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Alterations in the osmotic fragility of camel and donkey erythrocytes caused by temperature, pH and blood storage

Johnson Oyewale[1]*, Tavershima Dzenda[1], Lukuman Yaqub[1], Dayo Akanbi[1], Joseph Ayo[1], Olufemi Owoyele[1], Ndaza Minka[2], and Theophilus Dare[1]

[1]*Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[2]*College of Agriculture and Animal Science, Ahmadu Bello University, Mando Kaduna, Nigeria*

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ABSTRACT

The present study examined the changes in the fragility of camel and donkey erythrocytes caused by variations in temperature, pH and blood storage. Although the fragility did not differ between sexes in both species, camel erythrocytes were more osmotically resistant than donkey erythrocytes. At a constant pH of 7.4, the fragility decreased in the camel but increased in the donkey at lowered temperatures. However, at a constant temperature of 28 ºC, the fragility of camel and donkey erythrocytes increased in an acidic solution and decreased in an alkaline solution. Additionally, blood storage for 24 hours at 4 ºC increased the fragility of camel erythrocytes, but not donkey erythrocytes. However, the erythrocyte fragility increased in both species after 48 or 72 hours of blood storage at 4 ºC. In conclusion, our results suggest that variation in the temperature and pH of the erythrocyte environment and duration of blood storage may each play a significant role in the osmotic behaviour of camel and donkey erythrocytes.

Key words: camels, donkeys, erythrocyte fragility, temperature, pH, blood storage

Introduction

Camels (*Camelus dromedarius*) possess certain physiological, biochemical and pharmacological characteristics that distinguish them from other related ruminants. For instance, they have the exceptional ability to withstand considerable periods of dehydration (MacFARLANE et al., 1963; PERK, 1963) and the ability to rapidly replace water lost during prolonged periods of dehydration within a few minutes of access to

*Corresponding author:

Prof. Johnson Oyewale, DVM, MSc, PhD, Department of Veterinary Physiology and Pharmacology, University of Ibadan, Ibadan, Nigeria, Phone: +234 8023 256 541; Fax: +02 810 3043; E-mail: oyewalejohnson2003@yahoo.com

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drinking water (10 liters/min) (SCHMIDT-NIELSEN et al., 1956). It is known that camel erythrocytes are highly resistant to osmotic haemolysis, being able to expand to 240% of their original volume without rupturing in hypotonic solutions (PERK et al., 1964; PERK, 1966). Camel erythrocytes are more resistant (or less susceptible) to osmotic haemolysis than cattle, sheep, goat, mouse, pig and human erythrocytes (PERK et al., 1964; LIVINE and KUIPER, 1973). This may be due partly to the shape of camel erythrocytes, which is oval rather than the circular discs seen with other mammalian erythrocytes (TURNER et al., 1958; JAIN and KEETON, 1974) and partly to the composition of the erythrocyte membrane (LIVINE and KUIPER, 1973).

Many workers have attempted to study the ability of camel erythrocytes to withstand the osmotic challenges associated with severe dehydration and rapid rehydration (PERK, 1966; YAGIL et al., 1974) and the contribution of the erythrocyte and composition of its membrane to physiological adaptation to dehydration (LIVINE and KUIPER, 1973; RALSTON, 1975; EITAN et al., 1976; OMORPHOS et al., 1989; MIRGANI, 1992; AL-QARAWI and MOUSA, 2004).

In the present work, we studied the changes in the osmotic fragility of camel erythrocytes during variations in the temperature and pH of the surrounding hypotonic solution. The effect of blood storage on camel erythrocyte fragility was also studied, since it was not feasible to analyze all the blood samples collected in the field within minutes of collection and several hours might have elapsed between blood collection and analysis. Just as for the camels, these experiments were also carried out for normal healthy donkeys (*Equus asinus*) living in the same semi-arid zone of Northern Nigeria. The aim was to attempt to explain the unique abilities of camel erythrocytes to resist haemolysis and to quantify the magnitude of differences in osmotic behaviour, if any, between the camel and donkey erythrocytes in the above-mentioned aspects.

Materials and methods

Nigerian breeds of camels and donkeys were used for this study. Seventeen adult one-humped camels (*Camelus dromedaries*) (10 males and 7 females) and 22 adult Nubian donkeys (*Equus asinus*) (10 females and 12 males) were studied. They were all apparently healthy animals. Blood was collected from the jugular vein of the donkeys in Kaduna, Nigeria, while blood from the camels was obtained from the central abattoir at the point of slaughter in Kano, Nigeria. All blood samples were freshly collected, each into a bottle containing ethylene diamine tetraacetic acid (EDTA, 2 mg/μL of blood) as anticoagulant and transported in an ice-packed cooler to the laboratory for analysis in the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria.

One percent phosphate-buffered sodium chloride (NaCl) solutions (see SCHALM et al., 1975) of pH 7.4 at 28 ^{o}C were used to determine the effect of sex on the erythrocyte

fragility. For studying the effect of temperature, 1% phosphate-buffered NaCl solutions of pH 7.4 at 4 ºC and 28 ºC were used. The effect of pH was studied using 1% phosphate-buffered NaCl solutions of pH 6.5, 7.4 and 8.5 at 28 ^{o}C. For studying the effect of blood storage, the erythrocyte fragility test was determined using fresh blood samples as well as blood samples stored for 24, 48 and 72 hours at 4 ^{o}C. In an attempt to minimize the effect of temperature variation on erythrocyte fragility (OYEWALE, 1994), both fresh and stored blood samples were maintained at 4 ^{o}C until required for analysis. The osmotic fragility tests were performed as described previously (OYEWALE, 1992). Briefly, 5 mL of varying concentrations of each test solution of NaCl (0.0, 0.1, 0.3, 0.5, 0.7, 0.9%) was prepared in each of 6 centrifuge tubes and pre-incubated at the desired temperature for 30 minutes. Blood (0.02 μL) was added to each concentration of the test solution in each tube. The contents were mixed and incubated at the desired temperature for 30 minutes and then centrifuged at 3,000 g for 10 minutes. The haemoglobin content of the supernatant was determined spectrophotometrically at a wavelength of 540 nm using the Bausch and Lomb Spectronic 20 spectrophotometer (FL Sales Inc., Grafton, Ohio, U.S.A) with distilled water serving as the blank. The percentage of haemolysis in each concentration of NaCl was evaluated taking the tube with maximum haemolysis as 100%.

All results are given as means ± standard errors and analyzed statistically by Student's *t*-test, or where appropriate, by analysis of variance. A probability of $P<0.05$ was considered significant.

Results

Figures 1-5 show the results of the osmotic fragility of camel and donkey erythrocytes. The fragility of erythrocytes did not differ significantly ($P>0.05$) between sexes in the camels (Fig. 1A) and donkeys (Fig. 1B). However, as revealed in Fig. 2, donkey erythrocytes showed higher fragility than camel erythrocytes at NaCl-concentrations of 0.3% ($P<0.001$) and 0.5% ($P<0.01$).

Fig. 3A shows that the fragility of camel erythrocytes decreased at lowered temperatures. The fragility of camel erythrocytes at pH 7.4 was lower ($P<0.001$) at 4 ºC than at 28 ºC at the NaCl-concentration of 0.5%. In contrast, donkey erythrocytes (Fig. 3B) at pH 7.4 were more fragile at 4 ºC than at 28 ºC at NaCl-concentrations of 0.1% ($P<0.05$) and 0.5% ($P<0.01$).

As illustrated in Fig. 4A, the fragility of camel erythrocytes at the 0.3% NaCl-concentration was higher ($P<0.05$) at pH 6.5 than at pH 7.4 and also higher ($P<0.001$) at pH 7.4 than at pH 8.5. A decrease in osmotic fragility with an increase in pH, as observed with camel erythrocytes, was also seen with donkey erythrocytes (Fig. 4B). For instance, donkey erythrocytes were more fragile at pH 6.5 than at pH 7.4 at NaCl-concentrations of 0.5% ($P<0.0001$) and 0.7% ($P<0.01$). The fragility was also higher at pH 7.4 than at pH 8.5 at the NaCl-concentration of 0.5% ($P<0.0001$) (Fig. 4B).

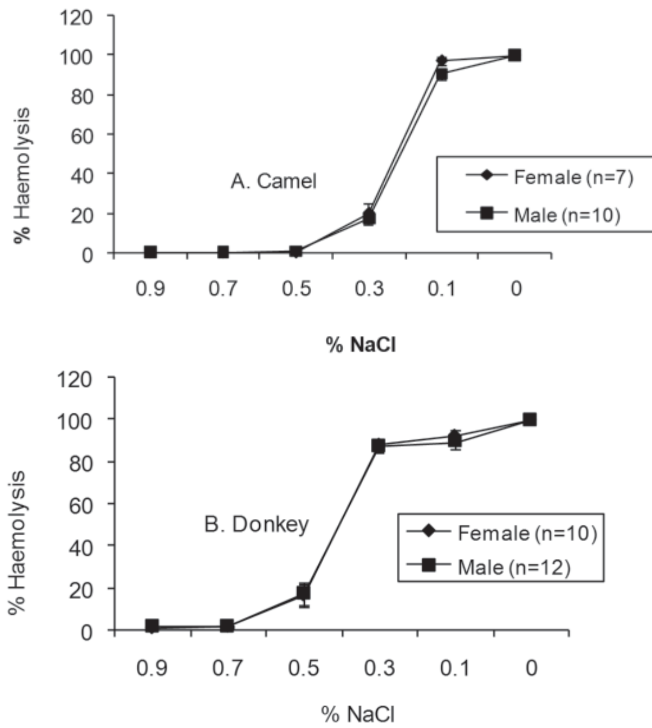

Fig. 1. Osmotic fragility of erythrocytes of 7 female and 10 male camels and 10 female and 12 male donkeys. Values are means ± SEM.

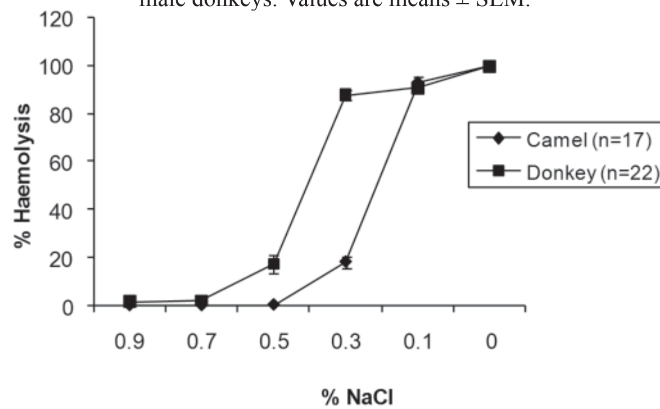

Fig. 2. Comparison of osmotic fragility of donkey and camel erythrocytes. Values are means ± SEM. n = number of animals.

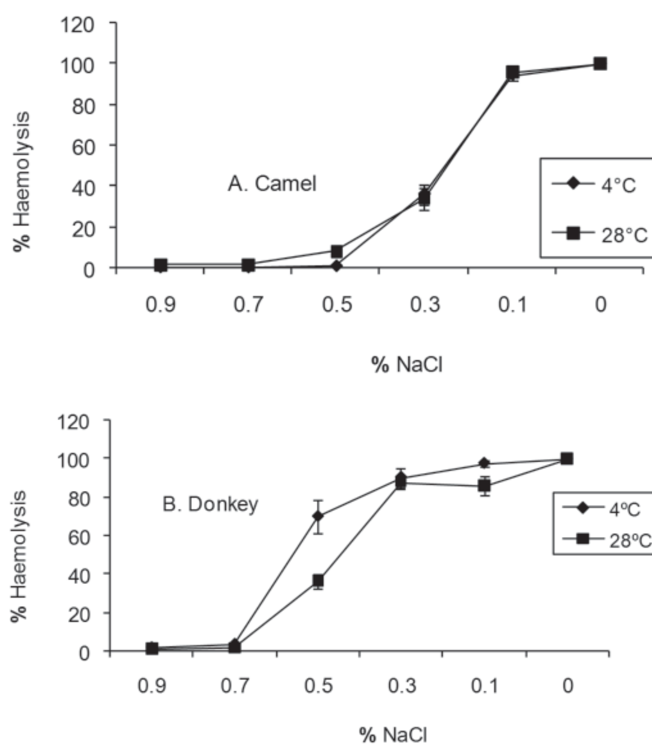

Fig. 3. Changes in the osmotic fragility of camel and donkey erythrocytes at pH 7.4 and 4 ºC and 28 ºC. Values are means ± SEM for 10 camels and 8 donkeys.

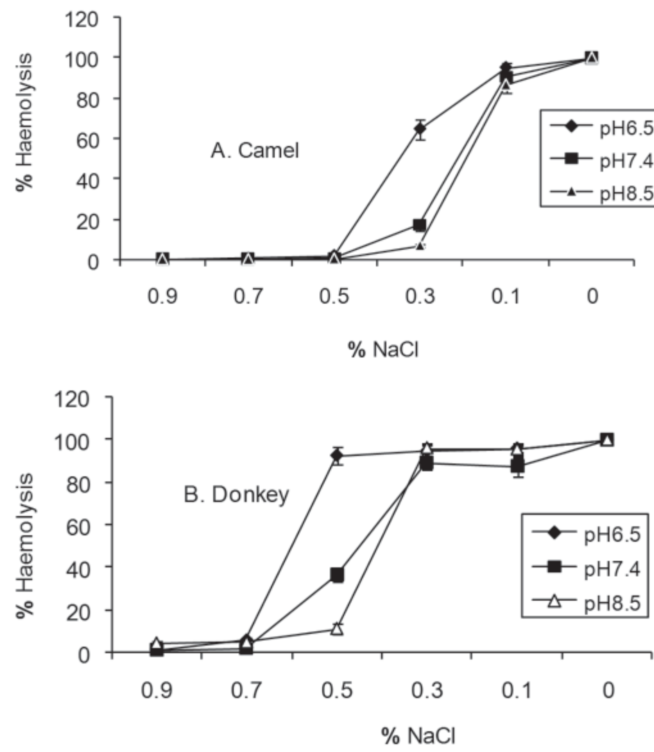

Fig. 4. Alterations in the osmotic fragility of camel and donkey erythrocytes at 28 ºC and pH 6.5, 7.4 and 8.5. Values are means ± SEM for 10 camels and 9 donkeys.

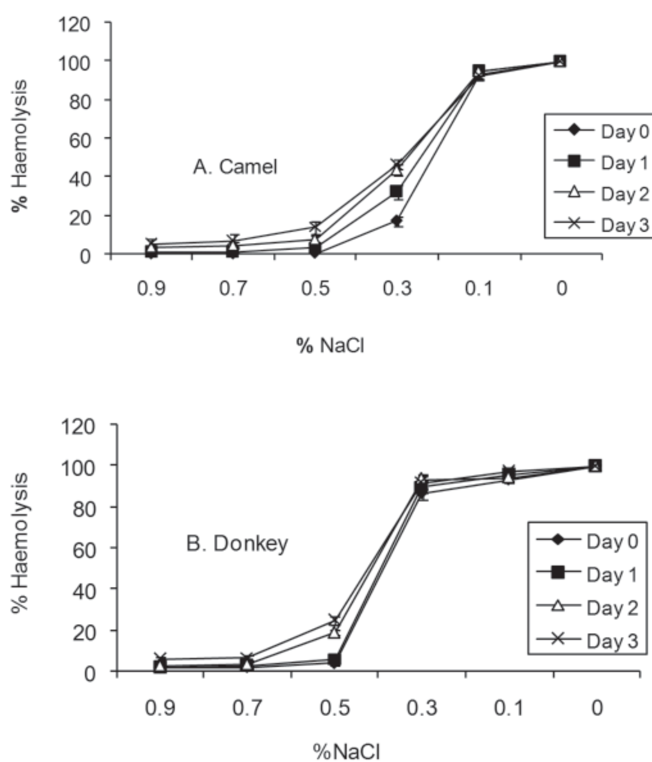

Fig. 5. Changes in the osmotic fragility of camel and donkey erythrocytes after blood storage for 1, 2 and 3 days. Values are means ± SEM for 14 camels and 12 donkeys.

Fig. 5A shows that blood storage for 24 to 72 hours at 4 ºC significantly increased (P<0.0001) the fragility of camel erythrocytes at 0.3% and 0.5% NaCl-concentrations. At the 0.3% NaCl-concentration, the increases in the fragility when compared with the pre-storage value (17.04 ± 2.83%) were significant following storage of camel blood for 24 hours (32.54 ± 3.76%, P<0.01), 48 hours (43.62 ± 2.95%, P<0.0001) and 72 hours (46.46 ± 2.29%, P<0.0001). Likewise, at the 0.5% NaCl-concentration, the fragility of camel erythrocytes increased significantly after blood storage for 24 hours (3.23 ± 0.96%, P<0.01), 48 hours (7.65 ± 1.57%, P<0.001) and 72 hours (13.93 ± 3.36%, P<0.01) compared with the pre-storage value of 0.33 ± 0.30% (Fig. 5A). However, the fragility of donkey erythrocytes, as depicted in Fig. 5B, did not change after 24 hours of blood storage. Increases in erythrocyte fragility were seen after 48 and 72 hours of storage

of donkey blood. The increases appeared at NaCl-concentrations of 0.3% ($P<0.05$) and 0.5% ($P<0.0001$) when compared with the corresponding pre-storage values (Fig. 5B). Following blood storage for 72 hours, the fragility of donkey erythrocytes increased at NaCl-concentrations of 0.5% ($P<0.001$), 0.7% ($P<0.01$) and 0.9% ($P<0.01$) compared with the corresponding pre-storage values (Fig. 5B).

Discussion

It has been established that erythrocytes from males are more susceptible to osmotic haemolysis than those from females in domestic fowls (MARCH et al., 1966; OYEWALE and DUROTOYE, 1988) and peafowls (OYEWALE, 1994). It is well known that with domestic fowl erythrocytes, oestrogen increases fragility, while androgen has no effect (MARCH et al., 1966). Since the fragility of camel and donkey erythrocytes, as obtained in the present study, lacks sexual difference (Figs. 1A and 1B), it suggests that oestrogen and androgen do not alter the fragility of erythrocytes in these species or that their effects are different from those seen with domestic fowl erythrocytes (MARCH et al., 1966). Our results in camels and donkeys also disagree with the findings in cattle (OLAYEMI, 2004 and 2007), in which erythrocyte fragility is higher in males and in African giant rats (*Cricetomys gambianus*, Waterhouse) (OYEWALE et al., 1998) and dogs (OLAYEMI et al., 2009) where the fragility is higher in females.

In the present study it was found that camel erythrocytes are more resistant to haemolysis (or less osmotically fragile) than donkey erythrocytes (Fig. 2). Erythrocytes of camels have also been shown to be more resistant to haemolysis when compared with cattle, sheep, goats, pigs, mice, man and other animals (PERK et al., 1964; LIVINE and KUIPER, 1973; AL-QARAWI and MOUSA, 2004). This greater osmotic resistance of camel erythrocytes may not be due to the morphological characteristics of the red cell alone (AGAR and BOARD, 1983**)**. Characteristics of the erythrocyte, such as ability to swell to twice its volume in hypotonic solutions, its resistance to the lytic effect of snake venom and resistance to sonic haemolysis (TURNER et al., 1958; CONDREA et al., 1964; PERK, 1966), indicate some unusual properties of the camel erythrocyte membrane. RALSTON (1975) has shown that the major proteins of camel erythrocyte membranes are similar to those of cattle and humans, with the major difference being in the major membrane protein "spectrin", which appears to be very tightly bound to the camel erythrocyte membrane. Concurrent with the total release of spectrin, camel erythrocytes undergo a change in shape, from flat ellipsoids to spheres, suggesting an important shape-maintaining role for spectrin in the erythrocytes of camels.

In humans (MURPHY, 1967; ALONI et al., 1977), sheep and goats (OYEWALE, 1991a), cattle, pigs, rats and rabbits (OYEWALE, 1992) and domestic fowls and guinea-fowls (OYEWALE, 1991b), the fragility of erythrocytes increases at lowered temperatures. This

agrees with our observation with donkey erythrocytes in the present study. It disagrees with our finding with camel erythrocytes, in which their fragility decreases at lowered temperatures. Our observation in camels confirms similar finding in this species by ALONI et al. (1977). It has been demonstrated that the lipids and proteins of the erythrocyte membrane are the sites for the effect of temperature on osmotic fragility (ALONI et al., 1977). The difference between species in the osmotic behaviour of erythrocytes with respect to temperature changes may therefore be related to the difference in the structural features of the cell membrane. For example, LIVINE and KUIPER (1973) have shown that camel erythrocyte membrane differs from that of humans in having higher protein:lipid ratios and also exhibiting some differences in amino acid composition.

In the present investigation, it was found that the osmotic fragility of camel and donkey erythrocytes increases in an acidic solution and decreases in an alkaline solution. Similar findings were reported with human erythrocytes (MURPHY, 1967) and erythrocytes of domestic fowls (OYEWALE, 1991b) and cattle, pigs, rats and rabbits (OYEWALE, 1992). However, the fragility of dog erythrocytes has been shown to increase in both acidic and alkaline solutions (MATSUZAWA and IKARASHI, 1979).

When blood was stored at 4 ºC for a day (24 hours) in the present study, it increased the fragility of camel erythrocytes, but did not alter that of donkey erythrocytes. It seems the finding with donkey erythrocytes is also true for sheep erythrocytes, because OYEWALE (1993) reported that blood storage at 10 ºC for 24 hours failed to alter the fragility of sheep erythrocytes. However, our observations in camels and donkeys are at variance with the findings in goats, pigs, cattle, and rats, in which blood storage at 10 ºC for 24 hours decreased the erythrocyte osmotic fragility (OYEWALE, 1993). On the other hand, we observed that blood storage at 4 ºC for 2 or 3 days (48 or 72 hours) increased the fragility of both camel (Fig. 5A) and donkey erythrocytes (Fig. 5B). In humans, BEUTLER et al. (1982) found that blood storage for prolonged periods (42 days) markedly increases the fragility of erythrocytes, which is attributable partly to the lactate produced by red cells and partly to the substitution of chloride ion for 2, 3 diphosphoglycerate in stored erythrocytes. It has also been shown that lactate-treated fresh blood from humans causes the same degree of increase in osmotic fragility of erythrocytes as in stored blood (BEUTLER et al., 1982). These considerations suggest that the observed increases in the osmotic fragility of camel and donkey erythrocytes after 2 or 3 days of blood storage could have resulted, at least, from the lactate produced by the stored erythrocytes.

Our findings in the present study may help to explain the osmotic behaviour of camel and donkey erythrocytes during infection by blood parasites or when confronted with conditions which affect the temperature and pH of blood. Also, our results suggest that the duration of blood storage prior to measuring the osmotic fragility of camel and donkey erythrocytes must be considered in the analysis of their osmotic behaviour.

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OYEWALE, J., T. DZENDA, L. YAQUB, D. AKANBI, J. AYO, O. OWOYELE, N. MINKA, T. DARE: Promjene u osmotskoj otpornosti eritrocita deve i magarca uzrokovane temperaturom, pH i pohranom krvi. Vet. arhiv 81, 459-470, 2011.

SAŽETAK

Cilj istraživanja bio je ustanoviti razliku u osmotskoj otpornosti eritrocita deve i magarca koja se javlja pod utjecajem temperature, pH i trajanja pohrane krvi. Iako nije ustanovljena razlika između muškoga i ženskoga spola u istraživanih vrsta, eritrociti deve pokazali su se osmotski otpornijima u odnosu na eritrocite magarca. Pri stalnoj pH vrijednosti od 7,4 otpornost eritrocita deve se smanjila, a eritrocita magarca povećala uz sniženu temperaturu. Pri stalnoj temperaturi od 28 ºC otpornost eritrocita deve i magarca povećala se u kiseloj, a smanjila u lužnatoj otopini. Pohranom krvi tijekom 24 sata na 4 ºC povećala se krhkost eritrocita deve, ali ne i eritrocita magarca. No krhkost eritrocita obiju vrsta povećala se tijekom pohrane od 48 do 72 sata na temperaturi od 4 ºC. Na temelju rezultata može se zaključiti da razlike u temperaturi i pH medija te duljina pohrane krvi mogu imati značajnu ulogu u osmotskoj otpornosti eritrocita deve i magarca.

Ključne riječi: deve, magarci, otpornost eritrocita, temperatura, pH, pohrana krvi