Introduction:

The foal is born immunocompetent but immunologically naïve. This reduced immune function means the neonatal foal is at risk of infection making infectious disease in the neonate common with an association of high morbidity and, less commonly, mortality. Immunoglobulin G (IgG) is one of four antibody types in the horse; it is made in the spleen, lymph nodes and the bone marrow and is of highest concentration in serum. IgG levels in a foal can be classified in four ways:

- Complete Failure of Passive Transfer (FPT) is an IgG level of less than 2g/l.
- Partial Failure of Passive Transfer (PFPT) is an IgG level of 2-4g/l.
- Moderate Failure of Passive Transfer (MFPT) is an IgG level of 4-8g/l.
- Complete Passive Transfer (CPT) is an IgG level of greater than 8g/l.

Hyperimmune Plasma:

FPT strongly predisposes the foal to developing a specific infection and/or a more generalised sepsis and a Hyperimmune plasma transfusion is always needed. **PFPT** results in an increased risk of developing a specific infection and/or a more generalised sepsis and a Hyperimmune plasma transfusion is needed. **MFPT** requires in depth consideration of factors such as environmental risk and general foal health. Sub-optimal IgG levels result in a grey area and blood tests can provide further information but in the majority of situations a plasma transfusion is warranted. It may be necessary to take several blood samples over time to assess IgG levels and ensure it is not declining despite initial satisfactory results. **CPT** means that no further action is normally necessary.

Other beneficial factors of plasma include improving neutrophil function and the provision of other useful proteins, complement, lactoferrin and cytokines. Hyperimmune plasma should ideally be administered prophylactically usually as a one litre transfusion. All potentially septic neonatal foals should receive plasma irrespective of their IgG levels and general health. This is due to the rapid rate at which IgG can be utilised in the sick neonate even when signs are subclinical. Some plasma can be targeted directly at reducing the incidence or severity of a specific disease e.g. *Rhodococcus equi* pneumonia (Caston *et al* 2006; Giguere *et al* 2011). It is now common for insurance companies to demand a Hyperimmune plasma transfusion if low IgG levels are apparent. The use of Hyperimmune plasma should form part of an expert management protocol, with excellent hygiene being essential in reducing environmental challenges to the neonate.

Plasma is also increasingly used in adult horses for a variety of conditions, such as an anti-endotoxin agent, colloidal support and to treat some coagulopathies (Tennent-Brown 2011).

Current Research on Adverse Reactions and Veterinary Immunogenics (VIL) Pharmacovigilance System:

The literature on the use of plasma in equine veterinary medicine is extensive, but there is little published with regard to adverse reactions. Their rare occurrence should be understood, anticipated and never underestimated.

As a company VIL has a well-established system set up with guidance from the Veterinary Medicines Directorate (VMD) on how to handle and investigate any reported adverse reactions; the system is referred to as Pharmacovigilance. Reactions are sub-divided into a Suspect Adverse Reaction (SAR)

where the animal recovers or a Serious Suspect Adverse Reaction (SSAR) where the animal dies. The VMD class the term adverse reaction as a type of adverse event and define it as: "Any observation in animals, whether or not considered to be product related, that is unfavourable and unintended and that occurs after any use of a veterinary medicine".

SAR's and SSAR's Incidence rate:

VIL decided to review the SAR's and SSAR's reported since the company started producing and selling plasma 20 years ago. The decision for the review was prompted by the publication from Hardefeldt *et al* (2010) which reported a transfusion reaction incidence rate of 0% in adult horses and 9.7% in neonatal foals. This was recorded over a 5 year period during which a 107 horses and foals were administered a plasma product from one commercially available source in the United States. VIL incidence rate figures (table one) are significantly less than the Hardefeldt *et al* (2010) study.

Table one: Incidence of SAR's and SSAR's reported to Veterinary Immunogenics.

Year	Total litres sold	Number of SAR's/year	Number of SSAR's/year	Total number of SAR's and SSAR's/year	Incidence rate (%)
2004	**3946**	2	0	**2**	0.05
2005	**4452**	0	3	**3**	0.07
2006	**4533**	3	0	**3**	0.07
2007	**4772**	1	3	**4**	0.08
2008	**4954**	3	3	**6**	0.12
2009	**4246**	3	2	**5**	0.12
2010	**3957**	0	0	**0**	0.00
2011	**4363**	5	0	**5**	0.11
TOTAL	35223	17	11	28	0.08

A direct comparison of VIL and Hardefeldt *et al* (2010) incident rate is inaccurate because:

- VIL has calculated a rate according to the number of litres sold (and assumed to have been used) as opposed to a relatively small sample group of horses in a single hospital.
- Hardefeldt *et al* (2010) added Heparin to the plasma prior to transfusion which is indicated in the discussion as a possible cause for adverse reaction on its own irrespective of the plasma transfusion.
- The rates are calculated over different time periods.

Clinical signs of an adverse reaction:

Plasma is a major investment for any stud manager or owner. A foal must be considered as an individual when a transfusion is considered. There is nothing routine about administering a litre of plasma to a neonate already enduring the complex and vulnerable adaptive period during which few body systems are untouched, notably the cardiovascular system.

Clinical signs are diverse and complex and a compromised foal will complicate a transfusion even further. Hardefeldt *et al* (2010) provide a definition of a transfusion reaction as "development of one or more of the following: anaphylaxis, hives, pruritus, oedema, tachycardia, tachypnoea, pyrexia, colic, changes in mentation, or evidence of haemolysis during or shortly after the transfusion" (Hardefeldt *et al* 2010). VIL classes transfusion reactions, anaphylaxis and volume overload as adverse reactions. Anaphylaxis is generally considered to be a rapidly progressing, life-threatening allergic reaction.

When looking through all of VIL's 28 cases it is clear that respiratory abnormality (18/28) and cardiac abnormality (9/28) dominate the list of clinical signs. The high number of respiratory abnormalities, generally tachypnoea, but also occasional froth at nostrils or mouth, is indicative of physiological volume overload resulting in pulmonary oedema. Details provided to VIL show evidence that rate of transfusion is often too fast and/or that the clinical state of the foal was not taken into account and the rate altered accordingly. Collapse is the next most common sign (7/28) reported along with colic signs (3/28).

Other, less frequently reported clinical signs are:

- Sleepiness
- Discoloured mucous membranes
- Facial and neck oedema
- Small airway inflammation evidenced by abnormal respiratory sounds
- Hyper-flexion of the head and neck
- Cyanosis
- Froth in mouth and nostrils
- Blood in mouth
- Scouring
- Fractious/distressed behaviour
- Hypothermia
- Fitting
- Pyrexia
- Depression
- Sweating

Clinical signs are often representative of a concurrent disease process e.g. sepsis and highlight the importance of pre and post transfusion clinical examinations with frequent monitoring in-between.

Immunological or Physiological:

Adverse reactions are immunologically or physiologically based. Anaphylaxis is always immunological in nature, whilst other reactions can be purely physiological e.g. volume overload. Clinical signs initiating towards the end of or post transfusion would more likely indicate a physiological based reaction, this is apparent in the majority of reactions reported to VIL. Physiological reasons for an adverse reaction include volume overload, complex interactions between drugs and error's in administration e.g. rapid infusion.

Only one SAR reported to VIL has been deemed an allergic or immunologically based reaction due to the presence of urticaria, facial oedema, small airway inflammation and a rapid improvement when corticosteroid was administered. The signs of this allergic reaction did not become apparent until 10 minutes into the transfusion and so was not an anaphylactic reaction.

Compromised foals:

The clinical state of the foal and the experience and knowledge of the clinician are fundamental to a successful plasma transfusion. If a foal is compromised and/or has had a difficult birth the way in which a plasma transfusion is approached should be reassessed. In VIL's experience a compromised foal has an increased likelihood of complications during a plasma transfusion, with 9/17 (53%) SAR's and 8/11 (73%) SSAR's involving compromised foals. It should be noted that foals may appear healthy on initial clinical examination but blood tests may identify elevated levels of inflammatory proteins such as Serum Amyloid –A (SAA). SAA is highly indicative of an underlying septic state and combined with IgG, glucose, fibrinogen, creatinine and lactate levels can be useful indicators of subclinical infection in neonates (Knottenbelt 2004; Castagnetti and Veronesi 2008).

Avoidable and predisposing factors:

Rate of transfusion and foal weight is vital and wherever possible should be measured accurately. It is recommended that a transfusion of one litre takes a minimum of 20 minutes yet on several occasions it has been stated that a full litre has been administered in less than 10 minutes. A post mortem (PM) should be carried out whenever possible following a SSAR, and frequently there are discrepancies between the estimated and accurate weight measurement reported, with errors up to 15kg. This can obviously affect transfusion rate detrimentally for the foal. On one occasion a miniature Shetland weighing only 20kg and in a severely compromised physiological state was administered a litre of plasma at a 50kg foal dose rate and it died several hours later. A 12 day premature, weak 6.5 hour old foal was reported as having received the full litre of plasma over 10 minutes and it was immediately followed by collapse and death of the foal. This was off label use; i.e. the product was used incorrectly, as the foal was under 24hours of age and the plasma was administered at too fast a dose rate. The post mortem report stated; "lesions are consistent with asphyxial damage during the birth process" and "crushing with haemorrhage" of the umbilical cord both provide clear evidence of a severely compromised foal at birth which was unable to manage a plasma transfusion 6.5hrs later.

Methods of restraint:

Where ever possible a foal should be manually restrained by a firm grip around the chest and buttocks or using the "ear and tail" restraint method (Knottenbelt 2004). Foals often become passive in such situations and this should not be mistaken for a sleepy foal. Lateral recumbency should be avoided and if the foal is unable to stand then sternal recumbency should be the preferred position. Sedatives (notably alpha-2-adrenoreceptors) should be avoided at all times due to their ability to alter cardiac function. In nearly 30% of adverse reactions reported to VIL sedative had been administered, some foals were even described as "profoundly sedated". It becomes very difficult to establish the cause of an adverse reaction when other drugs, especially sedatives, have been administered.

Conclusions:

A better understanding of the inherent vulnerability of a healthy neonatal foal as well as a compromised one would in no doubt reduce the incidence of adverse reactions. Provision of an expert, experienced and well prepared team will reduce the already minimal risk of an adverse reaction to a plasma transfusion and it will further ensure that foals have the maximum benefit of the plasma transfusion without further complication.

Volume overload due to avoidable, predisposing factors such as a reduced capacity to handle excess volume, a rapid infusion rate and inaccurate weight estimation are in VIL's experience the main reasons for adverse reactions associated with the administration of its Hyperimmune plasma.

Adverse reactions occur very rarely with plasma supplied by VIL and at no point has a defect in plasma quality ever been responsible for an adverse reaction.

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