ISSN: 1220-3173; E-ISSN: 2457-7618

BLOOD PROCESSING IN CATTLE: INSIGHTS FOR ALTERNATIVE THERAPIES

PROCESAREA SÂNGELUI BOVIN: PERSPECTIVELE UNOR TERAPII ALTERNATIVE

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ABSTRACT

Although the type of management in the cattle breeding industry makes it harder to apply treatment procedures, this should not limit the current medical alternatives. The increased prevalence of diseases that cause blood element loss required breeders to increase and save animal populations. This development gave rise to the need for specific therapies. Blood transfusion in cattle is becoming a common practise among practitioners. However, depending on the patient's needs, the use of unidirectional blood products became necessary due to the restrictions brought on by the occurrence of a post-transfusion response. Two blood units were obtained from 16 pregnant cows of three different breeds (Holstein, Romanian Black Spotted, and Montbéliard), which were further processed into plasma and erythrocyte concentrate. Gravimetric analysis of the obtained products yielded an average of 316.54 ± 18.57 g of blood plasma and 186.06 ± 25.85 g of erythrocyte concentrate, which were then preserved by freezing and refrigeration, respectively. The goal of the current study is to describe the technique used for bovine blood product processing, with an emphasis on how it can be used to successfully treat adult hypoproteinaemia, failure of passive immunoglobulin transfer (FPT) from mother to foetus, and diarrhoea in calves.

Key words: blood products, blood plasma, erythrocyte concentrate, cow

REZUMAT

Deși creșterea bovinelor reprezintă un sector în care aplicarea unor protocoale de tratament este vitregită din cauza tipului de management, acest lucru nu trebuie să limiteze posibilitățile medicale actuale. Ca urmare a extinderii patologiei cauzatoare de pierderi ale elementelor sangvine, a răsărit dorința din ce în ce mai mare a crescătorilor pentru sporirea și salvarea efectivului de animale, fapt ce a condus la aplicarea unor tratamente individuale. La ora actuală, hemotransfuzia la specia bo-vină începe să intre în rutina practicienilor. Cu toate acestea, limitările cauzate de apariția unei reacții post-transfuzionale a condus la folosirea unor produse sangvine unidirecționate, în funcție de necesitatea pacientului. De la un număr de 16 vaci gestante din trei rase diferite (Montbéliard, Bălțată cu Negru Românească și Holstein) au fost recoltate câte două unități sangvine ce au fost supuse procesării, obținându-se plasmă și concentrat eritrocitar. Analiza gravimetrică a produselor obținute a relevat o medie de 316.54 ± 18.57 g de plasmă sangvină și 186.06 ± 25.85 g de concentrat eritrocitar, care ulterior au fost conservate prin congelare, respectiv refrigerare. Studiul de față își propune să descrie procesul de obținere al produselor sangvine bovine, subliniind aplicabilitatea utilizării cu succes a acestora ca terapie alternativă în managementul diareii la viței, al eșecului transferului pasiv de imunoglobuline de la mamă la făt sau al hipoproteinemiilor la adulți.

Cuvinte cheie: produse sangvine, plasmă sangvină, concentrat eritrocitar, vacă

Despite the fact that human doctors have access to a variety of plasma-based products (including cryoprecipitate, albumin, platelet-rich plasma, and specific coagulation factors), bovine veterinarians are primarily limited to using whole blood frozen plasma and fresh frozen plasma for transfusions. In human medicine, there are just a few indications for using frozen or fresh frozen plasma, and there is often minimal proof of their effectiveness (1). Additionally, many human doctors are worried about the spread of illness and allergic reactions following the delivery of any plasma products (2). In bovine medicine, it can be used 1) to treat sepsis, coagulopathies, and FPT, 2) as "antiendotoxin" agents, and 3) to offer colloidal support (12). When the concentration of haemoglobin is low and/or the oxygen carrying capacity is decreased and there are insufficient physiological mechanisms of compensation, the transfusion of erythrocyte concentrate is recommended in order to quickly increase the supply of oxygen to the tissues (3).

The 11 blood groups in cattle make up the most

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complicated domestic animal blood group system, although most cattle do not have antibodies to additional blood group antigens unless they have previously received blood transfusions that have sensitised them (4). Due to potential (albeit rare) negative responses, the use of blood or erythrocyte concentrate must be carefully addressed prior to administration (5). In addition, Lefrère and Hewitt (2009) reported the transmission of prion diseases as a result of blood transfusion in cattle. The main benefit of using plasma is that negative responses will be limited because there won't be any antigen-antibody interactions (6).

The primary goal of this essay is to highlight all the procedures that must be followed in order to extract important blood components from cows. Secondly, we will try to emphasise the most critical actions to be taken in order to succeed, keeping in mind that this paper is the first to present these features in cattle.

MATERIALS AND METHODS

Animals, housing, and nutrition

The study was conducted in January 2023, on 16 late-term pregnant bovines belonging to 3 breeds (Holstein n = 3, Romanian Black Spotted n = 6, and Montbéliard n = 7), from 3 different farms located in south-east Romania. Each farm's breeding circumstances, food, microclimate, and animal welfare will be explained below.

Within the first establishment, Holstein cows are grown in paddocks. Barns feature both natural and artificial ventilation; manure is automatically removed; and the animals are fed using a feed mixer wagon.

The second farm houses Romanian Black-Spotted cows in traditional barns with windows and low ceilings but no natural or artificial ventilation. Milking is done with an automated system with milk stored in stainless steel canisters. One week before parturition, the cows are moved into the maternity pens, and the feed is tailored to the close-up period.

Montbéliarde cows, a mixed dairy-beef breed, are housed within the third farm, benefiting from a free stall system and natural ventilation via doors and windows. The feed mixer wagon feeds the animals, with the dung being automatically discharged. The cows are transported to a designated paddock two weeks before calving.

Blood collection

Due to farm vaccination protocols that imply later gestational stages of immunisation, all 16 selected donor cows were sampled in relation to immunisation dates between 210 and 245 days of pregnancy, respectively. This timing was backed up by older data suggesting that immunoglobulins approach satisfactory levels after 14 days (7), making the retrieved plasma product a more valuable immunological compound. In order to perform this procedure, each female was secured by placing her neck inside a head-lock feeding fence. A cattle nose plier was then fitted at the muzzle level, allowing the head to be immobilised. After locating the jugular groove, the hair was trimmed, betadine 10% (Egis Pharmaceutical, Hungary) was used to make the skin aseptic, and 10% Lidocaine spray (Egis Pharmaceutical, Hungary) was applied to the skin puncture site, in a similar way as van der Walt and Osterhoff (1969) acted. In 2006, to facilitate the blood collection, Bell sedated the donor with xylazine 0.05 mg/kg i.m. In order to facilitate vessel puncturing, he also performed a 2 cm skin incision at the level of the jugular vein. Sterile blood was collected using 600 ml total capacity double bags (Demophorius Healthcare, Cyprus), with a 400 ml satellite, along with a 16 G needle, and a main bag containing 63 ml citrate-phosphate-dextrose-adenine solution (CDPA-1). The blood bag was stirred throughout collection time in order to homogenise the blood with the anticoagulant solution uniformly, and it was periodically weighed in order to stay within the manufacturer's recommended amount of collected blood (Fig. 1).

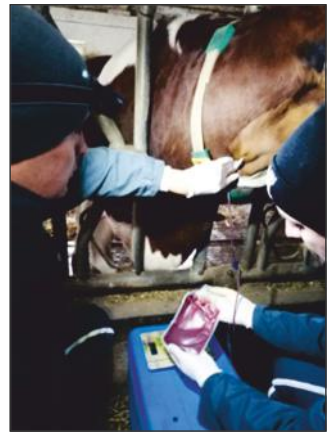

Fig. 1. Jugular vein blood collection in cow

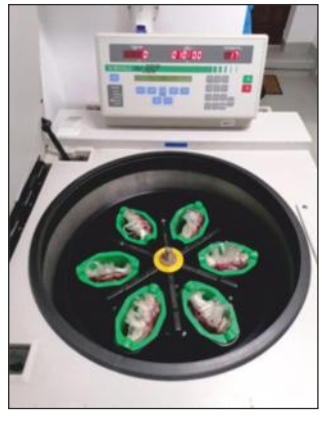

Fig. 2. SORVALL® RC3BPTM centrifuge and the blood units are ready for the centrifugation process

Fig. 3. Separation process after centrifugation

Fig. 4. Sealing the plasma unit

A scale was used to weigh blood for a more accurate estimate of volume than a visual estimate, as Balcombo and Foster already reported (2014). To collect the second unit of blood, the operation was repeated for the contralateral jugular vein. After the needle was removed, pressure was maintained at the puncture site with a betadine-soaked tampon. After collecting blood from all of the females, the units were identified, transported under refrigerated temperature conditions, and centrifuged in less than 8 hours from the time of collection.

Blood bag centrifugation

The SORVALL® RC3BPTM centrifuge (Thermo Fisher Scientific Inc., USA) was used to extract plasma, and erythrocyte concentrate. After the blood units were weighed, the centrifuge was adjusted to the desired schedule (3200 rpm for 7 minutes with chilling to 16°C) (Fig. 2). The centrifuged units were carefully retrieved and prepared for separation after the time permitted for centrifugation had elapsed. This was accomplished by the use of a semiautomatic plasma expressor (Bioline, India), which conducted to plasma and erythrocyte concentrate segregation (Fig. 3). The separation of the two blood products was accomplished under ideal conditions thanks to the spring included in the press. Further, the produced units were sealed with an automatic benchtop device (D-mach Automatic Bench®, Demophorius Healthcare, Cyprus) (Fig. 4). After inserting the tube, the sealing clamp automatically closes and establishes a seal in about 1.5 seconds. All plasma and erythrocyte concentrate units were stored at -20°C and -4°C, respectively, until use. The work was conducted in line with current EU law, specifically Directive 2010/63/EU of the European Parliament and Council on September 22, 2010, on animal welfare when employed for scientific purposes, as previously discussed by Furnaris et al. (2016). In order to reduce stress, animals were handled gently and gradually by the working staff. SPSS Statistics 26 (IBM Inc. Chicago, IL, USA) was used to analyse and interpret all of the study's data, and variables like mean, and standard deviation were computed for the blood samples that were collected.

RESULTS AND DISCUSSIONS

Even if the technique for extracting blood products in veterinary medicine is no longer a challenge, especially in the case of canids Ionescu et al. (2018), it is not well established in cows.

Table 1 displays the quantities of blood drawn from each patient as well as the quantities of erythrocyte concentrate and blood plasma collected after centrifugation. The average whole blood quantity collected was 527.53 ± 39.01 g. Out of the total, obtained erythrocyte concentrate weighed 186.06 ± 25.85 g, while plasma weighed 315.54 ± 18.57 g.

Proverbio et al. (2014), using a similar blood collection kit, obtained 300 g of plasma from each unit of blood, a value that is below our average.

In the current study, the cost of a bag of plasma was anticipated to be less than ten euros. By comparison, a bag of frozen bovine plasma costs between 185 and 205 euros at Midwest Animal Blood Services (Inc., Stockbridge, Michigan, USA), and its primary application is the treatment of hypogammaglobulinemia (failure of passive transfer) in calves, as well as a supplement to crystalloid therapy in severe systemic inflammatory diseases (9). Even when administered many days apart, the negative effects of repeated plasma delivery are modest (10).

Leukocyte adhesion defects in heifers have been treated by granulocyte transfusion; however, transfused cells vanish within five hours (11). For anaemic cattle in need of a transfusion who have heart failure or are in danger of intravascular volume overload if whole blood is given, erythrocyte concentrate is advised (10). Using blood transfusion, Arul et al. (2019), Braun et al. (2019), Muntean et al., (2021), Shah et al. (2021), and Bai et al. (2022) have successfully included blood transfusion in the treatment of various ailments such as type-2 abomasal ulcer, experimentally induced anaemia, anaplasmosis, or babesiosis. Although the use of bovine blood plasma is not well supported by the literature, intravenous blood product

Table 1

Whole blood, erythrocyte concentrate, and plasma obtained following centrifugation

Farm	Breed	Cows	Blood unit 1 (g)	Blood unit 2 (g)	Erythrocyte concentrate unit 1 (g)	Erythrocyte concentrate unit 2 (g)	Plasma unit 1 (g)	Plasma unit 2 (g)
Farm 3	Montbéliard	1	649	611	239	227	349	371
		2	562	547	191	180	339	335
		3	492	548	175	220	265	288
		4	538	548	192	193	311	319
		5	542	552	179	143	334	328
		6	575	555	193	179	345	334
		7	540	532	207	197	312	302
Mean			*556.86*	*556.14*	*196.57*	*191.29*	*322.14*	*325.29*
Standard deviation			*48.15*	*25.25*	*21.40*	*28.12*	*29.33*	*26.58*
Farm 2	Romanian Black Spotted	1	490	226	193	66	295	154
		2	533	492	188	173	346	318
		3	523	510	203	191	322	320
		4	491	528	148	244	308	290
		5	513	526	214	221	298	301
		6	450	495	171	203	280	303
Mean			*500.00*	*462.83*	*186.17*	*183.00*	*308.17*	*281.00*
Standard deviation			*27.29*	*106.80*	*21.58*	*56.89*	*21.19*	*57.71*
Farm 1	Holstein	1	549	542	164	168	334	328
		2	548	560	184	167	324	347
		3	560	509	209	186	326	311
Mean			*552.33*	*537.00*	*185.67*	*173.67*	*328.00*	*328.67*
Standard deviation			*5.44*	*21.12*	*18.41*	*8.73*	*4.32*	*14.70*
Overall Mean			***527.53***		***186.06***		***315.54***	
Overall standard deviation (SD)			***39.01***		***25.85***		***18.57***	

therapy has become a common medical practice because of the excellent results, notably in small animals (12) and equine medicine (15).

Erythrocyte concentrate, leukocyte-rich plasma, normal bovine plasma, and hyperimmune serum are a few examples of fractionated blood products used in farm animal transfusion treatment. Of them, only two bovine plasma and hyperimmune serum are regularly utilised in cattle (18).

Murphy et al. (2014) concluded that the findings of their study show that plasma products with sufficient immunoglobulins may fail to generate appropriate immunity when provided to calves after treating diarrhoea with intravenous blood plasma. Two years later, Boccardo et al. (2016) indicated that intravenous infusion of approx. 62 g of centrifugated plasma is an effective and safe method for providing adequate IgG concentrations for the passive transfer of immunity.

Considering that the literature on this subject is not particularly well-documented, the data from the current study offers helpful hints for blood product procurement in cattle. The current effort also aims to serve as inspiration for plasmapheresis-related future research.

CONCLUSIONS

Even though prophylaxis is essential in farm animal care and treatments are frequently administered in large groups, there are some circumstances in which priceless animals must be preserved individually. The use of blood products is also problematic, particularly in calves but also in adult cows. The intravenous infusion of blood plasma or whole blood is inescapably required due to the failure of passive transfer of immunity or certain neonatal diarrhoea. Through the lens of the obtained plasma and erythrocyte concentrate ratio, present results may serve for future research and may also represent a tool of significant benefit for practitioners.

Acknowledgment

This work was financially supported by University of Agronomic Sciences and Veterinary Medicine of Bucharest (UASVM Bucharest) by an Internal Research Project, Contract no. 1061/15.06.2022 COD 2022-0019 – "The development of eco-innovative therapies for the treatment and prophylaxis of calves' neonatal enteritis, in order to reduce the consumption of antimicrobials and ensure animal health".

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