathing with nasal flare or difficult breathing. Other localizing signs, such as nasal discharge, cough, or audible abnormalities, when present, are often significant.

**The foal's response** to sepsis can be highly variable, depending on the duration and intensity of the septic challenge. The initial response to infection should induce signs of decreased activity, malaise, increased periods of recumbancy, inability to follow the dam, decreased frequency of nursing and failure to gain weight.

All of these signs often are associated with the onset of fever. The recurring nature of increased body temperature necessitates ongoing monitoring or it may be missed. As the inflammatory response to infection intensifies, other signs of systemic disease appear such as increased heart rate, increased breathing rate, bilateral scleral injection, hyperaemia of the coronary bands, unpigmented skin and mucous membranes, petechial hemorrhages and oedema. Petechiae in the ears are a highly reliable indicator of sepsis in the foal and may develop as a result of either thrombocytopenia or inflamed blood vessels.

**The Spread of Septic Disease.** If the innate immune system is incapable of controlling the infection as septicaemia progresses, secondary sites of infection may develop. As the cardiovascular system is involved in the spread of septic disease all tissues of the body are susceptible to secondary infection. Tissues that receive a large portion of the cardiac output and experience turbulent, slow or unique blood supplies, are often involved first and include physes, synoviae, the uveal tract, meninges, endocardium, liver, kidney and skin/muscle.

Any neonatal foal that has joint swelling, lameness or prolonged recumbency should be carefully evaluated for sepsis. The cardinal signs of septicaemia inside the eye are uncontrolled blinking, epiphora, miosis, aqueal flare, oedema of the iris and hypopyon. These signs most often manifest bilaterally in septic foals, though unilateral presentation can occur.
Foals with meningitis often have an altered mental status, ataxia, seizures and a stiff, "guarded" neck and gait. Endocarditis is an infrequent complication of septicaemia in foals. Tachycardia, tachyarrhythmia, lethargy, murmurs, jugular pulsation and dependent edema may be signs of endocarditis.

Healthy neonatal foals commonly have a low-grade systolic murmur over the semilunar valves over the left heart base. However, loud murmurs over the semilunar valves, murmurs over the mitral or tricuspid valves or those that are accompanied by other signs of cardiac disease should be investigated further by echocardiography.

Hepatic, splenic and renal abscessation may occur secondary to septicemia, though secondary infection in these anatomic locations rarely cause localizing clinical signs.

When the systemic pro-inflammatory response to infection is uncontrolled and widespread, the clinical state of shock and Multiple Organ Dysfunction Syndrome (MODS) ensue.

Septic shock is defined as hypotension accompanied by signs of hypo-perfusion, such as altered mental status, hypothermia, hypotension, shivering, cold extremities, mucous membrane pallor, bradycardia or tachycardia, poor capillary or jugular vein refill, poor pulse quality, oliguria and ileus that is induced by the presence of sepsis, is the result of systemic vasodilation and persists, despite adequate fluid resuscitation.

The manifestations of MODS are vast and the signs reflect the organs that are predominantly affected. These may include mental deterioration, ataxia, seizures, oliguria, coagulopathy, dyspnea, tachypnea, tachycardia or bradycardia, colic and/or ileus. Ultimately, identification of clinical signs or physical findings of secondary infection, shock or MODS is significant. These conditions not only identify an advanced and improperly controlled disease state, but they also are associated with a poor prognosis.

Finally, when evaluating a neonatal foal for any reason, consider that any primary disease can induce a state of immunosuppression and/or cause loss of integrity of local protective barriers that subsequently welcomes infection. For example, other common diseases during the neonatal period, such as neonatal isoerythrolysis, neonatal encephalopathy, uroperitoneum, neonatal asphyxia syndrome, meconium impaction, diarrhoea or any cause of colic, may be complicated by concurrent infection.
Before considering plasma transfusion, carry out a full clinical examination with particular emphasis on cardiovascular and respiratory systems.

Consider stage of development:
- Premature <320 days
- Dysmature >320 days with signs of immaturity

A 50kg foal is not a miniature adult. In particular note that between birth and 1 month the sympathetic nervous system matures dramatically. Until then, the immature baroreceptor reflex (the body’s homeostatic mechanism for maintaining blood pressure) means 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.

**Expected clinical parameters in a normal foal:**

<table>
<thead>
<tr>
<th>Age</th>
<th>Heart rate (beats/min)</th>
<th>Respiratory Rate (breaths/min)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>&gt;60</td>
<td>Gasping</td>
<td>37.2-38.6°C 99-101.5°F</td>
</tr>
<tr>
<td>6-60 minutes</td>
<td>80 – 130</td>
<td>40 – 60</td>
<td>37.2-38.6°C 99-101.5°F</td>
</tr>
<tr>
<td>12 hours</td>
<td>80 – 120</td>
<td>30 - 40</td>
<td>37.2-38.6°C 99-101.5°F</td>
</tr>
<tr>
<td>24 hours</td>
<td>80 – 100</td>
<td>30-40</td>
<td>37.2-38.6°C 99-101.5°F</td>
</tr>
</tbody>
</table>


**RESTRAINT and the SEDATION of FOALS**

Foals up to 1 month of age can usually be restrained manually by a single person. Chemical restraint is to be avoided in equine neonates if at all possible. Consider only Benzodiazepines (Diazepam (Valium)) as these drugs are particularly useful because of their minimal side effects on the cardiovascular and respiratory systems.

**Preparing the Plasma for Administration.** (See page 17) Hypermune plasma products should be handled carefully when being unpacked and stored in the freezer. The bubble-wrap should not be removed as it protects the brittle frozen plastic which is susceptible to damage from careless handling such as being dropped or knocked in the freezer. When thawed it should be stored in a refrigerator and used within 24 hours.

Do not use after the expiry date stated on the label
The litre bag of plasma should be immersed only in warm water at not more than 40°C. A water bath such as a sink full of domestic warm water is ideal. As the plasma thaws and the water cools, more warm water may be added as required but hot water (i.e., greater than hand hot) must be avoided as it will denature the proteins. The entire litre of plasma should be brought slowly to body temperature, with frequent swirling of the bag before use to ensure all the cryoprecipitate is dissolved and plasma warmed thoroughly throughout. Under optimum conditions this whole process may take 2-2½ hours. Occasionally small amounts of fibrin may still be seen floating in the plasma. It is not significant but must be filtered out by the filter in the blood administration set. Inspect for leakage and if apparent on thawing the entire contents must be discarded.

The required dose, 20ml/kg body weight, is administered via a 16g catheter placed in the jugular vein using a blood giving set equipped with a mesh filter. The product should be administered slowly, particularly at the start and heart and respiration monitored closely. The administration should take a minimum of 15 – 20 minutes with constant monitoring throughout the administration for signs of adverse reactions.

Do not mix with any other medicinal products. It is recommended that no other immunological product other than Hypermune or Hypermune-RE be administered within 14 days following administration of Hypermune plasma apart from tetanus antitoxin which may be administered simultaneously but not intravenously.

Administration of more than two doses is not recommended.

If a second dose is required this should be administered after a minimum time interval of 24 hours from administration of the first litre.

**Plasma Administration:**
- Foals 24 hours to 6 days of age
- Blood giving set and 16 gauge catheter
- Thaw and warm plasma thoroughly (40°C)
- Dose 20ml/kg
- Start very slowly & diligently to monitor vital signs
- Administration should take at least 15 – 20 minutes
- Further dose may be given at not less than 24 hours
Transfusion Reactions. Careful monitoring, especially at the start and throughout the transfusion is essential. Distinction must be made between reaction to restraint and catheterisation and signs attributable to transfusion reaction.

Anaphylaxis. If tachycardia, hyperventilating or trembling occurs, the transfusion should be slowed down or stopped altogether. If these signs are severe, or other signs occur such as pyrexia, cardiac arrhythmias, urticaria and collapse, the transfusion should be stopped immediately and if necessary epinephrine (0.01mg/kg), corticosteroids and intravenous saline administered. These emergency drugs should always be on hand. Flunixin meglumine at 0.25mg/kg may be used prophylactically to reduce the incidence of side effects.

Volume Overload. Volume overload is a possible hazard of plasma transfusion especially if the administration is carried out too quickly. Careful monitoring throughout the transfusion is essential. If hyperventilation, respiratory distress or trembling occurs, the transfusion should be slowed down or stopped altogether. Diuretics may be used in severe cases.
It is very rare but not unknown for this company to receive reports of bags leaking plasma when thawing begins. The company’s response is to request the damaged bag be returned for inspection in an attempt to identify the cause of the problem. In 99.9% of cases this is due to the bag having been knocked, dropped or just cracked against the storage freezer or even another object or bag of plasma in the same freezer. The unique identity of the bag is also used to reveal the date of production and the date of supply and thus how long it has been stored on site and how long it has been stored in the customer’s facility.

Thousands of litres are handled on site by Veterinary Immunogenics and by its customers each year. Many customers use considerably large numbers of litres each season without experiencing any broken bags and do so by ensuring they have sufficient frozen storage space which can comfortably accommodate the volumes to be stored without having to cram or knock them when handling and storing and that all personnel using the bags are made aware of the need to handle the product with care. VIL’s personnel operate to written Standard Operating Procedures to ensure consistency in ensuring the bags are handled, packed and delivered in a way which eliminates the risks of accidental damage. In addition, in the very unlikely event that a bag is damaged before dispatch the slight thawing in transit would leak plasma and be seen to have spread under the bubble wrap and thus be visible on arrival.

It is paramount that all personnel handling, moving, unwrapping and thawing frozen plasma understand that frozen plastic is brittle and even two frozen bags being knocked together may fracture the plastic especially when retrieving plasma from storage for urgent use and particularly on the vulnerable corners.

A number of points should be considered when devising and adopting a protocol to store frozen plasma:

1. Is the bubble wrap kept in place when the bags are unpacked and moved from the shipping box to the freezer? **It is essential that it is kept in place.**

2. The plastic material used in the bags becomes increasingly brittle as the temperature decreases, especially once it is below −25°C. Is the storage freezer running excessively cold? It is particularly important not to keep even a domestic freezer on fast freeze all the time as the temperature may well drop to −30°C or even colder.
3. Is the storage freezer dedicated to storing only plasma? If not is the plasma held in a spacious dedicated container or area? This is important as whenever frozen plasma is moved about to find a particular bag, e.g. Hypermune-RE instead of Hypermune or something else in the same freezer the corners or spike ports are vulnerable to knocks even when bubble wrapped.

4. When removing the bags from storage for thawing and warming for use, personnel should familiarise themselves with the location of the ports to avoid knocking them and the corners, and should leave the bubble wrap in place for the initial stages of thawing. Once the bag softens the wrap can be removed without risk of damage to bag or ports.

For the very reason that Hypermune products are expensive because of the high production costs and regulatory compliance the company has produced a guide – PLASMA HANDLING a copy of which is included in this volume of HYPERMUNE-REview. This is to act as a source of information for new personnel and to act as a reminder for existing personnel particularly as it is a seasonal product being required in many equine practices for only a few months in the year.

If a broken bag is encountered the plasma need not go to waste. It can be used for oral supplementation in foals less than 8-10 hours of age where approximately 70% of the IgG is absorbed. Alternatively, one stud farm has reported its use for uterine irrigation of specific problem mares.
1. A veterinary surgeon reported that he had a foal of some 50kg with an IgG of 0.2g/l and he wondered if he should give it 2 litres of Hypermune straight away?

A similar question was received from two other vets. The company’s response is that the ideal is to administer 1 litre and then after approximately 24 hours administer the second litre. It is always emphasised that the main problem in giving two litres immediately, consecutively, is volume overload and therefore is to be avoided particularly bearing in mind the neonatal foals cardiovascular limitations and poor respiratory reserve.

Further discussion focussed on a more prudent approach by measuring the IgG using a validated test such as at Beaufort Cottage Laboratories and then making the decision regarding the second litre, taking into account the foal’s environment (considered to be low risk). It was stressed that if a second litre was not given it is still acceptable to give a second litre a week or two later if required.

In cases of very low IgG give 1 litre immediately and 1 later!

2. A veterinary surgeon phoned to express disappointment at measuring only a very small increase in IgG after transfusing 1 litre of Hypermune.

The company explained that each bag of plasma contained not less than 24g IgG (in most cases, considerably more) and that whatever the test result the foal had received a minimum and often more than 24g IgG. The possible explanations discussed were:

a) Dilution of the 24g by the foal’s circulating blood volume
b) Movement of IgG from the circulating blood.
c) Sub clinical sepsis increasing the rate of consumption of the IgG
d) The precision and accuracy of the test being used.

VIL explained that for all or some of these reasons the before and after serum IgG levels vary greatly and to emphasise the point emailed data from studies conducted at Newmarket involving approximately 60 foals.

3. A vet phoned to say that a foal which had not received plasma had developed R. equi disease. She asked if it was reasonable to give it plasma now. She also wanted to know if other foals of varying ages could be given prophylactic plasma.

It was explained that the published thinking is that plasma is most beneficial prior to exposure to infection. However, VIL had received anecdotal data to suggest that plasma can be helpful in foals already under challenge. The vet then said that some other foals in the same group had 1 litre of Hypermune-RE approximately a month ago but not had a second litre. She wanted to know if it would be beneficial to give the second litre now. The company explained the strategy in such places as California where foals born in January and February are often not given prophylactic plasma until the winter rains cease in March and the dry R. equi season starts. At that time all the foals, irrespective of age are given the plasma. Some vets in Newmarket also adopt a similar strategy of waiting until evidence of R. equi challenge is seen and then plasma is given to all the foals in the group.

4. A customer phoned to say that during the thawing and warming of two bags of plasma one was seen to contain many “lumps” in it and was unusable.

It was explained that if what he was seeing was stringy, “slimy” strands it was most likely to be fibrinous cryo deposit which indicates incomplete thawing and warming. The company went on to say that if what they were seeing looked like scrambled egg, multiple “lumps” of white/grey material then it suggests lumps of denatured protein due contact with excess heat. The customer agreed to make his own internal enquiries to see if it is possible that water from a hot tap, for instance, may have inadvertently contacted a bag.
Plasma Handling

Each bag of frozen plasma should be handled with the greatest of care as the plastic container becomes brittle at storage temperatures which assure product quality. Hypermune is a high protein product which is damaged by excess heat i.e. temperatures >40°C.

**Handle the product with the greatest of care**

**Use a dedicated freezer or a dedicated compartment/container in a freezer.** Use different sections for each of the Hypermune products.

**Thawing your Hypermune NO MICROWAVES**, never expose any Hypermune product to microwaves or temperatures >40°C. Quality will be compromised if this happens.

When thawing, use a water bath carefully, to ensure that no hot water flowing from the tap comes in direct contact with the Hypermune bag.

**Warming to body temperature and preparation for Transfusion**

Every 10 minutes over a period of an hour, pick up the bag and invert a few times so that the contents are mixed or ‘stirred’.

**Only when the Clammy feel has gone and nothing is floating is it ready for use.**

**Use a blood giving set with a large filter**
PLASMA PRODUCTS

HYPERMUNE is frozen equine plasma for intravenous transfusion, containing equine IgG ≥ 24g/l, equine Total Protein ≥ 50g/l, in a 1 litre human plasma bag and is for single use only. It contains the excipient Acid Citrate Dextrose-A to ensure citrate content 10 - 20mmols/l.

INDICATIONS FOR USE: For foals with Failure of Passive Transfer. To raise the level of circulating IgG in neonatal foals which have been shown to have low levels (less than 4g/l). The raised level has been demonstrated approximately 24 hours after administration but the duration of the effect is not known.

HYPERMUNE-RE is frozen equine plasma for intravenous transfusion after thawing, containing equine IgG ≥ 24g/l, equine Total Protein ≥ 50g/l, and antibodies to Rhodococcus equi ≥ 40% VIL standard in a 1 litre human plasma bag. It contains the excipient Acid Citrate Dextrose-A to ensure citrate content 10 - 20mmols/l.

INDICATIONS FOR USE: For foals with Failure of Passive Transfer. To raise the level of circulating IgG in neonatal foals which have been shown to have low levels (less than 4g/l). The raised level has been demonstrated approximately 24 hours after administration but the duration of the effect is not known.

For foals with Normal passive Transfer. To raise the level of Rhodococcus equi antibodies. The raised level has been demonstrated approximately 24 hours after administration and raised levels though declining generally last for up to 21 days.

TEST KITS - The Foal

GAMMA-CHECK-E is an inexpensive rapid foal side semi quantitative screening test for the detection of equine gammaglobulin (IgG) in whole blood or serum. There are six tests in a kit.

GAMMA-CHECK-E can be used to screen foals for IgG levels at any age. However, as most foals ingesting good quality colostrum shortly after birth have satisfactory IgG levels (>8g/l) by 4 - 8 hours of age, early testing permits the oral route for additional supplementation with colostrum or plasma. After 16 to 24 hours of age, supplementation of the foal with immunoglobulins, if required, must occur parenterally.

Negative foals should always be retested with a validated quantitative laboratory test prior to a plasma transfusion, eg Beaufort Cottage Laboratories, Newmarket or Leeds Veterinary Laboratories, Leeds.

TEST KITS - The Colostrum

GAMMA-CHECK-C test (six per kit) is a reliable and rapid means of semi-quantitatively measuring immunoglobulin in colostrum.
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LATEST NEWS

In 2009 there have been a number of personnel changes at VIL. Nichola Reynolds BSc (Hons) joined the team in June on a temporary basis but after spending a few weeks has taken up her permanent position of Graduate Technician.

Michael Fishwick started in August 2009 as livestock assistant and site maintenance.

Like most businesses in the UK, VIL has not been untouched by the national and global economic downturn but has employed strategies to minimise any adverse impact and is looking forward to the 2010 foaling season.

VIL has continued to support the industry by sponsoring CPD events and restraining prices. In 2010 VIL is involved with the Foal Care Course in Newmarket in January and will be involved in additional events.

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

www.veterinaryimmunogenics.com