Equine Antibody Products and Plasma: Current Regulatory Issues

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Cost: benefit of treating equine antibody products to assure pathogen-free product is not warranted currently in terms of treatment cost and resulting quality of treated product versus risk of contaminating viruses. It is desirable to require proof of antibody content of product, efficacy, and safety and to regulate plasma. Author’s address: Department of Large Animal Clinical Sciences, University of Florida, PO Box 100136, Gainesville, FL 32610-0136. © 2000 AAEP.

1. Introduction

The equine veterinary community is currently facing challenges regarding regulatory issues for equine antibody and plasma products. Equine veterinarians want assurance of products which are safe, efficacious, pure, composed of guaranteed provable content, and affordable. There has been widespread discussion among equine practitioners, manufacturers of equine antibody products, and the United States Department of Agriculture (USDA) regarding regulatory issues for equine antibody products. An inciting event was announcement by USDA of pending treatment (inactivation) requirements for veterinary antibody products. While technology exists to treat antibody products to assure pathogen-free product, requirements to treat antibody products for processing steps and inactivation by chemical or physical treatments to remove contaminating viruses is felt to be premature. The following provides a brief history of events, information about what it means to treat antibody products with examples of currently available methods, current USDA regulations for veterinary antibody products, and pertinent issues surrounding the recent change in USDA regulations.

2. Brief Historical Review

Management of the newborn foal was revolutionized by McGuire’s 1977 publication which led to study of the equine neonatal immune system, its relation to neonatal infection, and treatment methods. Understanding the importance of passively acquired humoral immunity was followed closely by testing for passive transfer as a routine procedure and use of intravenous plasma as a common therapeutic modality. Because equine plasma was not available commercially, innovative methods of collecting blood, separating reds cells, and administering plasma were explored and reported. Rapid, semi-quantitative, stall-side test kits to estimate serum IgG emerged. Attempts were made to “precisely” define failure of passive transfer (FPT) (< 200 mg/dl IgG), partial FPT (PFT; 400 mg/dl IgG), and a target for serum IgG of > 400 mg/dl. After Brewer and Koterba developed a sepsis scoring system and demonstrated that serum IgG of many septic foals was between 400 and 800 mg/dl, terms were redefined as FPT 400 mg/dl IgG, PFPT 400–800 mg/dl IgG,
and a target for serum IgG of > 800 mg/dl. Commercial IgG test methods were adapted. Plasma became commercially available, followed closely by specific antibody products, such as for Rhodococcus equi and endotoxin. Manufacturers of unregulated plasma and antibody products made claims about their products regarding IgG content and efficacy. Regulation of these products was and is in the best interest of the manufacturers, veterinarians, owners, and equine patients.

The USDA, as the agency which regulates veterinary biological products—such as live bacterial vaccines, inactivated bacterial products, live virus vaccines, killed virus vaccines, and blood origin products—was the appropriate regulatory agency for antibody products, i.e., veterinary biological products intended for administration of antibody. Early attention was focused on accuracy of IgG content claims, with a gold standard sought for IgG measurement to ensure consistency among manufacturers and reliability for the purchaser. A highly sensitive gold standard free from variation still has not been found; nonetheless, radial immunodiffusion (RID) is reasonably accurate and the method currently acceptable to Animal Plant Health Inspection Service (APHIS). Plasma, per se, is not considered a veterinary antibody product; therefore plasma remains unregulated.

The U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) is responsible for regulatory oversight of the human blood supply in the United States, including whole blood, transfusable components of whole blood, pharmaceuticals derived from blood cells or plasma, and related medical devices. During the 1980s the AIDS threat forced the medical community and the public to question safety of allogenic blood and blood products. Strict regulations, timely action, and a coordinated approach were required to protect against notable threats, such as new HIV variants, new hepatitis agents, human herpes viruses, Creutzfeld-Jakob Disease, human parvoviruses, and bacterial contamination.

Changing USDA Regulations
Bacteria, parasites, and viruses are pathogens of potential risk in blood products. While bacteria and parasites can be removed by sterile filtration, contaminating viruses require processing steps and inactivation by chemical or physical treatments. In response to concern about potential contaminating microorganisms, especially viruses, the USDA concluded that treatment of veterinary antibody products to inactivate potential contaminating microorganisms was desirable. Extrapolation from human products led to regulations which adapted to veterinary antibody products. Cobalt irradiation at 3 megarads and at 2.5 megarads resulted in a product containing considerable precipitates, which likely consist of fibrinogen, immunoglobulin, and other proteins. Precipitates are deemed unacceptable for many reasons, including alteration of product quality and risk of activation of pulmonary macrophages with subsequent adverse reactions.

As early as the late 1980s, the USDA expressed intent to require treatment of veterinary antibody products for inactivation of viruses and other microorganisms by irradiation. In 1995 manufacturers received official notification of impending new regulations and were given one year to reach compliance with a deadline of November 1996. An extension was granted until May 1997. In April 1997, an APHIS Veterinary Biologics Public Meeting included a topic on implementation of new standards for antibody products. In May 1997, a policy decision was made not to enforce the new regulations temporarily. Considerable discussion ensued within the equine veterinary community. In December 1998, a group of equine veterinarians was asked to represent AAEP and the equine practitioners’ view in a discussion with USDA officials in Maryland. The USDA was receptive to concerns of equine veterinarians and in April 1999 the Center for Veterinary Biologics hosted a Veterinary Antibody Products Public Meeting in Ames, Iowa, for the primary purpose of discussing regulations for veterinary antibody products and seeking information to aid in determining the disposition of veterinary antibody product regulations.

3. Treatment/Inactivation of Antibody Products
Treatment of plasma to avoid risk of virus transmission can be accomplished by physically removing or inactivating viruses, although it is considered impossible to completely eliminate all viral particles from a product. Methods used to remove viruses from plasma include precipitation, chromatography, and nanofiltration. Methods being used or being developed for use to inactivate viruses include heat (pasteurization, microwave, or dry heat), inactine, iodine/sepahex, methylene blue plus light, solvent/detergent, ultraviolet light in C band and rutin (UVC/rutin), psoralen, and ultraviolet light in A band (UVA). Virus inactivation issues include effect on viruses, effect on proteins in plasma, and quality of resulting product. There are advantages and disadvantages to each method, a few examples of which follow. Lipid envelope viruses can be inactivated by solvent and detergent methods. This method results in 20–30% reduction of all proteins in plasma and there is potential toxicity of residual solvent or detergent. Non-lipid-enveloped viruses can be inactivated by heat, gamma irradiation, iodine, methylene blue and light, UVC/rutin, psoralen, and UVA. Gamma irradiation inactivates all viruses, does not require addition of substances or subsequent removal of substances, but there is only 60–90% recovery of clotting factors. Methylene blue binds to nucleic acids more than to plasma proteins upon excitation with red light. The subsequent decrease in clotting proteins may be acceptable, but there is concern that byproducts may be
carcinogenic. UVC/rutin avoids carcinogenic compounds and preserves 70–80% of clotting factors, but this method requires a thin layer of plasma for adequate UVC exposure. Psoralens plus UVA are potentially carcinogenic and mutagenic. Iodine shows some promise, as it kills bacteria, fungi, molds, spores, and all viruses, including Scrapie. The process is relatively simple, and IgG remains functional. Inactin is a new compound which acts with nucleic acid, stops replication, and does not cause problem with plasma proteins.

Prothrombin is exquisitely sensitive to irradiation and other treatment methods. Therefore, prothrombin can be used potentially as a measure of protein degradation/inactivation by the various plasma treatment methods. If prothrombin is protected, other proteins are probably protected as well. More work is needed for human and veterinary antibody products.

4. Current USDA Regulations

General Requirements for Antibody Products

The following are excerpts of USDA regulation 113.450, General Requirements for Antibody Products, dated October 1996.

All animals used in production of antibody products shall be healthy. Their health status will be determined by physical examination by, or under the direct supervision of, a licensed veterinarian and by tests for infectious diseases. No animal shall be used while showing clinical signs of disease. Before first use and on a regular basis, all animals used in manufacture of antibody products shall be individually subjected to applicable tests for infectious diseases. Before first use, horses shall be tested as follows for: [1] EIA at laboratory approved by APHIS; [2] piroplasmosis, dourine, and glanders at the National Services Laboratories; [3] brucellosis at a laboratory approved by APHIS; [4] horses shall be re-tested annually for EIA and, if housed with other species, brucellosis. Blood derivatives [serum, plasma, etc.], lacteal secretions, and egg material used in production of antibody products shall be subjected to an appropriate procedure for inactivation of potential contaminating microorganisms.

Requirements for Antitoxin and Products for Treatment of FPT

Products of animal blood origin include antitoxin and products for treatment of FPT. The following are excerpts of USDA regulation 113.499 pertaining to products for treatment of FPT, dated October 1996.

A product for treatment of FPT shall contain a specified minimum quantity of IgG per dose and shall be recommended for use only in neonates of the same species as that of antibody origin. An IgG Reference Product shall be qualified by administering one dose of the reference serial of the product in accordance with label directions to at least 20 randomly-selected, newborn, colostrum-deprived animals. Animals are observed for 24 hours for any adverse reactions. Blood samples are taken before and 24 hours after administration. IgG concentrations are determined by a radial immunodiffusion (RID) method acceptable to APHIS. Concurrently, using the same method, 5 IgG measurements shall be made on an IgG Species Standard supplied or approved by APHIS. For an IgG Reference Product to be satisfactory, all animals must remain free of unfavorable product-related reactions and at least 90% of paired serum samples must reflect an increase in IgG concentration [post-tx minus pre-tx] equal to or greater than the IgG concentration of the Species Standard. Prior to licensure, functionality of product antibody must be demonstrated by neutralization study or other study acceptable to APHIS. Final bulk or container samples shall be tested for IgG content by RID, with 5 IgG measurements made on each, test serial and IgG Reference Product. If IgG level
5. Some Pertinent Issues Related to Changed USDA Regulations

Treatment (Inactivation) of Antibody Products

- Equine practitioners want regulation of antibody products: 1) a product which is free of red blood cells, anti-RBC antibody, endotoxin, bacteria, and other important contaminants; 2) guaranteed provable product content, as claimed, such as total IgG, and specific IgG; 3) donor free of agent and antibodies, including EIA, EVA, Babesia, and others; 4) donor to be removed from the donor pool, if its plasma is linked with immune-mediated reactions in recipients, especially repeatedly.

- Requirement for treatment (irradiation) of equine antibody products is acceptable, if the product is not altered adversely, there are documented needs for treatment, the treatment process is affordable for producers of product, the resulting product is affordable for veterinarians and clients, and the treatment does not adversely affect product availability.

- What is the real risk of viral contamination of antibody products from healthy, tested donors from a relatively closed herd? There are no reported cases in horses of viral transmission from antibody products.

- Irradiation alters antibody product. A precipitate forms which likely contains fibrinogen, immunoglobulin, and other proteins. Irradiated products pose a potential risk to recipients because of the precipitate, while being less efficacious. Comparison of serum IgG concentrations in colostrum-deprived foals receiving irradiated and non-irradiated IgG product (Hygamm-Equi, Lake Immunogenics Inc., Ontario, NY) showed lower serum IgG concentrations in foals receiving irradiated product.

- Actual cost of irradiation of antibody product to one manufacturer was $15–20 per unit (liter).

- Summary: It is premature to require irradiation of equine antibody products, even though it may be appropriate in the future.

Efficacy Requirements: IgG Species Standards

- IgG is an acceptable measure for antibody products for treatment of FPT—for now.

- Reasonable serum IgG concentrations in colostrum-fed foals are about 2500 mg/dl IgG. In one study, colostrum-deprived foals receiving 20 ml/lb of IgG product with 2000 mg/dl IgG (Hygamm-Equi, Lake Immunogenics Inc., Ontario, NY) had mean serum IgG concentration rises of 1042.74 mg/dl at 24 h, well above the 400–800 mg/dl serum IgG target.

- While FPT and sepsis are correlated in the newborn foal, some septic foals have serum IgG concentrations > 800 mg/dl and some foals with serum IgG < 200 mg/dl do not become septic.

- Summary: 400 mg/dl IgG is a reasonable IgG Species Standard for equine neonates.

Plasma

- Plasma is not regulated; many believe plasma should also be regulated by the USDA.

6. Summary and Future Expectations

The USDA is responsible for regulating veterinary antibody products, a role equine practitioners and product manufacturers support wholeheartedly. It has been gratifying to see equine veterinarians, manufacturers, USDA officials, and others come to the table in a positive spirit with a central goal of protecting the health and welfare of the horse. While antibody product treatment regulations were not widely supported by veterinarians or manufacturers, all recognize the good intentions of the USDA and look forward to the rewritten regulations. The USDA is currently in the process of rewriting these regulations.

References

