THE EQUINE NEONATE FAILURE OF PASSIVE TRANSFER AND USE OF PLASMA

FAILURE OF PASSIVE TRANSFER INTRODUCTION

All foals are born, because of their epitheliochorial placentation, with a deficiency of humoral immunity[1]. They must rely on the adequate intake of good quality colostrum within a few hours of birth to provide essential antibodies in order to withstand the challenges of infectious agents during the following weeks. Unfortunately, this transfer of antibodies from dam to offspring does not always occur successfully and "failure of passive transfer" (FTP) is said to exist, as depicted by the foal's serum gammaglobulin (usually specifically IgG) level being less than 400–800mg/dl (see page 2). If this deficiency is confirmed before the gut's ability to absorb antibodies ceases (12–18 hours of age), oral supplementation of good quality colostrum (or possibly plasma) may well rectify the situation. If, on the other hand, the foal is over 24 hours old when this deficiency is diagnosed, the foal must be considered at risk to infection, especially in the "high challenge" (see page 3) environment. In such circumstances, an intravenous transfusion of plasma should be considered, the benefits of which are well documented[2,3]. EQUIPLAS® and EQUIPLAS® PLUS are two forms of commercially available high quality equine plasma, specifically produced for this purpose.

THE DIAGNOSIS OF FPT

Accurately assessing the immunoglobulin status of the equine neonate on a clinical basis cannot be accomplished. Certain factors relative to the foaling process, such as prior leakage of "liquid" from the udder, failure to nurse soon after birth and the presence of agalactia will warn of the probability of low IgG levels. Denise Jones, DVM and Derek Brook, DVM recognized this problem and through a Research and development program devised the GAMMA-CHECK®C and GAMMA-CHECK®E tests to provide a simple cost-effective means of semi-quantitatively assessing colostrum and blood gammaglobulin levels. In addition, an accurate and standardized IgG test is available in the form of the EQUINE-RID kit. All of these tests can be easily performed by either the veterinarian or technical staff.

The GAMMA-CHECK®C test: This is a rapid, economical, semi-quantitative screening test to determine if there is an adequate gammaglobulin level in colostrum[4]. The test can be performed at foaling prior to nursing, or to evaluate colostrum prior to freezing, or after thawing colostrum to ensure quality. Details of the test are provided in our GAMMA-CHECK® info-sheet. Approximately 1.5–3.5 liters of colostrum with an IgG level greater than 3800mg/dl are required for the foal to reach optimal IgG levels. Each kit contains ten tests, everything needed to do the test and a complete set of instructions. It is important to know that mares older than 15 years often have colostrum of low IgG levels.

The GAMMA-CHECK®E test: This is a rapid, semi-quantitative screening test for equine IgG in whole blood or serum. It can be used to screen foals at any age, but is especially useful at 8–10 hours of age (before total gut closure to immunoglobulin), so that the oral route can still be used for additional supplementation with colostrum or plasma if required. There is now ample evidence that foals nursing a dam with good quality colostrum will reach an adequate IgG level by about 8–10 hours of age and therefore knowing the level is low at that time allows for action to be taken (see GAMMA-CHECK® info sheet for more details).

The EQUINE RID test: This is an accurate and easy means of determining equine IgG in serum or plasma using a radial immunodiffusion. Each kit comprises a plate containing agar gel, into which 24 wells have been punched, three equine reference sera of known IgG levels, graph paper with a measuring scale and instructions. Antibodies to equine IgG are incorporated into the agarose. The principle of the test is that these antibodies react with the foal's IgG in the test sample to form a visible, measurable ring of precipitate. The larger the diameter of the ring, the higher the IgG concentration in the sample.

CLINICAL USE OF THESE KITS

Without colostrum of adequate quality, the foal will probably exhibit a degree of FPT, even if she/he nurses and consumes a "normal" volume of low IgG colostrum. It behooves the person in charge of foaling (foal night watchman) to test the colostrum at birth, using the GAMMA-CHECK®C test.

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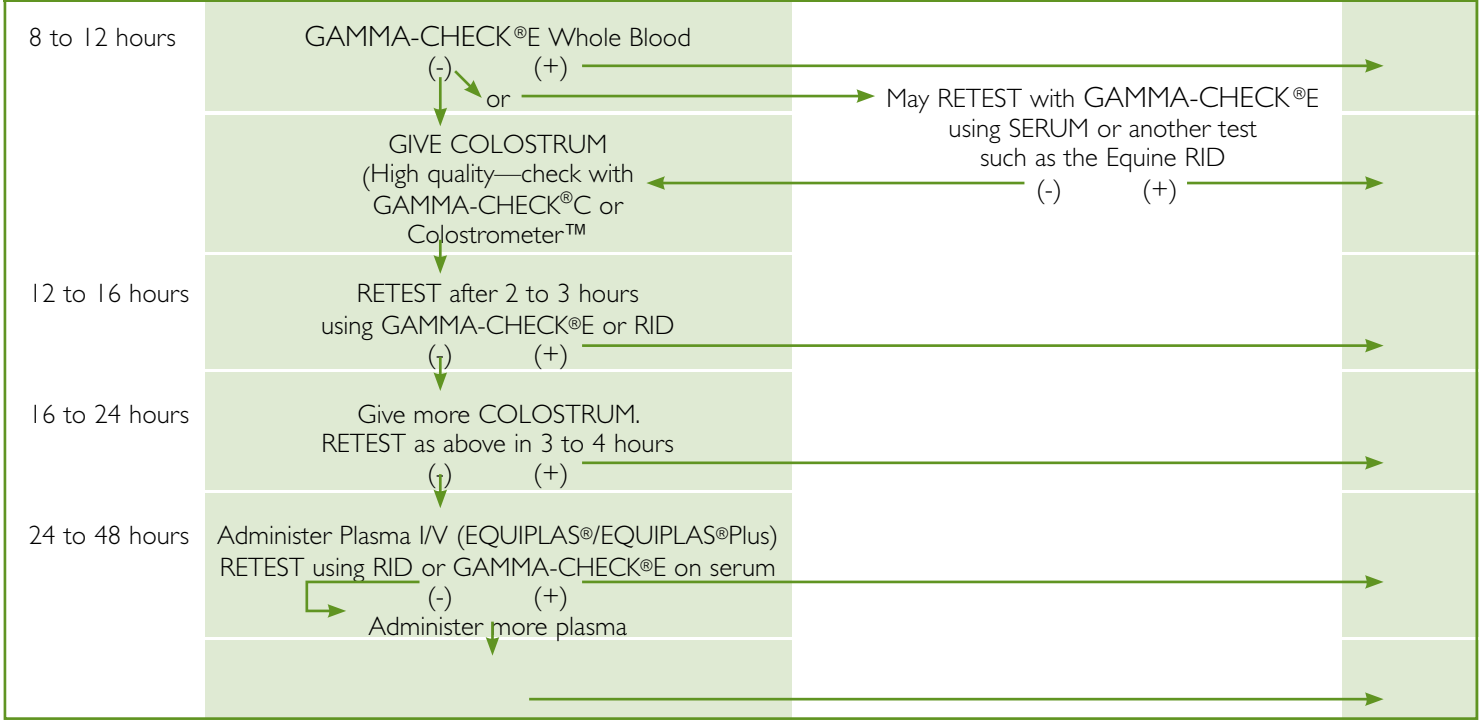
If the concentration is low, then every effort should be made to obtain high quality colostrum to be administered as soon as possible, preferably within the first 3–4 hours. Colostrum should always be tested prior to storage and only that with high IgG should be stored. High quality fresh or frozen colostrum is the ideal remedy in this type of FPT situation (see page 3 on the use of other forms of IgG).

Independent studies have shown[4] that foals nursing good colostrum in a timely manner will have high (over 800mg/dl) IgG levels by about 6 hours of age. Because the GAMMA-CHECK®E test is easy, economical and gives the results in 10 minutes, all foals should be tested if possible at about 10 hours of age. If they have low levels of IgG at that time, the gut is still able to absorb antibodies. Colostrum (or possibly oral plasma) should be given.

If it is not possible to test the foal before 18–24 hours of age, then the GAMMA-CHECK®E test is still of considerable help as a screening test. In a sick foal, occasionally a positive result, indicating a high IgG, can be misleading because of high fibrinogen levels. If this is the case, a more quantitative test should be used. False negatives do not usually occur and we strongly recommend checking every foal with this test. A second more accurate test (EQUINE RID) should be done before deciding that a plasma transfusion is necessary. The flow chart demonstrates this sequence of events.

Foals showing a negative result to the GAMMA-CHECK®E test, who are more than 24 hours old should be tested with the EQUINE-RID test and given plasma if IgG is low (see Treatment of FPT).

GUIDE TO THE DIAGNOSIS AND TREATMENT OF FAILURE OF PASSIVE TRANSFER IN THE FOAL



A positive (+) result indicates adequate levels of antibody. The interpretation of (+) is left to the practitioner for some of the tests. He or she may consider 400mg/dl adequate in some situations and 800mg/dl in others. The screening test "GAMMA-CHECK®E" has a cut off of 800mg/dl using whole blood or serum.

LEGEND

a.	GAMMA-CHECK®E	Plasvacc USA Inc.	Templeton, CA
b.	GAMMA-CHECK®C	Plasvacc USA Inc.	Templeton, CA
c.	Colostrometer™	Juergensen	Loveland, CO
d.	Equine RID	Plasvacc USA Inc.	Templeton, CA
e.	EQUIPLAS® and EQUIPLAS® PLUS	Plasvacc USA Inc.	Templeton, CA

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TREATMENT OF FPT

Foals under 18 hours old:

High quality colostrum (Spg. >1.060 or + GAMMA-CHECK®C) is the method of choice. Research has shown that 60g of good quality colostrum are needed to achieve a significant increase in the circulating IgG level. Presently in vogue are various non-colostrum oral forms of IgG and their efficacy awaits to be seen. What they do not contain are all the other essential components of colostrum, such as epithelial growth factor, transferrin, various cytokines, etc.

Foals over 24 hours old:

Plasma is the treatment of choice and has been for many years. Commercial plasma is well proven for efficacy and safety. Reactions are rare. With sophisticated vaccine strategies, antibodies to the common neonatal pathogens are present in the plasma, even though it might be produced 2000 miles from where the mare and foal are.

Other sources of IgG are now being made available. Serum products suffer from the frequent problem of being contaminated with endotoxin and prostaglandin metobolites[5–8], which may often cause severe reactions. Because of USDA requirements, these pasteurized serum products often contain damaged proteins and have abnormal electrophoretic patterns[8]. For these reasons they have to be diluted and administered very slowly[9, 10]. Many of the essential cytokines present in plasma are not present in serum.

Lyophilized IgG is now available, but again does not have the broad spectrum of components that plasma has. Whether it will provide adequate protection awaits to be seen. It is distinctly possible that we have become preoccupied with IgG and we are forgetting that there are many other essential components to the healthy immune system. It could well be that it is not sufficient just to increase the IgG level to cause the foal to stay healthy. Time will tell!

One leading equine practitioner in Kentucky[11] aptly described plasma as "life in a bag."

EQUINE IgG (EQUIPLAS® AND EQUIPLAS® PLUS)

Indications

Equine plasma is indicated where a foal that is 24 hours old or more has been diagnosed as having inadequate circulating immunoglobulin G levels. The definition of "inadequate" is open to some degree of interpretation and dependant on several factors[12]. In a recent research project, 85% of foals totally deprived of colostrum became ill within a very short period of time[13]. Other studies have shown that in the right environment foals can survive with very small amounts of maternally derived antibodies[14]. The "right" environment might, for example, be created by one foal being in a well-tended 20-acre field with only its dam to share the land. Conversely, a "high challenge" situation could be produced if 50 mares and foals shared the same field. The "right" environment is, unfortunately, not present in most situations and the majority of foals with FPT do succumb to some form of infection during the early weeks of life[2, 13].

Generally accepted veterinary medical standards (and often 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 supplemental antibodies.

Dosage

- **Intravenous:**

A 100lb foal has a blood volume of approximately 5 liters (or 3 liters of plasma) and therefore any transfused antibodies will be immediately distributed within this volume. The administration of one liter of plasma containing 25 grams IgG will initially raise the recipient's whole blood level by 5g (25 divided by 5). However, within the 24 hours following transfusion there is some movement out of the circulation and only about 50% remains in the vascular spaces after 24 hours. (This is why it is important to wait at least 24 hours before measuring the IgG level after transfusion). Therefore, after transfusion of the liter of plasma with 25g IgG, the foal's circulating whole blood level will be increased by about 2.5g/L (250mg/dl of whole blood). If we wish to express the concentration in the plasma, then the level will be 25 divided by 2 = 12.5g in the blood vessels which is now distributed within 3 liters of plasma, i.e. 4.17g per liter or 417mg/dl plasma.

When having tests done it is important to know whether the results are mg/dl of whole blood or plasma. As shown in the above example, there is a 65% difference. This has been well documented in the past[15]. By knowing the initial level and the desired final level, the amount of plasma to be administered can be calculated.

EQUINE PLASMA

- **Oral:**

Research has shown that the amount of IgG absorbed is directly dependent on the time of administration. Close to 100% could be absorbed if the plasma is given within the first hour or two. This rapidly diminishes, so that only 50% is absorbed at 10 hours and less than 20% at 15 hours. The dose required to correct complete lack of colostrum IgG is over 60g and this will be supplied by about two liters of EQUIPLAS® PLUS (see Treatment of FPT, page 3).

IT SHOULD BE POINTED OUT THAT **ANY** IgG ADMINISTERED TO A FOAL WILL BE OF **SOME** BENEFIT. It is also important to realize that passive immunoglobulin transfer is not a simple "yes" or "no" equation and there is no absolute level of circulating IgG that will ensure the foal's health. To afford maximum protection against disease, the immunoglobulin should be available at the place where an organism is invading. It must also be of the appropriate immunoglobulin type to attach to the specific invading organism and be present in sufficient quantity to neutralize the agent[16].

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In the presence of sepsis, immunoglobulins are rapidly consumed having, therefore, a very short 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 emphasized that the sick foal would have had a lower IgG if plasma had not been given. To keep IgG levels up in the face of rapid consumption, severely compromised foals can require many liters over a few days.

Non-antibody factors in EQUIPLAS®

As well as specifically increasing resistance to infection by providing antibodies, plasma also provides non-specific protection against diseases. The protection is provided by cytokines, lymphokines and other bioactive peptides[17]. These compounds increase the activity of neutrophils and enhance phagocytosis. Plasma also enhances the benefits of fluid therapy because of its albumin content. It is indicated for intravenous use in horses of any age with hypovolemia and/or hypoproteinemia, and as supportive therapy in major equine surgery.

Production

Plasvacc USA Inc. produces normal equine plasma in California from a closed herd of donor horses, under the regulations of the USDA and the California Department of Agriculture.

Prior to acceptance into the herd, the animals are screened by special arrangement with the Department of Serology, University of California, Davis, the USDA and the Californian Animal Health and Food Safety Laboratory System. The tests ensure that these horses are essentially free from diseases and that they are cleared as plasma donors.

To ensure the presence of high levels of immunoglobulin (specifically IgG), a special vaccination protocol is utilized. Accurate IgG measurements are done on each batch using a standardized radial immunodiffusion test. The level of IgG in the plasma ranges from 1600–>3500mg/dl. In addition, the total protein is measured during the production stage by refractometer and ranges from 50g/L to 68g/L.

Collection of plasma is by the process of plasmapheresis, using equipment initially devised for human donors, but with modifications to facilitate its use in large animals. This completely closed process ensures sterility and freedom from endotoxins. Nevertheless, each batch is also subjected to sterility tests using thioglycollate broth. Plasma is collected into one liter bags which are immediately labelled, batch numbered, packaged and placed in the freezer at -18°C.

Specific Antibodies

Plasvacc USA Inc. produces plasma with high levels of antibodies to specific organisms, as well as providing plasma with a range of antibodies against common neonatal pathogens. EQUIPLAS®REA (Rhodococcus Equi Antibody) is a USDA licensed plasma product with high titers to *Rhodococcus equi.* The product is used as an aid in the management and control of respiratory disease associated with *Rhodococcus equi* in foals under 6 months of age[21, 22].

Endotoxemia is a serious problem in horses of all ages and, whilst its treatment is a controversial topic, we produce plasma containing gram negative core antibodies by using a *E. coli* bacterin. In human medicine it has been documented that anti-endotoxin plasma is most beneficial when there is a concomitant bacteremia[23]. In horses, good results have been obtained in a double blind study[24] at U.C. Davis, California. EQUIPLAS®J containing antibodies to J-5 strain of *Escherichia coli* is presently licensed in California.

Diarrhea has long been a concern in neonates and young animals. Rotavirus has been implicated as a cause of viral diarrhea[18, 19]. Consequently, Plasvacc USA Inc. immunizes some of the plasma donors with the Equine rotavirus vaccine. Plasma with a high rotavirus titer has been used for a number of years in Europe both orally and IV to treat severely ill and dehydrated foals.

In young foals, *Clostridium perfringens* has been implicated in acute, hemorrhagic diarrhea[20]. Some plasma donors are vaccinated with this antigen. Other bacteria associated with diarrhea are various species of Salmonella and *Clostridium difficile*. A *Clostridium sp.* toxoid and Salmonella vaccine have also been incorporated into our vaccination program.

Plasvacc USA Inc. produces EQUIPLAS® (Clostridium Botulinum Type B Antitoxin). This product is licensed by the USDA. *Clostridium Botulinium*, Type C toxoid has also been used in our donor herd to produce antitoxin to this organism.

Storage

Plasma must be handled carefully when frozen, as the blood transfer bags are somewhat brittle in this state and might crack if knocked or dropped. It is advisable, therefore, to keep it packaged (as supplied) until required. The shelf life of the frozen product is three years.

Administration

Plasma is best thawed immediately prior to administration. The most convenient means is to place the bag into warm water with a temperature of about 40°C (this will feel like a warm shower—**if you cannot keep your hand in it, it is too hot**.) Thawing in water too hot will denature certain proteins and cause excessive fibrin precipitation. Keep adding more warm water as the plasma thaws. At the correct temperature, the whole process will take about 20 minutes. Plasma that has been thawed slowly, but not heated, will have a large amount of precipitate in it.

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This is called cryoprecipitate and contains several important clotting factors. By allowing plasma to warm to about body temperature, this precipitate will dissolve and the liquid should be relatively clear before administering. Occasionally, some fibrin strands are present and these are filtered out by the administration set.

Plasma should be given USING A BLOOD ADMINISTRATION SET WITH APPROPRIATE FILTER (40–180 microns). It should be used straight from the bag with nothing added. Foals may be mildly sedated if necessary and the jugular site prepared aseptically. It is also recommended that the skin be anaesthetized and a 16G x 2" catheter or similar be used. One liter of plasma can be safely administered to a 50kg foal in 15–20 minutes.

Plasma that has been thawed and not brought to body temperature can be stored in the fridge for up to one week or refrozen. We do not recommend that you keep plasma or refreeze it, if it has been warmed to body temperature.

Adverse Reactions

As with any biological (or pharmaceutical) product, reactions can occur. Fortunately, the incidence with commercially produced plasma is very rare. Over almost twenty years we have been selling this product (amounting to thousands of liters), we have had only a hand full of reactions reported. Some of those, such as reactions caused by not using a filter, are easily avoided.

There are potentially several types of adverse reactions:

1. Volume overload—as the newborn foal has a total blood volume of about 5 liters, it is easily to understand how volume overload might occur. A healthy foal is able to handle the transfusion of 1 liter of plasma in about 15–20 minutes, but 2 liters should be given over a minimum of two hours. A sick and compromised foal will require more time for the administration of the same volume. Signs of volume overload include hyperventilation, tachycardia and sweating. The rate of administration must be immediately slowed down if these signs appear and stopped completely if these signs do not quickly abate.
2. Anaphylaxis—there are two types:

   A—This usually occurs when the foal is in some way sensitized to the plasma being transfused. In the neonate, the likely cause of this is by receiving sensitizing antibodies through the colostrum[25]. We are aware of several instances where this appears to have occurred in mares with previous histories of severe dystocia. It is theorized that the dystocia leads to the mare being exposed to foreign (foal) antigens and then producing antibodies to these. Signs include hyperventilation, frothing at the mouth and gurgling sounds from the lungs. The transfusion should be stopped immediately. Epinephrine and anti-inflammatory drugs should be administered. Forced ventilation, thoracic massage and oxygen might be needed. Mares producing such foals do so year after year and if a transfusion is essential in a subsequent foal, intradermal skin testing could be performed with the plasma prior to transfusion.

   B—If using plasma from a donor not screened for red cell antibodies, then agglutination and/or hemolysis might occur. Signs include hyperventilation, but not profound bubbling. This is not a problem with commercial plasma from screened donors.
3. Fibrin entering the system—by not using a filter. Symptoms include rapid dilation of the pupil, apnea and sudden death. It is likely that a fibrin clump enters the cardiac circulation causing myocardial ischemia.

All these problems are easily avoided by using properly harvested plasma from screened donors. The only occasion which might cause an unexpected problem is when a foal is transfused that was born to a mare with unknown hypersensitivity from a previous foaling.

Delivery

Plasma is shipped frozen in insulated containers by express courier to reach destinations in most parts of the USA by noon the following day. (Orders must be placed by 2pm. PST to ensure next day delivery). Special arrangements can be made for weekend delivery and when emergency situations arise.

We are happy to discuss your specific requests at any time.

Call 800-654-9743

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