Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(12): 331
https://doi.org/10.31083/j.fbl2812331

Short Communication

Examination of Bovine Red Blood Cell Death *in Vitro* in Response to Pathophysiologic Proapoptotic Stimuli

Bailey Kennedy[1], Sonia Mirza[2], Tania Mandiangu[2], Rosi Bissinger[3], Theresa E. Stotesbury[1,4], Holly Jones-Taggart[1,2], Julia Green-Johnson[1,4], Syed M. Qadri[1,2,*]

[1]Applied Bioscience Program, Ontario Tech University, Oshawa, ON L1G 0C5, Canada
[2]Faculty of Health Sciences, Ontario Tech University, Oshawa, ON L1G 0C5, Canada
[3]Department of Internal Medicine, Division of Endocrinology, Diabetology and Nephrology, University Hospital Tübingen, 72076 Tübingen, Germany
[4]Faculty of Science, Ontario Tech University, Oshawa, ON L1G 0C5, Canada
*Correspondence: syedm.qadri@ontariotechu.ca (Syed M. Qadri)
Academic Editor: Ioanna-Katerina Aggeli
Submitted: 20 September 2023 Revised: 18 October 2023 Accepted: 26 October 2023 Published: 6 December 2023

Abstract

Background: Interspecies variations in mammalian red blood cells (RBCs) are observed in circulating RBC lifespan, cell size, fluidity, aggregation, water permeability, metabolism, lipid composition, and the overall proteome. Bovine RBC cell membrane is deficient in phosphatidylcholine and exhibits anomalies in the arrangement of phosphatidylethanolamine within the lipid bilayer. However, like human RBCs, virtually all the aminophospholipid phosphatidylserine (PS) is found within the cytoplasmic side of the cell membrane of intact circulating bovine RBCs. During apoptotic cell death of human and murine RBCs, PS translocates to the outer leaflet of the cell membrane via Ca^{2+}-dependent and -independent signaling mechanisms. However, little is known about this process in bovine RBCs. **Methods**: Using cytofluorometry analyses, we characterized and compared the cell death responses in bovine and human RBCs *in vitro* exposed to various pathophysiologic cell stressors. **Results**: Ionic stress, by ionophore treatment, and oxidative stress enhanced cytoplasmic Ca^{2+} levels and cell membrane PS expression in both bovine and human RBCs. Fever-grade hyperthermia and energy starvation promoted Ca^{2+} influx and elevated reactive oxygen species levels in both human and bovine RBCs. However, bovine RBCs displayed minimal increases in PS expression elicited by hyperthermia, energy starvation, and extracellular hypertonicity as compared to human RBCs. In response to decreased extracellular osmolality, bovine RBCs exhibited significantly enhanced fragility as compared to human RBCs. **Conclusions**: Bovine RBCs display differential cell death patterns as compared to human RBCs, only partly explained by increased Ca^{2+} influx and oxidative stress. Premature removal of circulating RBCs could potentially contribute to the pathogenesis of anemia in cattle caused by a wide range of factors such as systemic diseases, parasitic infections, and nutritional deficiencies.

Keywords: red blood cells; cell death; bovine; calcium; anemia; cell stress; hemolysis

1. Introduction

Evolutionary differences in the circulatory systems of vertebrates are paralleled by functional variations in red blood cell (RBC) morphology, numbers, and biophysical properties [1,2]. Mammalian RBCs are characterized by enucleation of erythroid cells towards the end of their maturation [3]. Interspecies differences in mammalian RBCs are observed in RBC cell size, aggregation, metabolism, membrane lipid composition, and the overall proteome [4–8]. These variations dictate differences in hemorheological properties and the RBC's ability to adapt to various extracellular environmental challenges in the circulation [9–12]. RBC lifespan varies across mammalian species with bovine RBCs known to circulate longer (~160 days) than human RBCs (~120 days) [13]. Premature cell death of circulating RBCs prior to completion of their expected lifespan *in vivo* has been demonstrated in a wide range of human diseases associated with anemia, as well as in mouse models of different clinical conditions [14]. However, little is known about this phenomenon in cattle.

During their lifespan, RBCs accrue a wide range of physical injuries together with enzymatic alterations leading to their senescence and subsequent clearance from the circulation by phagocytic catabolism [13–15]. In the context of various systemic diseases in humans, RBCs may be afflicted by oxidative injuries leading to reduced survival [16]. Other pathophysiologic cell stressors, such as fever-range hyperthermia, extracellular tonicity alterations, and energy starvation, can trigger RBC dysfunction and premature cell death [14]. When excessive loss of RBCs is not compensated by enhanced erythropoiesis in the bone marrow, anemia ensues [14]. Apoptotic cell death in RBCs, also known as eryptosis, is characterized by the breakdown of the phospholipid asymmetry in the cell membrane leading to the flipping of the aminophospholipid, phosphatidylserine (PS), towards the exterior leaflet of the cell membrane [14,17]. While a wide array of mechanisms are operative during RBC cell death, Ca^{2+}-mediated signaling has been considered as a canonical pathway regulating this process in human RBCs [14].

Structurally, ruminant RBCs exhibit anomalies in their cytoplasmic membrane phospholipid architecture as compared to RBCs from other mammalian species such as the presence of N-acylated aminophospholipids [18]. Bovine RBCs are deficient in phosphatidylcholine and possess high levels of sphingomyelin in the cell membrane [19]. Strikingly, unlike human RBCs, the outer leaflet of bovine RBC cell membrane is reported to have a significantly lower proportion of phosphatidylethanolamine (2% versus 20%) [19]. Differences in composition of the lipid membranes of bovine and human RBCs have been linked with differential RBC membrane fluidity [20] and water permeability [21] between the two species. Despite the unique lipid organization in the bovine RBC membrane PS is found entirely in the inner leaflet of the lipid bilayer of intact cells, like human RBCs [19].

In bovine RBCs, the possibility of PS translocation to the outer leaflet in response to proapoptotic signals, as in human and murine RBCs, remains elusive. One important clue on the clinical significance of apoptotic cell death in stressed bovine RBCs comes from a previous study showing increased RBC PS expression in anemic cattle infected with the intraerythrocytic parasite, *Theileria sergenti* [22]. Infected cattle have been reported to display enhanced clearance, hemolysis, and morphological changes in RBCs [22–24]. Systemic bovine diseases involving RBC dysfunction and anemia can influence the overall health and productivity of livestock. Thus, characterization of the apoptotic cell death process in bovine RBCs would provide novel insights into the pathogenesis of bovine anemia resulting from accelerated loss of circulating RBCs.

2. Materials and Methods

2.1 Blood Samples and Reagents

Bovine blood was procured from a local abattoir (Windcrest Meat Packers Ltd., Port Perry, ON, Canada). Cow blood was collected into an anticoagulant-containing tube immediately following an incision in the neck as described previously [25]. For bovine RBC isolation, whole blood was centrifuged for 10 min at 1000 $\times g$ to remove cell-free supernatant and buffy coat. Phosphate-buffered saline (PBS) was then added to the RBC pellet at ~1:10 ratio and the samples were washed three times. Human RBCs (4 ♀ and 4 ♂ donors) used in this study were obtained from concentrates produced by Canadian Blood Services Blood4Research program (Vancouver, BC, Canada). Leukoreduced RBC concentrates in SAG-M were shipped to Ontario Tech University in containers validated to maintain core temperatures between 1 and 10 °C and were refrigerated on receipt. Prior to experimentation, human RBCs drawn from blood bags were washed three times in PBS to remove residual storage solution. Bovine and human RBCs (stored at 4 °C for <3 days post-collection) were incubated at 37 °C (0.5% hematocrit) in isotonic Ringer's solution (pH 7.4) prepared as described previously [26]. Supraphysiologic intracellular Ca^{2+} overload was artificially induced using *Streptomyces conglobatus* ionomycin and oxidative stress using tertbutyl-hydroperoxide (TBOOH; Sigma, St. Louis, MO, USA). Extracellular osmolality of Ringer's solution was increased with addition of 600 mM sucrose.

2.2 Flow Cytometry

Cellular markers of RBC apoptosis were examined using flow cytometry (BD Accuri C6 Plus) analysis in the FL1 channel (excitation wavelength of 488 nm and an emission wavelength of 530 nm) following previously described protocols [26]. RBC PS expression was quantified using annexin V FITC (1:100 dilution; BioLegend, San Diego, CA, USA; verified reactivity with PS in all mammalian species according to manufacturer). Relative intracellular Ca^{2+} levels in RBCs were determined using Fluo 4/AM staining (2 µM in Ringer's solution; Tocris Bioscience, Toronto, ON, Canada). Relative levels of reactive oxygen species (ROS) were examined using dichlorodihydrofluorescein diacetate staining (DCFDA, 25 µM in PBS; Sigma). RBC viability was quantified by estimating the activity of intracellular esterases using Calcein-AM (2 µM in PBS; Cayman Chemical, Ann Arbor, MI, USA) staining as described previously [27]. Mean fluorescence intensity (MFI) data were normalized to control or baseline values.

2.3 Osmotic Fragility Tests

For the determination of osmotic resistance, 2 µL of RBCs were added to 1000 µL of different mixtures of isotonic PBS and hypotonic distilled water with varying osmolalities. Relative hemolysis was measured in terms of absorption measured at 405 nm as described previously [26].

2.4 Statistics

Data are expressed as means ± standard error of mean (SEM). Statistical analyses were performed using paired Student's *t* test or ANOVA as appropriate (GraphPad Prism 8 Software, GraphPad Software, Inc., San Diego, CA, USA) where $p < 0.05$ was considered statistically significant.

3. Results

Firstly, we examined the effects of artificially enhancing intracellular Ca^{2+} content in human and bovine RBCs using ionomycin, a well-established Ca^{2+} ionophore. As shown in Fig. 1A–C, ionomycin treatment similarly enhanced Ca^{2+}-dependent Fluo 4 fluorescence in both bovine and human RBCs. While extracellular Ca^{2+} uptake responses are similar for RBCs from both species, PS expression in bovine RBCs was achieved with a higher ionomycin concentration as compared to human RBCs, potentially indicating differences in downstream enzymatic activation in RBCs from the two species (Fig. 1D–F).

Oxidative damage to human RBCs is paralleled by increased cation channel activation leading to increased

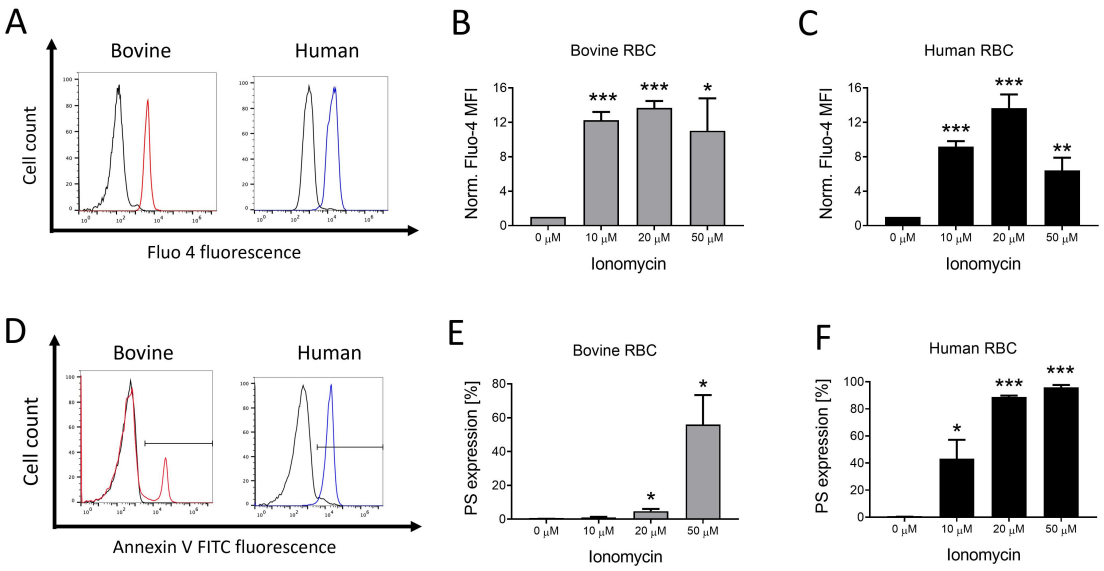

Fig. 1. Ionophore-mediated Ca^{2+} influx and cell death in bovine and human red blood cells (RBCs). Representative histogram (A,D; Black: 0 μM ionomycin; Red and Blue: 20 μM ionomycin). Means ± standard error of mean (SEM) of Fluo 4 mean fluorescence intensity (MFI) (B,C; n = 4) and percentage of phosphatidylserine (PS) expression (E,F; n = 4) in bovine and human RBCs following 1-hour incubation in Ringer's solution containing 0–50 μM ionomycin at 37 °C. *, ** and *** indicate significant difference ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively) from the absence of ionomycin treatment.

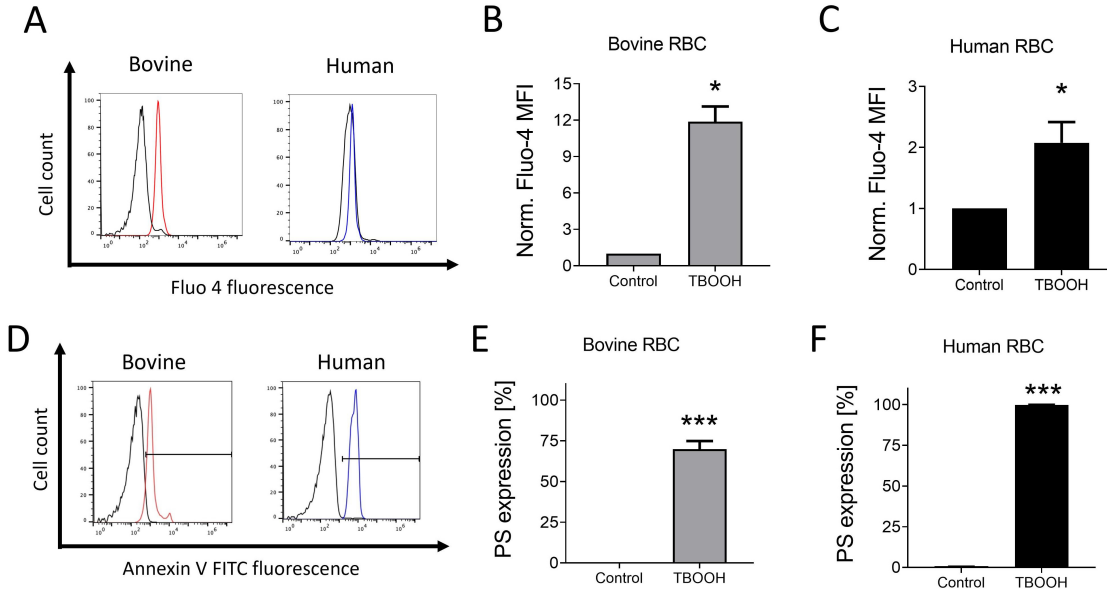

Fig. 2. Oxidative stress stimulates Ca^{2+} entry into and cell death in bovine and human RBCs. Representative histogram (A,D; Black: Control; Red and Blue: 0.2 mM tertbutyl-hydroperoxide (TBOOH)). Means ± SEM of Fluo 4 MFI (B,C; n = 4) and percentage of PS expression (E,F; n = 4–6) in bovine and human RBCs following 30-minute incubation in Ringer's solution containing 0.2 mM TBOOH at 37 °C. * and *** indicate significant difference ($p < 0.05$ and $p < 0.001$, respectively) from the absence of TBOOH (Control).

Ca^{2+} influx [28]. Acute exposure of RBCs to the oxidant, TBOOH, elicited significantly enhanced Fluo 4 fluorescence in both bovine (Fig. 2A,B) and human RBCs (Fig. 2A,C). We then interrogated the extent to which TBOOH-induced Ca^{2+} increases translated into increases in cell membrane PS exposure. As illustrated in Fig. 2D–F, oxidant treatment significantly upregulated PS expression in both bovine and human RBCs. We further tested RBC viability after oxidant treatment by determining intracellular esterase activity using quantification of Calcein fluorescence. Human RBCs exposed to oxidant treatment showed reduced Calcein fluorescence confirming a recent

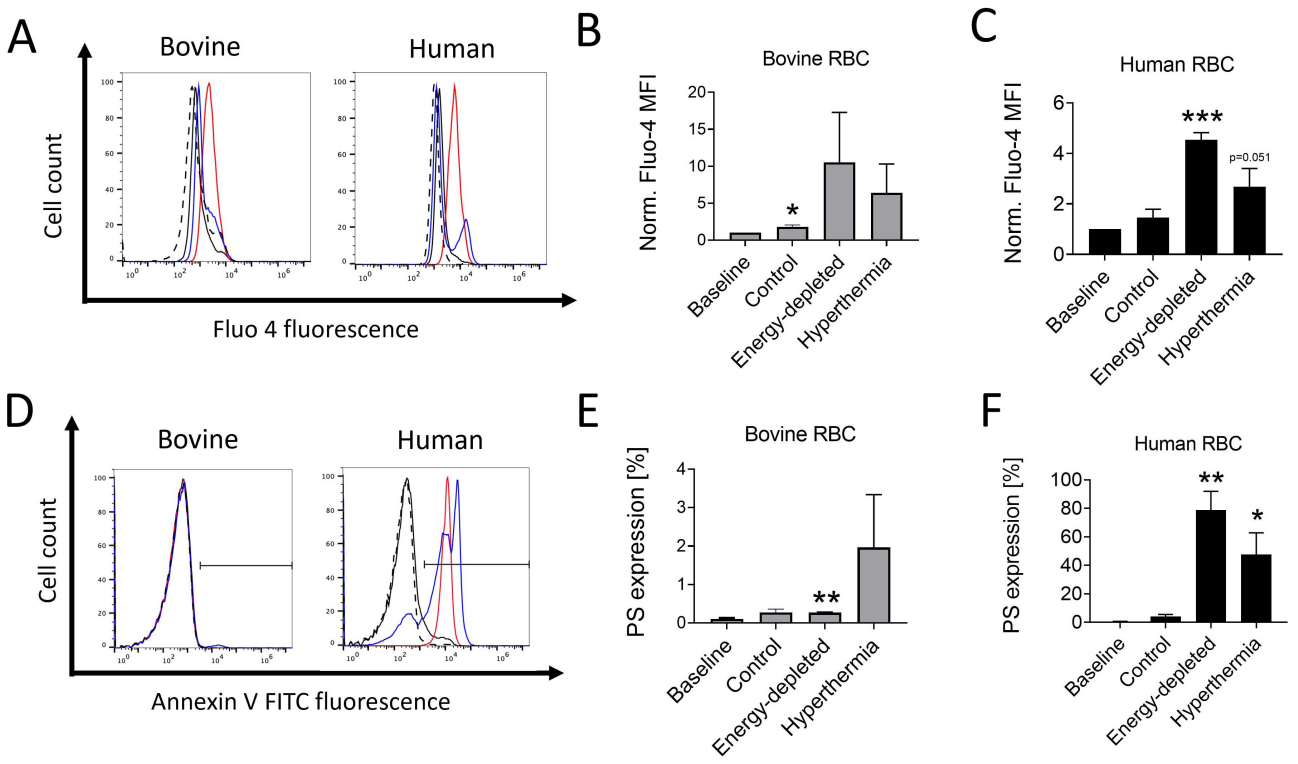

Fig. 3. Effects of hyperthermia and energy starvation on cytoplasmic Ca^{2+} levels and PS expression in bovine and human RBCs. Representative histogram (A,D; Dotted: Baseline; Black: Control; Red: Energy-depleted; Blue: Hyperthermia). Means ± SEM of Fluo 4 MFI (B,C; n = 4) and percentage of PS expression (E,F; n = 4) in bovine and human RBCs following 72-hour incubation in Ringer's solution without glucose (energy-depleted) at 37 °C or in Ringer's solution at 41 °C (hyperthermia). Baseline readings were determined in refrigerated RBCs. *, ** and *** indicate significant difference ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively) from baseline values.

report [27]. However, bovine RBCs did not show decreases in Calcein fluorescence (data not shown) possibly due to reduced cellular uptake of this dye.

Next, we evaluated bovine and human RBC survival under the conditions of fever-grade hyperthermia and glucose deprivation. As shown in Fig. 3A–C, a 72-hour incubation of bovine RBCs in Ringer's solution, glucose-devoid conditions, or exposure to 41 °C temperature upregulated intracellular Ca^{2+} levels in human and bovine RBCs as compared to intracellular Ca^{2+} levels in refrigerated RBCs (baseline). Due to large data scatter, the effects were not statistically significant in bovine RBCs. We then sought to elucidate whether enhanced cytosolic Ca^{2+} levels translated into breakdown of cell membrane phospholipid asymmetry. In contrast to human RBCs, bovine RBCs displayed only a subtle increase in PS expression in response to energy starvation and hyperthermia (Fig. 3D–F).

Additional experiments were conducted to assess whether fever-grade hyperthermia or energy starvation induced oxidative stress in bovine and human RBCs. As shown in Fig. 4A–C, DCF fluorescence, indicating increased ROS levels, was significantly enhanced in both bovine and human RBCs after a 72-hour incubation, under glucose-devoid conditions or incubation in hyperthermic conditions (41 °C), suggesting that despite starvation- and hyperthermia-associated redox imbalances in RBCs from both species, bovine RBCs displayed a minimal increase in membrane PS expression.

Deviations in extracellular tonicity are known to trigger ionic and fluid shifts in RBCs. Hemolysis in bovine RBCs was significantly higher at 30–50% reductions in osmolality as compared to human RBCs (Fig. 5A). Hypertonicity (addition of 600 mM of sucrose into the medium) significantly enhanced PS expression in human RBCs after 4 hours (Fig. 5B,E). In contrast, a modest but significant increase in PS exposure was observed in bovine RBCs after a 48-hour, but not 4-hour, incubation in hypertonic Ringer solution (Fig. 5B–D) suggesting that bovine RBCs are relatively more resistant to hypertonic stress-induced cell death as compared to human RBCs.

4. Discussion

Clinically, the pathogenesis of anemia in cattle has been ascribed to various factors such as immune-mediated RBC destruction [29], parasitic infections [30–32], and nutrient deficiency [33,34]. *In vitro* and *in vivo* studies provide some mechanistic evidence that increased erythrophagocy-

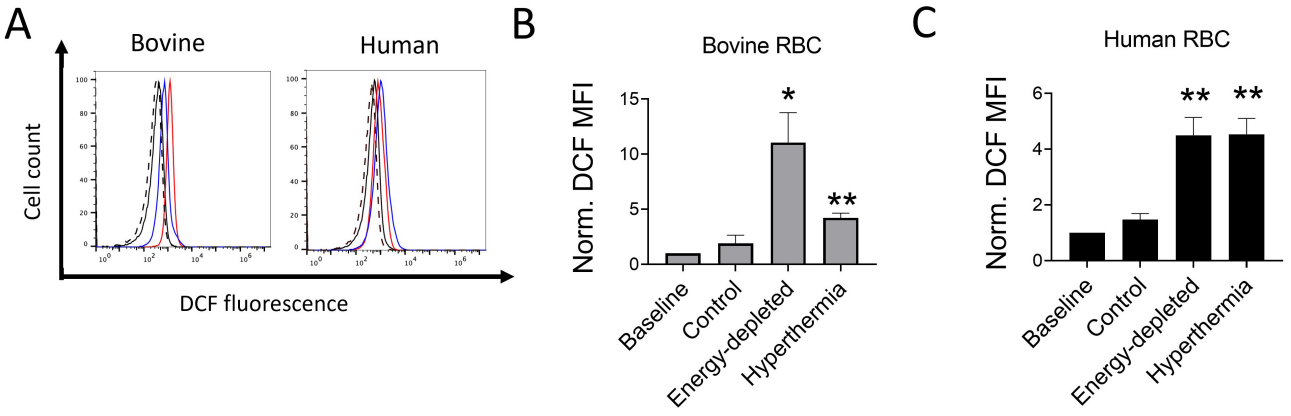

Fig. 4. Effects of hyperthermia and energy starvation on reactive oxygen species (ROS) production in bovine and human RBCs. Representative histogram (A; Dotted: Baseline; Black: Control; Red: Energy-depleted; Blue: Hyperthermia). Means ± SEM of dichlorodihydrofluorescein (DCF) MFI (B,C; n = 4) in bovine and human RBCs following a 72-hour incubation in Ringer's solution without glucose (energy-depleted) at 37 °C or in Ringer's solution at 41 °C (hyperthermia). * and ** indicate significant difference ($p < 0.05$ and $p < 0.01$, respectively) from baseline values.

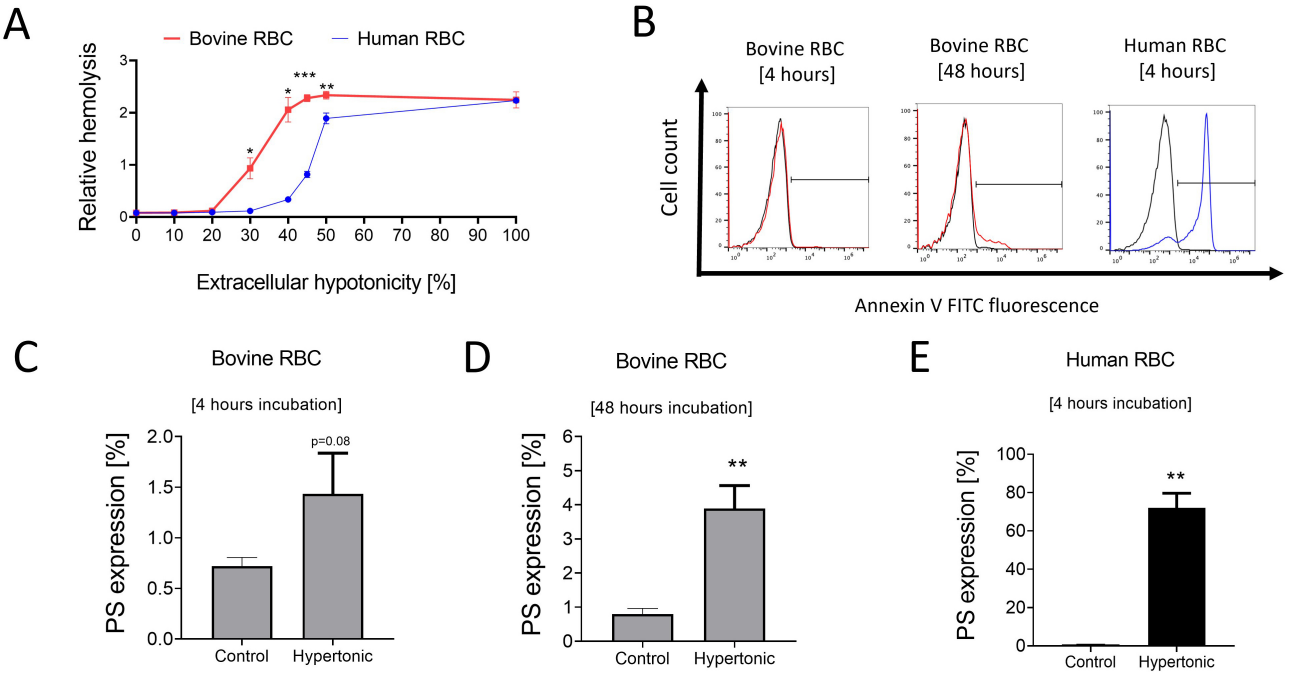

Fig. 5. Impact of extracellular osmolality changes on bovine and human RBCs. Means ± SEM of relative hemolysis (n = 4; 405 nm absorbance; A) in bovine RBCs (red line) and human RBCs (blue line) after exposure to different extracellular osmolalities. *, ** and *** indicate significant difference ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively) from human RBC at the respective osmolality. Representative histogram (Black: Control; Red and Blue: hypertonic; B) and means ± SEM of percentage of PS expression in bovine RBCs (C,D; n = 4–6) and human RBCs (E; n = 4) following incubation in Ringer's solution containing 600 mM sucrose for 4 hours or 48 hours at 37 °C. ** indicates significant difference ($p < 0.01$) from Control.

tosis and extravascular RBC catabolism are responsible for Trypanosoma-elicited anemia, a common parasitic infection in cattle [35,36]. Despite the morphological and compositional peculiarities of bovine RBCs, our *in vitro* data indicate that bovine RBCs undergo a cell death process with some mechanistic similarities to that observed in human [14], murine [14], and canine RBCs [37].

Supraphysiologic Ca^{2+} overload in human RBCs induces metabolic reprogramming, ion channel modulation, posttranslational protein modifications, and affects the RBC cytoskeleton [14,38]. Remarkably, bovine RBCs showed higher resistance to Ca^{2+} ionophore-induced cell death as compared to human RBCs suggesting a potentially higher molecular threshold for RBC cell death in bovine

diseases. Unlike humans and other species, physiological levels of ionized Ca^{2+} in bovine RBCs remain unknown [39]. It may be surmised that possible interspecies variations in basal Ca^{2+} concentrations may dictate differences in downstream signaling responses to acute Ca^{2+} elevations. Removal of extracellular Ca^{2+} mitigates metabolic alterations in human RBCs such as changes in glutathione synthesis and lipid remodeling [38]. Oxidative stress is an important activator of Ca^{2+}-permeable cation channels in human RBCs leading to their dysfunction and eventually cell death [16,40]. Our data showed enhanced ROS levels in bovine RBCs after incubation in glucose-depleted medium, despite very low membrane PS expression. A potentially plausible explanation for this observation comes from a recent study showing several metabolic anomalies in bovine RBCs during prolonged refrigerated storage [4]. Bovine RBCs were reported to display reduced levels of glycolytic metabolites, S-adenosylmethionine, adenosine triphosphate (ATP), and glutathione, and enhanced purine oxidation products as compared to RBCs from other mammalian species, suggesting slow energy metabolism and high oxidative stress during storage [4].

Heat stress in cows can affect their physiological homeostasis and reduce livestock productivity [41]. Cattle suffering from some types of infectious febrile conditions such as anaplasmosis and trypanosomiasis, often display anemia [42,43]. Normal bovine body temperatures have been reported to vary between 38–39.5 °C depending on age and breed [44]. Our data show that exposure of bovine and human RBCs to hyperthermia increased ROS levels. However, bovine RBCs showed a minimal increase in PS expression despite prolonged hyperthermia exposure, suggesting that bovine RBCs potentially have a higher acute adaptability, possibly underpinned by interspecies metabolic variations, to hyperthermic challenge. Interestingly, exposure of bovine RBCs to extracellular hypertonic stress for relatively shorter periods did not elicit increased PS exposure as compared to human RBCs and only a prolonged incubation of bovine RBCs modestly enhanced PS expression. However, in contrast to human RBCs, bovine RBCs displayed markedly increased hemolysis in response to reduction in extracellular osmolality. Indeed, RBCs from cows have previously been reported to display higher levels of hemolysis in response to various challenges compared to RBCs from various mammalian species [5,45]. A limitation of the present study is that the relatively small sample size did not permit us to determine the effects of biological variables such as age, sex, and cow breed. Notably, age, sex, and genetic heterogeneity have been documented to influence RBC phenotype in other mammalian species [46–48]. Nevertheless, the strength of our study is that it provides the first published evidence of the cell death process in bovine RBCs and paves the way for follow-up studies in the future, relevant to veterinary medicine and the livestock industry.

5. Conclusions

From our data and previous findings taken together, it may be conjectured that structural and metabolic variations between human and bovine RBCs confer differential responses to stress-induced cell death patterns. Accordingly, further studies are required to fully discern the cell death machinery in bovine RBCs, which may potentially be an important mechanism underlying bovine anemia.

Availability of Data and Materials

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

BK and SMQ designed the experiments. BK, SM, and TM performed the acquisition and analysis of data. BK, SM, and SMQ wrote the manuscript and prepared the figures. RB, TES, HJT, JGJ, and SMQ advised on the research, provided resources, and/or interpreted the data. All authors have sufficiently participated in the research, reviewed the manuscript draft, edited the changes, approved the final version, and agreed to take responsibility for this work.

Ethics Approval and Consent to Participate

Use of bovine and human blood for research was cleared by Ontario Tech University's Biosafety committee (permit# BSC28). RBC concentrates were supplied by CBS Blood4Research (CBS REB #2022.034).

Acknowledgment

The authors thank Masoomeh Eghtedari and Shivani Patel for their technical support, and Windcrest Meat Packers Ltd. staff (Port Perry, ON) for providing bovine blood samples.

Funding

SMQ received research funding from the Natural Sciences and Engineering Research Council of Canada (NSERC; RGPIN-2022-04382). RB was supported by a grant from the German Research Foundation (BI 2149/2-1). SM and TM were recipients of the NSERC Undergraduate Student Research Award.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Tutwiler V. To deform or not to deform: the evolutionary basis of mammalian red blood cell deformability. Biophysical Journal. 2021; 120: 3539–3540.

[2] Yeo JH, Lam YW, Fraser ST. Cellular dynamics of mammalian red blood cell production in the erythroblastic island niche. Biophysical Reviews. 2019; 11: 873–894.

[3] Ji P, Murata-Hori M, Lodish HF. Formation of mammalian erythrocytes: chromatin condensation and enucleation. Trends in Cell Biology. 2011; 21: 409–415.
[4] Miglio A, Maslanka M, Di Tommaso M, Rocconi F, Nemkov T, Buehler PW, *et al*. ZOOMICS: comparative metabolomics of red blood cells from dogs, cows, horses and donkeys during refrigerated storage for up to 42 days. Blood Transfusion = Trasfusione Del Sangue. 2023; 21: 314–326.
[5] Yamaguchi T, Hirakawa R, Ochiai H. Correlation between sphingomyelin and the membrane stability of mammalian erythrocytes. Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology. 2023; 265: 110833.
[6] Pasini EM, Lutz HU, Mann M, Thomas AW. Red blood cell (RBC) membrane proteomics–Part II: Comparative proteomics and RBC patho-physiology. Journal of Proteomics. 2010; 73: 421–435.
[7] Engen RL, Clark CL. High-performance liquid chromatography determination of erythrocyte membrane phospholipid composition in several animal species. American Journal of Veterinary Research. 1990; 51: 577–580.
[8] Windberger U, Bartholovitsch A, Plasenzotti R, Korak KJ, Heinze G. Whole blood viscosity, plasma viscosity and erythrocyte aggregation in nine mammalian species: reference values and comparison of data. Experimental Physiology. 2003; 88: 431–440.
[9] Kiss F, Toth E, Peto K, Miko I, Nemeth N. The investigation of interspecies diversity of erythrocyte aggregation properties by two different photometric methods in four animal species. Journal of Animal Physiology and Animal Nutrition. 2015; 99: 1074–1083.
[10] Zeidler RB, Kim HD. Effects of low electrolyte media on salt loss and hemolysis of mammalian red blood cells. Journal of Cellular Physiology. 1979; 100: 551–561.
[11] Plasenzotti R, Stoiber B, Posch M, Windberger U. Red blood cell deformability and aggregation behaviour in different animal species. Clinical Hemorheology and Microcirculation. 2004; 31: 105–111.
[12] Varga A, Matrai AA, Barath B, Deak A, Horvath L, Nemeth N. Interspecies Diversity of Osmotic Gradient Deformability of Red Blood Cells in Human and Seven Vertebrate Animal Species. Cells. 2022; 11: 1351.
[13] Roland L, Drillich M, Iwersen M. Hematology as a diagnostic tool in bovine medicine. Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 2014; 26: 592–598.
[14] Qadri SM, Bissinger R, Solh Z, Oldenborg PA. Eryptosis in health and disease: A paradigm shift towards understanding the (patho)physiological implications of programmed cell death of erythrocytes. Blood Reviews. 2017; 31: 349–361.
[15] Lutz HU, Bogdanova A. Mechanisms tagging senescent red blood cells for clearance in healthy humans. Frontiers in Physiology. 2013; 4: 387.
[16] Bissinger R, Bhuyan AAM, Qadri SM, Lang F. Oxidative stress, eryptosis and anemia: a pivotal mechanistic nexus in systemic diseases. The FEBS Journal. 2019; 286: 826–854.
[17] Alghareeb SA, Alfhili MA, Fatima S. Molecular Mechanisms and Pathophysiological Significance of Eryptosis. International Journal of Molecular Sciences. 2023; 24: 5079.
[18] Matsumoto M, Miwa M. Study on the new phospholipid, N-acyl-I-alkyl glycerophosphorylethanolamine, from bovine erythrocytes. Biochimica et Biophysica Acta. 1973; 296: 350–364.
[19] Florin-Christensen J, Suarez CE, Florin-Christensen M, Wainszelbaum M, Brown WC, McElwain TF, *et al*. A unique phospholipid organization in bovine erythrocyte membranes. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98: 7736–7741.
[20] Spengler MI, Bertoluzzo SM, Catalani G, Rasia ML. Study on membrane fluidity and erythrocyte aggregation in equine, bovine and human species. Clinical Hemorheology and Microcirculation. 2008; 38: 171–176.
[21] Benga G. Comparative studies of water permeability of red blood cells from humans and over 30 animal species: an overview of 20 years of collaboration with Philip Kuchel. European Biophysics Journal: EBJ. 2013; 42: 33–46.
[22] Shiono H, Yagi Y, Chikayama Y, Miyazaki S, Nakamura I. Oxidative damage and phosphatidylserine expression of red blood cells in cattle experimentally infected with Theileria sergenti. Parasitology Research. 2003; 89: 228–234.
[23] Yagi Y, Furuuchi S, Takahashi H, Koyama H. Abnormality of osmotic fragility and morphological disorder of bovine erythrocytes infected with Theileria sergenti. Nihon Juigaku Zasshi. the Japanese Journal of Veterinary Science. 1989; 51: 389–395.
[24] Yagi Y, Ito N, Kunugiyama I. Decrease in erythrocyte survival in Theileria sergenti-infected calves determined by non-radioactive chromium labelling method. The Journal of Veterinary Medical Science. 1991; 53: 391–394.
[25] Orr A, Gualdieri R, Cossette ML, Shafer ABA, Stotesbury T. Whole bovine blood use in forensic research: Sample preparation and storage considerations. Science & Justice. 2021; 61: 214–220.
[26] Qadri SM, Chen D, Schubert P, Perruzza DL, Bhakta V, Devine DV, *et al*. Pathogen inactivation by riboflavin and ultraviolet light illumination accelerates the red blood cell storage lesion and promotes eryptosis. Transfusion. 2017; 57: 661–673.
[27] Sudnitsyna J, Skverchinskaya E, Dobrylko I, Nikitina E, Gambaryan S, Mindukshev I. Microvesicle Formation Induced by Oxidative Stress in Human Erythrocytes. Antioxidants (Basel, Switzerland). 2020; 9: 929.
[28] Duranton C, Huber SM, Lang F. Oxidation induces a Cl(-)-dependent cation conductance in human red blood cells. The Journal of Physiology. 2002; 539: 847–855.
[29] Jalali SM, Ghorbanpour M, Razi Jalali M, Rasooli A, Safaie P, Norvej F, *et al*. Occurrence and potential causative factors of immune-mediated hemolytic anemia in cattle and river buffaloes. Veterinary Research Forum: an International Quarterly Journal. 2018; 9: 7–12.
[30] Farikou O, Simo G, Njiokou F, Kamé Ngassé GI, Achiri Fru M, Geiger A. Trypanosome Infections and Anemia in Cattle Returning from Transhumance in Tsetse-Infested Areas of Cameroon. Microorganisms. 2023; 11: 712.
[31] Kim S, Yu DH, Chae JB, Choi KS, Kim HC, Park BK, *et al*. Pathogenic genotype of major piroplasm surface protein associated with anemia in Theileria orientalis infection in cattle. Acta Veterinaria Scandinavica. 2017; 59: 51.
[32] Jaimes-Dueñez J, Triana-Chávez O, Mejía-Jaramillo AM. Parasitological and molecular surveys reveal high rates of infection with vector-borne pathogens and clinical anemia signs associated with infection in cattle from two important livestock areas in Colombia. Ticks and Tick-borne Diseases. 2017; 8: 290–299.
[33] Morris JG, Cripe WS, Chapman HL Jr, Walker DF, Armstrong JB, Alexander JD Jr, *et al*. Selenium deficiency in cattle associated with Heinz bodies and anemia. Science (New York, N.Y.). 1984; 223: 491–493.
[34] Joerling J, Doll K. Monitoring of iron deficiency in calves by determination of serum ferritin in comparison with serum iron: A preliminary study. Open Veterinary Journal. 2019; 9: 177–184.
[35] Taiwo VO, Anosa VO. In vitro erythrophagocytosis by cultured macrophages stimulated with extraneous substances and those isolated from the blood, spleen and bone marrow of Boran and N'Dama cattle infected with Trypanosoma congolense and Trypanosoma vivax. The Onderstepoort Journal of Veterinary Re-

search. 2000; 67: 273–287.
[36] Stijlemans B, Brys L, Korf H, Bieniasz-Krzywiec P, Sparkes A, Vansintjan L, *et al*. MIF-Mediated Hemodilution Promotes Pathogenic Anemia in Experimental African Trypanosomosis. PLoS Pathogens. 2016; 12: e1005862.
[37] Katahira I, Neo S, Nagane M, Miyagi S, Hisasue M, Bhuyan AAM. Characterization of Suicidal Erythrocyte Death (Eryptosis) in Dogs. Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology. 2020; 54: 605–614.
[38] Nemkov T, Qadri SM, Sheffield WP, D'Alessandro A. Decoding the metabolic landscape of pathophysiological stress-induced cell death in anucleate red blood cells. Blood Transfusion = Trasfusione Del Sangue. 2020; 18: 130–142.
[39] da SilveiraCavalcante L, Acker JP, Holovati JL. Differences in Rat and Human Erythrocytes Following Blood Component Manufacturing: The Effect of Additive Solutions. Transfusion Medicine and Hemotherapy: Offizielles Organ Der Deutschen Gesellschaft Fur Transfusionsmedizin Und Immunhamatologie. 2015; 42: 150–157.
[40] Maruyama T, Hieda M, Mawatari S, Fujino T. Rheological Abnormalities in Human Erythrocytes Subjected to Oxidative Inflammation. Frontiers in Physiology. 2022; 13: 837926.
[41] Rhoads ML. Review: Reproductive consequences of whole-body adaptations of dairy cattle to heat stress. Animal: an International Journal of Animal Bioscience. 2023; 17: 100847.
[42] Değirmençay Ş, Küçükler S, Özdemir S, Kaman R. Evaluation of erythrocyte arginase activity, plasma nitric oxide concentration and oxidative stress status in cattle with anaplasmosis. Veterinary Parasitology. 2023; 314: 109855.
[43] Da Silva AS, Molosse VL, Deolindo GL, Cecere BG, Vitt MG, Nascimento LFN, *et al*. Trypanosoma vivax infection in dairy cattle: Parasitological and serological diagnosis and its relationship with the percentage of red blood cells. Microbial Pathogenesis. 2022; 166: 105495.
[44] Liu J, Li L, Chen X, Lu Y, Wang D. Effects of heat stress on body temperature, milk production, and reproduction in dairy cows: a novel idea for monitoring and evaluation of heat stress — A review. Asian-Australasian Journal of Animal Sciences. 2019; 32: 1332–1339.
[45] Matsuzawa T, Ikarashi Y. Haemolysis of various mammalian erythrocytes in sodium chloride, glucose and phosphate-buffer solutions. Laboratory Animals. 1979; 13: 329–331.
[46] Zimring JC, Smith N, Stowell SR, Johnsen JM, Bell LN, Francis RO, *et al*. Strain-specific red blood cell storage, metabolism, and eicosanoid generation in a mouse model. Transfusion. 2014; 54: 137–148.
[47] Cloutier M, Cognasse F, Yokoyama APH, Hazegh K, Mykhailova O, Brandon-Coatham M, *et al*. Quality assessment of red blood cell concentrates from blood donors at the extremes of the age spectrum: The BEST collaborative study. Transfusion. 2023; 63: 1506–1518.
[48] Kanias T, Sinchar D, Osei-Hwedieh D, Baust JJ, Jordan A, Zimring JC, *et al*. Testosterone-dependent sex differences in red blood cell hemolysis in storage, stress, and disease. Transfusion. 2016; 56: 2571–2583.