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ASSESSMENT OF ERYTHROCYTE OSMOTIC FRAGILITY IN CATTLE DUE TO HAEMOPROTOZOAN DISEASES

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Abstract: The Erythrocyte Osmotic Fragility (EOF) in case of anaemic cattle mostly affected with haemolytic anaemia were evaluated in this study. As haemolytic anaemia is a consistent finding in different blood protozoan diseases like theileriosis and trypanosomiasis, determination of EOF in these cases shows the level of osmotic stress in the affected animals. The present study was also aimed to determine the influence of temperature, storage time and season on EOF in the anaemic cattle. The EOF was determined by a standard method using NaCl concentrations ranging from 0.1% to 0.9%. The result revealed a significant difference in percentage haemolysis recorded at NaCl concentrations of 0.2%, 0.3% & 0.4% respectively in cattle. The EOF value was at a higher level in the animals affected with theileriosis or trypanosomiasis in comparison to other anaemic cases. The result of present study could be used as a determinant of osmotic stress in anaemic cattle.
Keyword: erythrocyte osmotic fragility, haemolytic anaemia, theileriosis, trypanosomiasis

Introduction

Erythrocyte Osmotic Fragility (EOF) which denotes the sensitivity to change in osmotic pressure an erythrocyte is exposed to, has been shown to vary within different conditions (Oyewale and Durotoye, 1988). Different factors tend to influence osmotic fragility in animals. These include storage conditions, temperature and season of the year (Oladele *et al*., 2003). Erythrocyte osmotic fragility has been used as an indicator of oxidative stress in animals (Adenkola and Ayo, 2009; Abdul Wahab *et al*., 2010; Ambali *et al*., 2010). Haemolysis usually recognised by free haemoglobin in red blood cells suspended in a media. The erythrocyte osmotic fragility test is used to determine the osmotic stress imparted due to haemolysis of red cells. The extent of osmotic stress is dependent upon red cell volume, surface area and functional integrity of cell membrane (Islah *et al*., 2016). Therefore EOF test is frequently applied for the diagnosis of haemolytic anaemia and oxidative damage due to large scale destruction of RBCs (Jain *et al*., 1983; Hanzawa *et al*., 2000 and Adenkola *et al*., 2010). It was also reported that haemolytic anaemia has been a consistent finding in different

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blood protozoan diseases like theileriosis and trypanosomiasis. The EOF of the erythrocyte was approximately 40% higher in trypanosome infected animals as compared to the healthy animals (Alfredo *et al*., 2009). Erythrocyte membrane damage has been a regular finding in trypanosomiasis due to adhesion of the RBCs. Moreover there is also mechanical damage to the erythrocyte occur due to the lashing action of the locomotory flagella during high parasitaemia leading to haemolytic anaemia (Vickerman and Tetley, 1978). Damage of the erythrocytic cell membrane occurs due to adhesion of erythrocytes to the surface of trypanosome via sialic acid receptors (Bungener and Muller, 1976; banks, 1980; Anosa and Kaneko, 1983; Shehu *et al,* 2006). High body temperature due to parasitaemia leads to enhanced osmotic fragility in animals (Nwosu and Ikeme, 1992; Igbokwe, 1994; Mbaya *et al*., 2009 a). During trypanosomiasis, erythrocytes with weak cell membrane become fragmented and lyses which leads to large destruction of RBCs ultimately leading to high EOF (Anosa and Kaneko, 1983; Murray and Dexter, 1988). Living and dead trypanosomes can also procedure various forms of toxins that can lyses erythrocyte and cause haemolytic anaemia (Tizzard and Holmes, 1976;1977; Zwart and Veenendal, 1978; Naessens *et al*., 2005). The RBCs of cattle infected with theileriosis shows greater morphological alterations than healthy RBCs leading to its damage (Jain and Kono, 1972). Highly positive cases of theileriosis lead to alterations in the membrane phospholipids of erythrocytes (Singh *et al*., 2001). If not treated, high parasitaemia produce marked increase in reticulocytes, a kind of immature RBC in the circulation which can easily destroyed and cause haemolytic anaemia due to oxidative stress (Kanaya, 1985; Haider, 1992). This ultimately leads to high osmotic fragility in such conditions.

Osmotic fragility test is common test in haematology, and, is often performed to aid with diagnosis of haemolytic anaemia mostly due to theileriosis and trypanosomiasis in cattle erythrocyte. Mostly these diseases cause anaemia in cattle but it's not clearly determined by the traditional haemoglobin estimation to know whether it's associated with haemolytic anaemia or not. Osmotic fragility test can be indicated in such cases to know the degree of haemolytic anaemia for better management of erythrocyte stress. This will also be helpful for effective treatment regimen of the affected animals. Therefore the present study has been undergone to determine the osmotic fragility values of RBCs under different conditions in cattle. Efforts have made to compare the EOF values on different storage conditions, ie, storage time, temperature of storage. Season of the year, Spring and Summer was also taken to know the alterations in the osmotic fragility values.

Materials and Method

Animals

To investigate the effect of all factors considered here on EOF, total number of117 cattle blood samples were taken irrespective of age, sex and breed.

Blood sampling

The blood samples were presented to the Department of Veterinary Pathology, Bhubaneswar for examination purpose. All the samples presented here were declared anaemic by haemoglobin estimation. Samples were presented in the EDTA vials. Out of total 117 samples, 101 samples were positive for theileria and rest 16 samples were positive for trypanosome.

Preparation of the mixture and Osmotic Fragility test

At first different saline concentrations of NaCl starting from 0.1% to 0.9% (total nine concentrations) were prepared by mixing NaCl (AR grade) with distilled water. The pH was maintained at 7.4. 500 ml each of the different concentrations of NaCl stock solution was prepared. The method followed here is of Faulkner and King (1970). A set of 10 test tubes, each containing 5 ml of NaCl solution of concentrations ranging from 0.1 to 0.9% were arranged serially in a test tube rack. One set was used to analyse each sample. The test tubes were labelled with corresponding saline concentrations. 1 ml pipette was used to transfer one drop of EDTA mixed blood (0.02 ml) to each of the 10 test tubes. The samples were inverted five times gently to mix the contents. The test tubes were allowed to stand for 30 min in room temperature (26-27 ^{0}C). Thereafter the contents were remixed and centrifuged at 1500xg for 15-20 mins. The supernatant of each test tubes were transferred into a cuvette and absorbance was taken at 540 nm using a colorimeter. The same procedure was repeated for every blood samples of the cattle used in this study. The percentage haemolysis was calculated by using the formula (Faulkner and King, 1970).

Percentage of haemolysis (%H) = (optical density of test/optical density of distilled water) x 100.

Erythrocyte Osmotic Fragility curve was determined by plotting the percentage of haemolysis against different saline concentrations.

Effect of storage time

The blood samples were stored at 4^{0}C for 10 consecutive days and the osmotic fragility was tested at each period of 24 hr duration. Tests were performed at room temperature.

Effect of incubation temperature

The effect of incubation temperature was analysed at 4^0 C, 18^0 C, 38^0 C, 42 ^{0}C and 50^0 C for 30 mins.

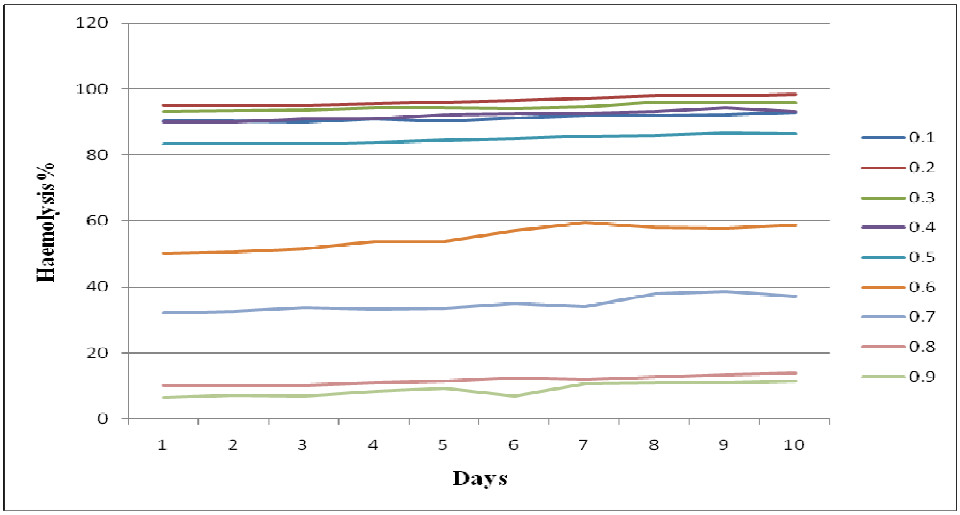
Season

The results were analysed in spring and summer season for all the samples.

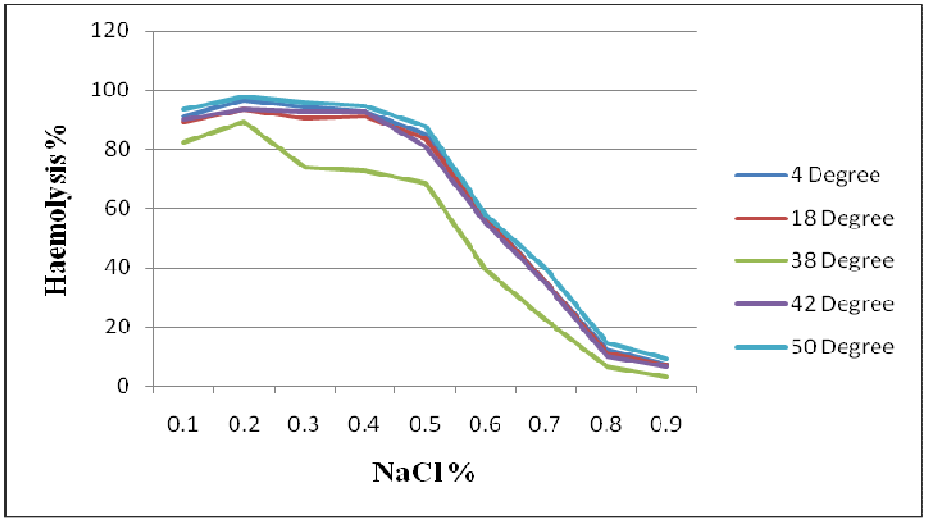
Results

The absorbance of the 117 samples were taken in colorimeter and then converted into percentage of haemolysis. The result showed that, there's highest degree of haemolysis in 0.2% concentration of NaCl followed by 0.,3%, 0.4% and 0.5%. then it gradually declined up to 0.9%. the average value of % of haemolysis in 0.5%, 0.4%, 0.3% and 0.2% NaCl induced was 85.1±2%, 92.54 ±2%, 94.08± 3%, 96.55± 3% and 91.12± 4% respectively. The value of % of haemolysis was lowest in 0.9% of NaCl, ie, 7.32± 2%.

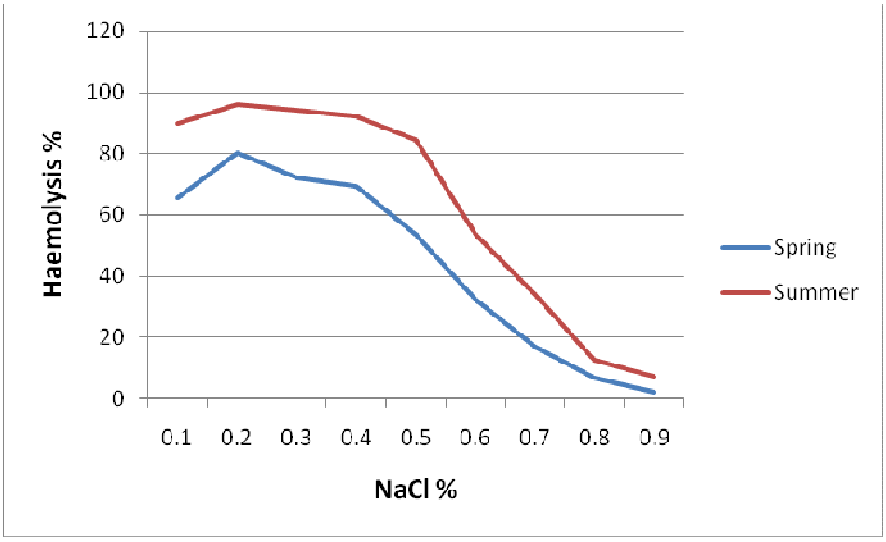
Effect of storage time- The storage time didn't showed any significance during the first 5 days of storage at 4^0 C. However, it showed a significant variation in % of haemolysis at 7^{th}, 8^{th} and 10^{th} days of storage. There was a stiff rise in curve for 0.4%, 0.3% and 0.2% NaCl indicating an decreased osmotic resistance and increased haemolysis of RBCs (Fig.1).For example, for 0.3% NaCl the average % of haemolysis at 1,7,8 and 10 days was 93.24± 3%, 94.57± 4%, 95.85± 4%, 95.69± 5% respectively.



Effect of incubation temperature- The incubation temperature showed a variation in osmotic fragility. Osmotic fragility curve of the samples didn't showed any significant variation at 18^0 C and 42^0 C. However it was higher at 4^0 C and 50^0 C. Thus in 0.3% NaCl at 4^0 C, 38^0 C and 50^0 C, average % of haemolysis was 94.08± 3%, 73.78± 4%, 95.65 ± 4% respectively (Fig .2).



Season- The values of osmotic fragility in terms of % of haemolysis were analysed in spring and summer. The % of haemolysis was higher in summer in comparision to spring for different saline concentrations. Thus, 0.4% and 0.3% NaCl solutions induced an average % of haemolysis of 92.33± 2%, 94.34± 3% respectively in summer as compared to 69.32± 3% and 72.09± 3% in spring season of the year (Fig.3).



Discussion

The osmotic fragility test is a measure of degree of haemolysis of erythrocytes. In the present study, it was noticed that there is highest percentage of haemolysis was observed in the 0.2%, 0.3%, 0.4% and 0.5% conc. of NaCl which gradually declined upto 0.9% conc of NaCl.

In this study haemolysis of the RBC increased with storage time. The variation was significant after 7th, 8th and 10 th day of incubation. Storage conditions causes significant

alterations in the membrane integrity, so, it's more prone tolysis (Arun *et al*., 1999). Proteases released by leukocytes during storage have been reported to cause RBC lysis (Humbert *et al*., 1991). Long storage of erythrocytes causes a gradual increase in haemolysis (Makroo *et al*., 2010).

Incubation temperature showed its effect on the haemolysis of RBC. The % of haemolysis was more at very low temp.(4^0C) or very high at 50^0C. This is in accordance to the findings of Islah *et al*.,(2016).

Season of the year has also influence on osmotic fragility values. In our study, it was observed that % of haemolysis was more in summer in comparision to spring. EOF is greatly influnced by heat as it causes massive destruction of erythrocytes. This was also studied by Islah *et al*.,(2016).

So osmotic fragility test can rightly be employed to know the degree of haemolytic anaemia in cattle. We have taken the blood samples of the anaemic cattle mostly affected with trypanosomiasis and theileriosis. But this may also be useful in other anaemic cases to know the osmotic stress to determine the osmotic fragility of erythrocytes. Osmotic fragility is very much beneficial in determining the haemolytic anaemia in cattle associated with different disease conditions. This will also be helpful for effective treatment regimen of the affected cattle.

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