In vitro feasibility of bovine whole blood and commercially prepared canine whole blood and packed red blood cells as a source of xenotransfusion in swine (Sus scrofa domestica) (#109345)

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In vitro feasibility of bovine whole blood and commercially prepared canine whole blood and packed red blood cells as a source of xenotransfusion in swine (Sus scrofa domestica)

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Since sourcing porcine blood for transfusions to porcine recipients is diûcult, bovine or canine blood products might represent alternative sources. The primary objective of this study was to determine the frequency of incompatible major (CMMa) and minor (CMMi) crossmatches by the standard saline agglutination tube method (SSA) between 1) bovine whole blood (bWB) and whole blood from commercial pigs (pWB), and 2) canine whole blood or commercially-prepared packed red blood cells (pRBCs) with whole blood from companion pigs. A secondary objective was to determine the agreement between the reference method (SSA) and a quick slide (QS) crossmatch. Blood was collected from 12 heifers, 7 companion pigs, and 8 commercial-cross pigs. A0 blood typing was performed for all porcine samples. Bovine blood was pooled into 8 bags each containing 3 crossmatch-compatible individuals. Canine blood included whole blood from 3 canine blood donors (DEA 1.1, 5, 7 negative, and DEA 4 positive), and 3 bags each of DEA 1.1 negative and DEA 1.1 positive pRBCs. Sixty-four and sixty-three pairs of crossmatches were performed for bovine-to-porcine and canine-to-porcine samples, respectively. Incompatibility was deûned as any macroscopic or microscopic agglutination or hemolysis on either CMMa and CMMi and reported separately. Complete incompatibility was deûned as incompatibility of both CMMa and CMMi on the same pair. Kappa statistics tested the agreement between SSA and QS (signiûcance at P <0.05). For bWB and pWB, agglutination was observed in 9.4% of CMMa and 100% of CMMi via SSA. Incompatibility on CMMa of bWB was more frequent with porcine blood type <0= (P =0.0107) than with type <A=, whereas porcine blood group had no eûect on CMMi results. All canine-to-porcine CMMa were incompatible with SSA and showed hemolysis severe enough to prevent evaluation of agglutination. The accuracy of QS at detecting incompatibilities was 87.5% in

CMMa and 98.4% in CMMi in bovine-to-porcine samples. Agreement between SSA and QS methods was fair (» = 0.36) for bovine-to-porcine CMMa but could not be calculated for CMMi due to lack of compatible matches. Because all canine-to-porcine CMMa were incompatible, the eûects of the porcine blood group on incompatibility, accuracy of QS, and agreement between SSA and QS could not be calculated for CMMa. For CMMi, the agreement between tests was poor (» = 0). When a xenotransfusion to a pig is indicated, bWB appears to be suitable based on *in vitro* CMMa testing, whereas canine blood products are contraindicated for *in vivo* administration to swine based on absolute CMMa incompatibility and incidence of hemolysis. *In vivo* studies are needed to elucidate the clinical signiûcance of CMMi incompatibilities. Based on these results, QS cannot be accurately used as a surrogate of SSA in pretransfusion testing due to the increased risk of false compatible results as QS can only be identiûed as agglutination, not hemolysis.

In vitro feasibility of bovine whole blood and commercially prepared canine whole blood and packed red blood cells as a source of xenotransfusion in swine

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Short running head: *Crossmatch for xenotransfusion in swine*

Keywords: Blood, Bovine, Canine, Crossmatching, Dog, *ln vitro*, Pig, Swine, Xenotransfusion

Abstract

Background. Since sourcing porcine blood for transfusions to porcine recipients is difficult, bovine or canine blood products might represent alternative sources. The primary objective of this study was to determine the frequency of incompatible major (CMMa) and minor (CMMi) crossmatches by the standard saline agglutination tube method (SSA) between l) bovine whole blood (bWB) and whole blood from commercial pigs (pWB), and 2) canine universal donor whole blood or commercially-prepared packed red blood cells (pRBCs) with whole blood from companion pigs. A secondary objective was to determine the agreement between the reference method (SSA) and a quick slide (QS) crossmatch.

Methods. Blood was collected from l2 heifers, 7 companion pigs, and 8 commercial-cross pigs. A0 blood typing was performed for all porcine samples. Bovine blood was pooled into 8 bags each containing 3 crossmatch-compatible individuals. Canine blood included whole blood from 3 canine blood donors (DEA l.l, 5, 7 negative, and DEA 4 positive), and 3 bags each of DEA l.l negative and DEA l.l positive pRBCs. Sixty-four and sixty-three pairs of crossmatches were performed for bovine-to-porcine and canine-to-porcine samples, respectively. Incompatibility was defined as any macroscopic or microscopic agglutination or hemolysis on either CMMa and CMMi and reported separately. Complete incompatibility was defined as incompatibility of both CMMa and CMMi on the same pair. Kappa statistics tested the agreement between SSA and QS (significance at $P<0.05$).

Results. For bWB and pWB, agglutination was observed in 9.4% of CMMa and l00% of CMMi via SSA. Incompatibility on CMMa of bWB was more frequent with porcine blood type "0" ($P=0.0l07$) than with type "A", whereas porcine blood group had no effect on CMMi results. All canine-to-porcine CMMa were incompatible with SSA and showed hemolysis severe enough to

prevent evaluation of agglutination. The accuracy of QS at detecting incompatibilities was 87.5% in CMMa and 98.4% in CMMi in bovine-to-porcine samples. Agreement between SSA and QS methods was fair (» = 0.36) for bovine-to-porcine CMMa but could not be calculated for CMMi due to lack of compatible matches. Because all canine-to-porcine CMMa were incompatible, the effects of the porcine blood group on incompatibility, accuracy of QS, and agreement between SSA and QS could not be calculated for CMMa. For CMMi, the agreement between tests was poor (» = 0).

Discussion. When a xenotransfusion to a pig is indicated, bWB appears to be suitable based on *in vitro* CMMa testing, whereas canine blood products are contraindicated for *in vivo* administration to swine based on absolute CMMa incompatibility and incidence of hemolysis. *ln vivo* studies are needed to elucidate the clinical significance of CMMi incompatibilities. Based on these results, QS cannot be accurately used as a surrogate of SSA in pretransfusion testing due to the increased risk of false compatible results as QS can only be identified as agglutination, not hemolysis.

0ver the last three decades, pigs have become established companion pets, in addition to remaining a fundamental food source and one of the most prominent experimental pre-clinical models for translational research (Swindle 2007). This population growth unfortunately has not

been accompanied by a proportional increase in evidence-based medical practices dedicated to this species, and clinical decisions are often made based on anecdotal knowledge, or by applying guidelines and practices established for other species. Therefore, species-specific evidence-based information is needed to meet the clinical standards of care received by species like canine, feline, and equine.

Transfusions of blood products are administered with the intent to replace blood components, either cells, plasma, or coagulation factors. When whole blood (WB) or packed red blood cells (PRBCs) are selected for administration to a recipient, an additional aim is to increase the patient's oxygen carrying capacity, which may have been compromised by conditions such as chronic or acute loss of blood, hemolysis, ineffective erythropoiesis, immune-mediated hemolytic anemia, chronic inflammation, and/or neoplasia (Kumar 20l7). In swine, metabolic conditions such as mineral and vitamin deficiencies (e.g., iron, copper, cobalt, B3, B5 , B6 , B9, and Bl2), toxicities (e.g., mycotoxins and rodenticides), infectious diseases (e.g., *Mycoplasma suis*), parasitism (e.g., *Ascaris suum* and others), and other gastrointestinal pathologies (e.g., esophageal ulcers, gastric ulcers, hemorrhagic ileitis, proliferative enteritis) may cause anemia and decreased oxygen carrying capacity (Clark and Coffer 2008). Elective surgical procedures such as ovariohysterectomy and castration can be another cause of hemorrhagic anemia in pigs and are being performed with increasing frequency as the population of pigs presenting to veterinary practitioners increases. In a retrospective study, surgical procedures involving the neoplastic reproductive tract have been shown to yield high percentages of hemorrhage, which in some cases has led to a patient's cardiovascular collapse and death (Cypher et al. 20l7; Mc0nie et al. 202l). Although significant efforts have been made to successfully use pigs as a xenotransplantation source for humans (Cooper 2003; Roux, Sa", and

Deschamps 2007b; 2007a; Wang et al. 20l6), evidence-based clinical guidelines for transfusion of blood products in pigs are non-existent. In fact, this species consistently fails to appear on review articles published on the matter due to a lack of evidence-based recommendations (Kumar 20l7; Credille and Epstein 20l6). The reasons for this lack of evidence-based information are multiple. Firstly, pigs have only recently acquired "pet" societal status, encouraging the owner's financial commitment. Secondly, ideal crossmatching techniques in pigs have not been established, hindering blood donor selection when treating an anemic pig with transfusions. Furthermore, the significance of the incompatibility of porcine A0 blood groups in promoting major transfusion reactions has not been established. Finally, there are no commercially available blood products for pigs (Credille and Epstein 20l6) and blood collection from a porcine donor is notoriously difficult due to the hardship in gaining reliable venous access in this species and invasive due to the need for general anesthesia and potential surgical cut-downs to harvest blood (Elane et al. 2024). As seen in our veterinary practice, lack of access to blood to restore circulating volume and oxygen carrying capacity may contribute to perioperative morbidity and mortality in this species (Cypher et al. 20l7; Mc0nie et al. 202l).

Xenotransfusion is the practice of administering blood products harvested from a subject of one species to a subject of another (Roux, Sa", and Deschamps 2007b). This practice has been investigated in several species as a mean of providing a one-time emergency transfusion based on the assumption that the recipient would not have been previously exposed to blood from the donor species, and therefore it would lack antibodies against those foreign (*xeno*-) antibodies (James et al. 2022; Buck et al. 20l8; Bovens and Gruffydd-Jones 20l3; Euler et al. 20l6; Le Gal, Thomas, and Humm 2020; 0ron et al. 20l7; J. S. Smith et al. 202l). For example, the current evidence on using dogs as donors for feline recipients is that "canine blood can be administered

to cats in genuine emergency situations when no other options exist, provided the cat has not received dog blood previously" (Caroline 20l6). Due to the current difficulties in sourcing swine blood, we are evaluating xenotransfusions to pigs to verify if this practice could represent a viable clinical practice with the potential to provide therapeutic benefit in the emergency settings due to its ability to temporarily stabilize the patient's cardiovascular system. This would allow additional time for essential diagnostic and surgical procedures to be performed, and a more suitable species-specific donor to be identified (Bovens and Gruffydd-Jones 20l3; Euler et al. 20l6). Bovine blood has been investigated as a potential source of blood to produce blood products for humans, due to its low chance of adverse reaction to the recipient's blood (Johnstone et al. 2004) and bovine hemoglobin-based oxygen carrier products exist for therapeutic tissue oxygenation (Gupta 20l9). Furthermore, cattle have easily accessible blood vessels, which makes the collection of large amounts of WB logistically and technically easier than harvesting blood from a donor pig. The volume of blood that can be safely collected from cattle is also greater than in pigs, allowing the administration of larger volumes during transfusion to more effectively raise the packed cell volume regardless of the size of the recipient pig. However, since some small animal veterinary practices may occasionally treat pet pigs, blood products available to small animal practitioners such as canine whole blood (cWB) and canine packed red blood cells (cPRBCs) are also of interest as xenotransfusion sources. To our knowledge, there are currently no veterinary studies assessing the feasibility of xenotransfusion of either WB from bovine donors (bWB) or of canine blood products to porcine recipients. Crossmatching is a serological method that, along with blood typing, constitutes the base of pretransfusion testing (Sidhu and Shah 2020). The purpose of these tests is to assess the potential for adverse transfusion reaction by evaluating for agglutination and/or hemolysis

between the donor's and recipient's blood. Two types of crossmatches are typically performed. The major crossmatch, which tests whether antibodies in the serum of the recipient cause agglutination or hemolysis of the donor red blood cells, is the most clinically relevant test, and an incompatibility contraindicates use of that donor (Wardrop 2022). Incompatibilities in the minor crossmatch, which tests recipient serum against donor red blood cells, usually do not cause life-threatening transfusion reactions (Bovens and Gruffydd-Jones 20l3).

Therefore, the primary aim of this study was to determine the frequency and degree of incompatible crossmatch (minor and major) reactions via a standard tube method (SSA) and quick slide technique (QS) using l) bWB and porcine whole blood (pWB), and 2) different types of canine blood products and companion swine whole blood (cpWB), where the types of canine blood products evaluated included cWB (DEA l.l negative), cPRBCs (DEA l.l negative), and cPRBCs (DEA l.l positive). The null hypotheses were that crossmatching assessed by SSA and QS would yield fewer than 40% incompatible reactions (micro- and macro-agglutination or hemolysis) for bWB (donor) and pWB (recipient) crossmatches or for canine blood products (donor) and cpWB (recipient) crossmatches. A secondary aim of this study was to determine the level of agreement between the results obtained by SSA and those obtained via QS to verify if the latter would constitute a quicker and easier alternative to the classic crossmatching procedure via SSA to be used in the emergency setting. The null hypothesis was that the value of the K statistics between the results produced by SSA and QS would be higher than 0.4.

Materials & Methods

Design

This experiment was designed as a prospective observational *in vitro* study conducted on a total of l27 crossmatching paired tests.

Animals

This study was approved by the Institutional Animal Care and Use Committee at the University of Tennessee (Protocol # 2965-0323). Client consent (in supplemental documents) was obtained for blood sampling from all porcine subjects prior to collection. All pigs were client-owned, and they presented to the University of Tennessee, Veterinary Medical Center due to unrelated, concomitant procedures. They ~~were~~ underwent individual housing and care as per hospital standard operating procedures for client-owned animals. Due to the lack of similar studies and existing data on this topic, there was insufficient information to calculate a sample size via power analysis. Based on the currently known distribution of blood groups in pigs (D. M. Smith et al. 2006; C. Hampton et al. 2023), a sample size of 4 pigs per major A0 blood group system was deemed to be needed (a total of 8 commercial pigs and 8 pet pigs, acting as the "recipients") to be representative of the blood group population. Inclusion criteria for swine were age (greater than 5 weeks) and (weight greater than l kg). There are ll major blood group systems in cattle, and more than l00 antigens are currently identified. Some groups are particularly rare (M, R, T, TF, Z) (Rocha et al. l998). Blood typing is no longer performed in cattle by commercial laboratories due to the complexity of the antigenic system. Therefore, the sample size of bovine donors was based on previous references (Dell, Holleran, and Ramakrishnan 2002) which indicated that assuming that ⎩ was 0.05 with the occurrence of bovine blood types being 4/ll (e.g. A, B, C, L) and maximize antigen exposure (Rocha et al. l998), a minimum of 5 bags of pooled blood from at least ll cattle would be needed. Enrolled heifers were procured from the Little River Unit at the University of Tennessee, UT Institute of

Agriculture. Cattle were housed in a heard of 24 subjects in an outdoor covered barn setting, with natural light cycle, and they were fed a roughing and grain-based diet appropriate for their age. Inclusion criteria for cattle and dogs were normal physical examination prior to blood sampling. Enrichment was not provided due to the short nature of the animal enrollment in this study. Analgesia was not provided as part of this study due to its non-invasive nature. Euthanasia was not an endpoint of this study. Dogs and pigs were discharged from the hospital and returned to owners, and cattle transferred to a separate study protocol.

ln vitro "Donor" Blood Samples

Bovine whole blood was used as a "donor" sample for crossmatching with samples from commercial-bred pigs. Approximately l0 mL of blood was collected from the jugular or coccygeal veins of l2 Holstein heifers using a 20G l" hypodermic needle connected to a plastic syringe, and tested for compatibility via a complete crossmatching technique. Compatibility of blood from the three heifers pooled in the same bag was verified prior to commencing the porcine study and prior to the pooling of bovine blood. The contribution of each heifer was limited to two pooled bags, to allow for identification of the subject that would have caused a potential reaction of incompatibility (e.g., if both bags in which the blood from the same subject reacted with porcine blood). After a 2-month period, an additional 60 mL of blood was collected from the same compatible heifers and pooled into 8 bags (Feline blood collection bag, l00 mL with ACD J0520R Jorvet) with added acid citrate dextrose (ACD) at an optimal ratio (l2.5%) (0rr et al. 202l) and stored at 4≡C for a maximum of 7 days before usage for crossmatch. The combination scheme of the pooled blood is exemplified in Table l.

The "donor" samples for the crossmatch procedures to be performed against blood samples from companion pigs included 3 canine whole blood (cWB) universal donor samples

(DEA l.l negative, DEA 4 positive, DEA 5 negative, DEA 7 negative, n = 3), and commercially purchased canine universal donor (n = 3) and DEA+ (n = 3) packed red blood cells (cPRBCs) bags. Canine whole blood (l0 mL) was sampled from 3 client-owned healthy dogs (two desexed male and one female) for which client consent had been obtained. The dogs had previously been blood typed and profiled with an IDEXX Blood Typing Complete Panel. The dogs were admitted to the Veterinary Medical Center for blood sampling. They had no food or water restriction for the day of sampling. They were housed for a maximum of 4 hours in individual pens at the Veterinary Medical Center and receive standard case for admitted canine patients. Samples were collected using a 20G l" hypodermic needle connected to a plastic syringe, and stored at 4≡C prior to performing CMs. Bags of cPRBCs were purchased and stored at 4≡C for a maximum of l5 days before usage for the crossmatching procedure.

ln vitro "Recipient" Blood Samples

Blood samples (l0 mL) from eight commercial pigs (6 barrows and 2 sows, weight 255.9 ± 72.9 kg, age 7.l ± 2.7 years) and seven companion pigs (l boar, 4 sows, 2 gilts, weight 69.6 ± 30.9 kg, age 8.2 ± 5.8 years) were opportunistically collected using a 20G l" hypodermic needle connected to a plastic syringe under sedation or general anesthesia from various collection sites (vena cava, jugular, tarsal, auricular veins) and placed in EDTA (Blood Collection Tube, EDTA 7.5%, Lavender, Cardinal Health) and serum collection tubes (Blood Collection Tube, Serum, Red Lid, No Additives, Cardinal Health). Blood typing was performed via a clinically validated method (EldonCard 25ll; Eldon Biological A/S, Gentofte, Denmark) (C. Hampton et al. 2023; C. E. Hampton, Zhu, and Giori 2023) according to the A0 blood group system, and used as the "recipient" samples for the crossmatch procedure. Four blood type "A" commercial and companion pigs, and 4 blood type "0" commercial and companion pigs were included in the

study. Health status was not an exclusion criterion based on previous references (C. E. Hampton, Zhu, and Giori 2023). Three mL of WB samples were placed in labeled round-bottom borosilicate tubes (Fisherbrand round bottom disposable tube, Fischer Scientific) and centrifuged at 2600 g for 3 minutes, and plasma (at least 750 µL) was transferred to a new labeled tube.

Washed Red Blood Cell Procedure

All red blood cell (RBC) samples were washed and diluted to a 4% suspension. Fifty µL of the donor's and recipient's RBCs were placed in a round bottom tube and filled with approximately 2 mL of phosphate buffered saline (PBS, Thermofisher Chemicals). The samples were centrifuged at l000g for l minute and saline aspirated and discarded. This washing procedure was repeated 2 more times with a final l.2 mL of PBS added to create the final 4% suspension.

Standard Saline Tube Agglutination

A single blood sample was collected from each "recipient". Each sample from commercial pigs was crossmatched with eight pooled bags of bovine blood (n = 64 pairs). Each sample from companion pigs was crossmatched with three samples of cWB (n = 2l pairs), three samples of cPRBCs DEA l.l positive (n = 2l pairs), and three samples of cPRBCs DEA l.l negative (n = 2l pairs). Complete crossmatching was performed on all donors and recipient samples, including one auto-control per sample within 4 hours of blood collection from the "recipients". Sample preparation included making a 4% RBC suspension as previously described. The CMMa was performed using l00 µL of recipient serum and 50 µL of donor's 4% RBCs. The CMMi was performed using l00 µL donor plasma and 50 µL recipient 4% RBCs. The auto-control was performed using l00 µL recipient serum and 50 µL recipient 4% RBCs. Some control samples (QS: porcine plasma + porcine pRBC; SSA: porcine serum + washed

253 porcine pRBC) were performed with the use of with rabbit or guinea pig complement. All
254 samples were incubated for 30 minutes at 37ºC. After incubation, the tubes were centrifuged at
255 l000g for 30 seconds. Agglutination was first evaluated macroscopically based on a graded scale
256 (Table 2) (Guzman, Streeter, and Malandra 20l6), and if none was detected, it was evaluated
257 microscopically. The presence of microscopic agglutination was reported as binary (positive or
258 negative). Samples were evaluated for hemolysis in the supernatant and compared to the auto-
259 control with a standardized scoring system shown in Figure l and Table 3, Newman, ALW
26O 20l4). Incompatibility was defined as any macroscopic or microscopic agglutination or
261 hemolysis. All CMs, including macroscopic and microscopic evaluations were performed by the
262 same individual (VMD), who was extensively trained prior to the commencement of the study.
263
264 *Quick slide procedure*
265 Complete crossmatching via QS was performed on all donors (bovine and canine) and
266 porcine samples with one auto-control for every porcine sample. The CMMa was done by
267 mixing 2 drops of recipient plasma with l drop of donor pRBCs on a microscope slide. The
268 CMMi was done by mixing two drops of donor plasma with one drop of recipient pRBCs on the
269 microscope slide. The slides were rotated for 2 minutes to allow thorough mixing of plasma and
27O RBCs while assessing for macroscopic agglutination. If CMMa and CMMi slides were free from
271 macroscopic agglutination, they were evaluated for microscopic agglutination by performing a
272 saline dilution test to distinguish agglutination from rouleaux. A microscopic dilution of the
273 auto-control was also performed for comparison. In brief, four drops of saline from a transfer
274 pipette were placed on a microscope slide with a small amount of the slide mixture from a
275 pipette tip to create an approximate l:50 dilution of RBCs. The slide was gently rotated to mix

blood and saline. A coverslip was placed over the sample and evaluated for microscopic agglutination.

Statistical Analysis

Results from CMs and microscopic evaluations were recorded manually and then transcribed and stored in a commercially available spreadsheet (Excel, Microsoft, Redmond, WA). Incidence of compatibility was reported in percentage for complete crossmatching, CMMa, and CMMa for both techniques. Mann-Whitney U test was used to determine the difference between porcine blood groups in terms of compatibility with bovine blood. The accuracy of QS was calculated via the following formula:

$$\left[\frac{(\#\ddot{y}\acute{y}\ 2\ \#\ \acute{y}\ddot{y}\ \acute{y}\ddot{y}\acute{y}\ddot{y}\acute{y}\ddot{y}\acute{y}\ddot{y}\ \ddot{y}\acute{y})}{\#\ddot{y}\acute{y}}\right] 7\ l00$$

Kappa statistics was used to test the level of agreement between SSA and QS. Kappa statistics were categorized as poor (0-0.20), fair (0.2l-0.40), moderate (0.4l-0.60), good (0.6l-0.8), or very good (0.8l-l.00). Parametric and non-parametric data are reported as appropriate. Significance was set at $P < 0.05$. MedCalc® Statistical Software version 22.009 was used for all the statistical analysis (0stend, Belgium; https://www.medcalc.org; 2023).

Results

Twelve healthy Holstein heifers aged 237 ± 32 days, three dogs (2 male castrated and one spayed female; 3.9 ± 2.3 year old), eight commercial pigs (6 male castrated and 2 intact females; 7.l ± 2.7 year old; 255.8 ± 72.9 kg), and seven companion pigs (l male castrated, 4 intact female, 3 female spayed; 8.2 ± 5.7 year old; 69.6 ± 30.9 kg) were enrolled in the study. Breeds are specified in Table 3. Collection of samples from companion pigs was stopped after 7 pigs

298 since all samples were highly incompatible. Results regarding agglutination and hemolysis are
299 summarized in Table 4.

3OO *Bovine-to-porcine Whole Blood Crossmatches*

3O1 Sixty-four pairs of CMs were performed on bWB and pWB samples via SSA and QS.
3O2 Briefly, agglutination was observed in 9.4% of CMMa, and in l00% of CMMi via SSA of bWB
3O3 and pWB, and in 9.4% and 98.4% via QS on the same samples. Therefore, *in vitro* compatibility
3O4 between bWB and pWB based on CMMa was 90.6%. Some control samples resulted in
3O5 agglutination and hemolysis despite being from the same patient when complement was added to
3O6 the crossmatching procedure. When using saline in place of the complement, there were no
3O7 agglutination or hemolysis noted.

3O8 *Canine-to-porcine Whole Blood and pRBCs Crossmatches*

3O9 Sixty-three pairs of CMs were performed on cWB and cpWB samples via SSA and QS.
31O All canine-to-porcine CMMa on SSA and QS were incompatible with all tested canine products,
311 independent of the DEA antigen profile. In the canine-to-porcine CMs, hemolysis was severe
312 enough to prevent the evaluation of agglutination. Two/6 auto-control were scored as a l+ on
313 SSA for agglutination, and 0/6 presented hemolysis.

314 *Effect of Porcine Blood Type on Compatibility*

315 The compatibility of bWB with porcine blood type "A" on CMMa was greater than that
316 of pBW type "0" (P = 0.0l07). Porcine blood group had no effect on CMMi results. The effects
317 of the porcine blood group on incompatibility could not be calculated for canine-to-porcine
318 CMMa due to the absolute lack of compatible matches.

319 *Performance of Quick Slide Method*

Incompatibility reactions based on agglutination and hemolysis on SSA were compared to incompatibility based on agglutination only on QS due to the fact that this technique is unable to detect hemolysis. The accuracy of QS was 87.5% for detection of incompatibilities in CMMa and 98.4% in CMMi in bovine-to-porcine samples. Agreement between SSA and QS methods was fair (» = 0.36) for bovine-to-porcine CMMa but could not be calculated for CMMi due to lack of compatible matches. The accuracy of QS and agreement between SSA and QS could not be calculated for canine-to-porcine CMMa due to the absolute lack of compatible matches. For CMMi, the agreement between tests was poor (» = 0). The QS control samples contained individual porcine plasma and porcine pRBC. The QS still resulted in a positive microscopic agglutination (l+). However, when performed on the SSA, there were no signs of reaction (hemolysis or agglutination), once again showing that the SSA is the more reliable testing method.

Discussion

This is the first study investigating the *in vitro* compatibility of porcine blood with blood products from two other species, bovine and canine. 0ur findings suggest that due to the high frequency of compatibility on major crossmatching between bovine donors and porcine recipients, the practice of xenotransfusion between these species may be supported. 0n the contrary, due to the high incidence of hemolysis on both major and minor crossmatching between canine products and porcine samples, we strongly discourage *in vivo* xenotransfusions involving a canine donor and a porcine recipient.

The aim of the CMMa procedure is to evaluate for antibodies in the recipient's plasma that react with the donor's RBCs (Sidhu and Shah 2020; Wardrop 2022). Incompatibility on

CMMa is considered a contraindication for the administration of blood products from one individual to another, no matter the species. Even though this concept has been challenged by some recent findings, that is, cats being given type specific blood with and without crossmatching (reaction criteria being l+ agglutination and hemolysis of l-2+) (Sylvane et al. 20l8), the current recommendation is to use plasma and RBCs for canine-to-feline xenotransfusions that are at least compatible on a CMMa, since this type of crossmatch best mimics host immunity (Wardrop 2022; Weltman, Fletcher, and Rogers 20l4). Bovine blood has already been evaluated as a potential transfusion source for humans (Johnstone 2004). In a recent in vitro crossmatching study, bovine red blood cells were commonly compatible on the major crossmatch with canine plasma (Salazar 2024). Similarly, in our study, the majority of crossmatches between bovine red blood cells and porcine plasma were also compatible, providing more support for the use of bovine blood in xenotransfusions. Because there are a large number of bovine blood groups, it is statistically probable that the bovine donors used in our study expressed a variety of red cell antigens, yet the majority of crossmatches with porcine plasma were still compatible, suggesting that it is unlikely that pigs produce naturally-occurring antibodies to bovine red cell antigens. However, the minor crossmatch between porcine red blood cells and bovine plasma was always incompatible in our study, supporting that cows do have naturally-occurring antibodies to both type A and type 0 pig blood. Heifers were used to prevent any potential allo-antibodies that could be circulating from prior sensitization to a fetus with a different blood. We did not evaluate whether or not there would be any incompatibility with the use of cows vs. heifers with any *in vitro* samples. Multiparous cows were the main donors for xenotransfusions to goats, and there were no extreme reactions (J. S. Smith et al. 202l).

366 The aim of the CMMi procedure is to verify the compatibility of the donor's plasma and the
367 recipient's RBCs (Sidhu and Shah 2020; Wardrop 2022). Most paired bovine-to-porcine samples
368 were incompatible on CMMi, which would test for the presence of anti-porcine antibodies in
369 transfusion-na"ve bovine donor plasma. Incompatibility on CMMi is considered less clinically
37O significant than the CMMa unless transfusing blood products rich in plasma content (Tocci and
371 Ewing 2009), as they are less likely to cause a transfusion reaction, since the donor plasma
372 would be diluted *in vivo*, leading to less exposure of the recipient red blood cells to foreign
373 plasma (Bovens and Gruffydd-Jones 20l3). Supporting this, two studies of canine to feline
374 xenotransfusions showed that cases with incompatible minor crossmatches had no clinically
375 detectable adverse effects despite administration of transfusion (Bovens and Gruffydd-Jones
376 20l3; Le Gal, Thomas, and Humm 2020). However, another study showed that the average
377 lifespan of canine erythrocytes transfused into cats was very short, less than 4 days (Euler et al.
378 20l6). Therefore, based on the minor crossmatch incompatibilities found in this study, using
379 solutions of bovine pRBCs where some of the plasma is removed might reduce the risk of
38O transfusion reactions in the porcine recipient. However, further studies would be needed to
381 determine if the circulating lifespan of bovine erythrocytes in xenotransfusion is similarly short,
382 but the results highlight that xenotransfusion is likely to be most useful for short-term
383 management of critical patients, such as those with acute blood loss where compatible same-
384 species donor blood is not available (Bovens and Gruffydd-Jones 20l3).

385 In contrast to our results using bovine blood, all major crossmatch results between canine
386 erythrocytes and porcine plasma were incompatible. Additionally, many of the minor
387 crossmatches were also incompatible, typically showing both hemolysis and agglutination. This
388 supports the idea that both pigs and dogs have naturally-occurring antibodies to red cell antigens

389 of other species. For this reason, administration of canine blood to swine patients is not
39O recommended, even in the immediate need for life-saving xenotransfusion.

391 In applicable clinical conditions as deemed by the clinician and patient presentation,
392 xenotransfusions have been performed between a wide variety of species. In these situations, the
393 nearest similar species has usually been the donor of choice. While the resources are slim for
394 species beyond the major veterinary species (canine, feline, and equine) for transfusion therapy,
395 there are cases of *in vivo* xenotransfusions in lesser studied species. The majority of
396 xenotransfusions in large animals are with bovine donors (J. S. Smith et al. 202l; Buck et al.
397 20l8; Brown and Vap 20l2; James et al. 2022). Despite the lack of literature on porcine
398 xenotransfusions, there is enough evidence from other species studies that even with an
399 incompatible result (in this case the minor), a xenotransfusion can still be life-saving. Recipient
4OO sensitization occurs in rabbits similar to other mammals (Dannemiller et al. 2024; Bovens and
4O1 Gruffydd-Jones 20l3; Kisielewicz and Self 20l4; Kumar 20l7). *In vivo* studies would be needed
4O2 to evaluate whether simply having crossmatch compatible blood is effective on post-transfusion
4O3 significantly improved PCV without having type-specific blood (Weltman 20l4).

4O4 0ur results support that the quick slide method should not be used as a surrogate of SSA
4O5 in pre-transfusion testing due to the increased risk of false compatible results between donor and
4O6 recipient. Statistically, the test is not capable of detecting as many complete incompatibilities as
4O7 the reference method. There is then a risk of false compatibility of the CM in an incompatible
4O8 donor/recipient pair if only QS is used. Rather, a complete crossmatch procedure by saline tube
4O9 agglutination or using a crossmatch kit is recommended.

41O Several limitations deserve mention in this discussion. Causes of incompatible
411 crossmatch could be due to patient or donor unit factors, but also to technical or clerical errors as

412 previously reported by Sidhu and Shah in 2020 and potential and inherent errors may have
413 affected our results. However, we followed rigorous standard operating procedures created *ad*
414 *hoc* for this study to minimize these errors. The detection of hemolytic reaction in large animal
415 species usually requires the addition of complement, in this case commercially available guinea
416 pig or rabbit serum (Clark and Coffer 2008; Wardrop 2022), since incompatibilities in these
417 species may sometimes result only in hemolysis without agglutination. However, in our study,
418 the addition of guinea pig or rabbit complements hemolyzed all CM pairs, indicating that the
419 animals that were the source of the complement had antibodies against pig erythrocytes. These
42O cross-reacting antibodies can be removed from the complement reagent through an absorption
421 procedure (Brown and Vap 20l2). However, the addition of complement with this absorption
422 procedure is expensive and time-consuming and may not be feasible for many laboratories
423 performing crossmatching procedures. Therefore, we acknowledge that hemolysis may have
424 been under-detected in this study. This was easily detected when the SSA was performed with
425 the canine blood, even without the addition of complement. Another limitation is the presence of
426 positive results of the auto-control which may affect our ability to accurately determine whether
427 incompatibility is a normal attribute compared to the control or an actual positive result. This
428 phenomenon only occurred with the quick slide method and therefore, has limited clinical
429 significance as this method has been shown to be unreliable in the current study. Finally, testing
43O for bovine blood groups is clinically not available. Therefore, our study cannot point to blood
431 groups or antigens that were responsible for incompatibility. This limitation was handled by
432 pooling bovine blood groups to increase antigen exposure to porcine blood. To our point, we
433 were able to isolate two individual donors that had at least one incompatible result (Appendix l).

However, having a variety of individuals to do a crossmatch would be ideal in order to find a compatible donor for a transfusion.

Conclusions

This study contributes to the current body of knowledge in the field of xenotransfusion. Based on these *in vitro* results, for any clinical scenario in which a blood product transfusion is indicated, bovine whole blood holds potential as a donor source for swine and appears to be suitable based on *in vitro* pre-transfusion testing, whereas canine blood products appear to cause severe hemolysis and are therefore considered contraindicated for administration to pigs. *ln vivo* studies are needed to elucidate the clinical significance of the incompatibility of major and minor crossmatch in pigs. In order to improve the compatibility of bovine-to-porcine xenotransfusions based on CMMi, bovine pRBCs may be a suitable blood product for administration to swine recipients. Further *in vivo* investigation is warranted.

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569

57O

Table 1 (on next page)

See individual tables

Pool Bag Code	Subject Combination
A	B1/B2/B3
B	B1/B4/B5
C	B2/B6/B7
D	B3/B8/B9
E	B4/B10/B11
F	B5/B6/B12
G	B7/B9/B11
H	B8/B10/B12

Table 1. Scheme of bovine blood pooling to increase antigen exposure with porcine blood samples during *in vitro* crossmatching. B = Bovine; 1-12 = Subject.

Grade	Description
0	No agglutination visible microscopically or macroscopically
1+	No macroscopic agglutination, but weak or transient microscopic adherence of RBCs where there are groups of 2-3 cells that appear loosely aggregated and may be difficult to distinguish from rouleaux. Dilute the sample further by mixing equal parts of sample and saline and reevaluate to see if the associations disperse.
2+	No macroscopic agglutination, but small microscopic agglutinates present (4-10 RBCs per agglutinate)
3+	No macroscopic agglutination, but at least 1 large microscopic agglutinate (>10 RBCs per agglutinate)
4+	Macroscopic (grossly visible) agglutination

Table 2. Scoring scale for agglutination grading (Guzman, Streeter, and Malandra 2016).

Grade	Description
0	No visually detectable hemolysis
1+	Slight hemolysis (*tube l in the referenced image*)
2+	Moderate hemolysis (*tubes 2-3 in the referenced image*)
3+	Marked hemolysis (*tubes 4-6 in the referenced image*)

Table 3. Scoring scale for hemolysis grading (Newman, ALW 2014).

Species	Number of Subjects	Breed
Bovine	12	Holstein

Canine	1	Bulldog Mix
	1	Husky Mix
	1	Labrador Retriever
Porcine (Commercial)	3	Large White
	2	Wild Boar
	2	Spotted
	1	Yorkshire Cross
Porcine (Companion)	3	Vietnamese Potbellied Pig
	2	American Mini Pig
	2	Kune Kune

Table 4. Summary of breeds and number of subjects enrolled in the present study.

Figure 1

Grading scale for evaluation of hemolysis as described in Table 1.