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DOCUMENTATION OF HEINZ BODIES IN ERYTHROCYTES OF MICROCHIROPTERAN FALSE VAMPIRE BAT *MEGADERMALYRA LYRA*(Geoffroy) DURING THE ANNUAL CYCLE

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ABSTRACT

The presence of red blood cell abnormalities can give important diagnostic clues for the well being of any species. It is important therefore, that all features of the erythrocytes be assessed including number, color, size, polychromasia, the presence of precursors, alterations in shape, inclusions,the presence ofRBC parasites and arrangement. Keeping this in mind an annual study (April 2009-March 2010) was done on the peripheral blood of the false vampire bat,*Megadermalyralyra* (family:Megadermatidae) from both the genders collected from the underground mines of Mansar/Kandri near Nagpur, Maharashtra (20°92"N 78°95"E). Presence of inclusion bodies or "Heinz bodies" in a number of red cells particularly in the spherocytes were evident on supravital staining which are aggregates of denatured precipated hemoglobin produced when hemoglobin (Hb) protein globin chains are denatured through oxidative damage by reactive oxygen species since oxidative damage to RBCs is ongoing due to the continuous generation of free oxygen radicals from cellular metabolic pathways. 1 to 2 µm Heinz bodies peripherally located on the erythrocytes (protruded) were of common occurrence in the blood samples from femalescollected in the month of June (4%), July (8%), August (4%), September (15%) and December (8%) concomitant with male bats collected during June (3%), October(6%) and December (12%).Similarly single, smooth, rounded inclusions were observed during June (3%), July (4%), August (2%), September (6%) and December (4%) from femalesconcomitant with male bats collected during June (2%), October (4%) and December (6%). Their nonoccurrence during other months of the year may be justified to the presence of antioxidant pathways that protect against oxidative processes and the production of Heinz bodies. Some of the erythrocytes also revealed protruded "Heinz bodies", may be a means of cell healing. From the foregoing it is concluded that occurrence of such inclusion bodies may be correlated to systemic diseases and some injury to the erythrocyte, a decrease in oxygen carrying capacity,and therefore shortening of RBC life and hence on the wild population conservation since bats play a greater role in maintenance of ecological balance of nature.

INTRODUCTION

Certain morphologic changes in the erythrocytes, first described accurately by Heinz in 1890, have been noted by many investigators both in experimental animals and in man when provoked by aromatic compounds possessing amino, nitro, or hydroxyl groups of which the most notorious being aniline, nitrobenzene and allium from onion and garlic(Webster,1949;Webster et al. 1949;J and l,1963;Pierce et al.1972; Greenberg,1976;Kobluk etal.1995;Murphy,1996; Knight and Walter,2001).Heinz bodies are aggregates of denatured, precipitate hemoglobin within red blood cells or reflect the heightened precipitability of heme-deficient globin, attached to red cell membrane thiol groups presumably through mixed disulfide bodies (Jacob et al. 1968).

These are single, smooth, rounded inclusions or protrusions often with a pale ring of cytoplasm around the projection.Hemoglobin protein globin chains are denatured through oxidative damages by reactive oxygen species. Oxidative damage to RBCs is ongoing due to the continuous generation of free oxygen radicals from cellular metabolic pathways. Reactive oxygen species include superoxide anion (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) (Desnoyers, 2000). Similarly, oxidation of reactive sulfhydryl (S-H) groups creates disulfide bonds that change

the conformation of the globin protein chains, resulting in precipitation of the hemoglobin molecule when the phosphatase dehydrogenase gets low enough (Duncanet al. 1994;Tarigo-Martinie and Krimer,2002).

In RBC energy is generated almost exclusively through the breakdown of glucose. The Embden Meyerhof pathway is responsible for about 90% of the cells glucose use. The other pathway is functionally deficient or when environmental oxidants exceed its reducing capacity, haemoglobin precipitates (due to globin denaturation) to form "Heinz bodies" along the inner surface of red blood cell membrane. Its limited metabolism is barely enough to sustain it during its 120 days life span in circulation. All these metabolic pathways are closely related and must function in proper coordination if erythrocytes is to transport oxygen normally and survive in circulation (Miale,1962; Valdivieso and Tamsitt,1971;Pearson and Halloran,1972and Desnoyers,2000).

MATERIAL AND METHODS

Collection of specimens

The specimens of *Megadermalyralyra*were collected with the help of a mist net placed at the entrance of the underground mines atMansar / Kandri near Nagpur, Maharashtra (20°92"N 78°95"E) once every calendar month throughout the complete reproductive cycle from April 2009-March 2010.

Blood sampling

The bats were held in hands and no anesthesia was used at the time of sample collection. 2 ml of blood was collected into sterile Eppendorf tubes with no anticoagulants (neither EDTA nor heparin) after puncturing a wing vein. After blood sampling each bat was released.

Preparation of a blood film

Blood from males and femaleswere used without any anticoagulant directly from the syringe or needle immediately after collection by applying the "wedge smear" or the "slide and coverglass" technique. A routine preparation of at least three smears per blood sampling were performed. The air-dried smears were kept in a dustfree environment until they were stained. **Giemsa staining of blood smeared sli**

The occurrence of Heinz bodies in red blood cells were studied throughout the annual cycle from April 2009 to March 2010 by staining blood smears with Giemsa for 24 hours(Giemsa,1904; Wintrobe et al.1974).

Statistical Analysis

The data was analyzedstatistically,standard errors were calculated on the basis of which graphs were plotted to compare the seasonal variations (Delgaard, 2008).

OBSERVATIONS

In the present study Heinz bodies of 1 to 2 µm, darker and distinct were seen protruding from the abnormal erythrocytes," Spherocytes" membrane during the months June (4%), July (8%), August (4%), September (15%) and December (8%) from females and during June (3%), October (6%) and December (12%) from males (Table-1, fig.1 Panel A and B). Each protruded Heinz body appeared slightly paler than Spherocytes. Similarly single, smooth, rounded inclusions with a pale ring of cytoplasm were observed during June (3%), July (4%), August (2%), September (6%) and December (4%) from femalesand during June (2%), October (4%) and December (6%) from males (Table-1, fig. 2, Panel C and D).

Table 1 - Pertinent data regarding collection of *Megadermalyralyra* used for the present study. The number in parenthesis is for animals used.The observed data is also tabulated in the same table.

Specimen (n = 3)	Date of collection	Time of collection	Body weight (g)	Occurrence of Heinz bodies(%)
Female	22/04/2009	3.00 p.m.	39	Non occurrence
Male			30	Non occurrence
Female	19/05/2009	2.30 p.m.	36	Non occurrence
Male			32	Non occurrence
Female	25/06/2009	10.30 a.m	34	Protruded, 4%; Heinz inclusions, 3%
Male			31	Protruded, 3%; Heinz inclusions,2%
Female	30/07/2009	11.00 a.m.	34	Protruded, 8%; Heinz inclusions,4%
Male			32	Non occurrence
Female	16/08/2009	10.30 a.m.	34.5	Protruded, 4%; Heinz inclusions, 2%
Male			31	Non occurrence
Female	02/09/2009	3.30 p.m.	33	Protruded, 15%; Heinzinclusions, 6%
Male			32	Non occurrence
Female	04/10/2009	11.25 a.m.	33	Non occurrence
Male			32	Protruded, 6%; Heinz inclusions,4%
Female	05/11/2009	11.30 a.m.	34	Non occurrence
Male			31.5	Non occurrence
Female	08/12/2009	11.00 a.m.	33.5	Protruded, 8%; Heinz inclusions,4%
Male			31.5	Protruded, 12%; Heinzinclusions,6%
Female	07/01/2010	11.30 a.m.	34	Non occurrence
Male			30	Non occurrence
Female	02/02/2010	2.00 p.m.	34	Non occurrence
Male			32.5	Non occurrence
Female	03/03/2010	11.00 a.m.	36	Non occurrence
Male			32	Non occurrence

Fig. 1 -% of protruded Heinz bodies in males and females of *Megadermalyralyra* from the observed data.

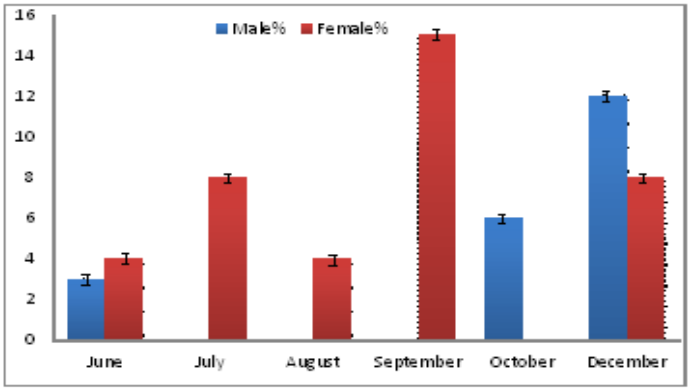
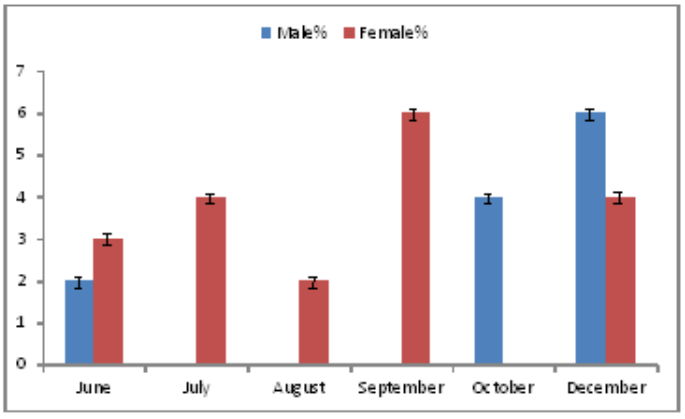


Fig. 2- Heinz bodies (inclusion) from male and female *Megadermalyra lyra*from the observed data .



DISCUSSION

In the present study Heinz bodyinclusion as well as protrutedwere documented in the natural collection of *Megadermalyralyra*during the months(June, July, August, September and Decemberfrom females) and (June, October and December from males), however, administration of a toxic element allium from onion and garlic as well as aromatic compounds causes oxidation of hemoglobin which 'bubbles' out on the outside as a Heinz body (Webster,1949; Webster et al.1949;Jandle,1963; Pierce et al.1972;Greenberg,1976;Kobluk et al.1995;Murphy,1996; Allison et al. 2000; Knight and Walter, 2001).Other possibility in the formation of Heinz bodies may be a greater dissociability of Hb from tetramers to diamers or presence of eight reactive sulfahydryl groups per hemoglobin molecule as described in cats (Taketa et al. 1967; Hamilton and Edelstein, 1972; Harvey and Kaneko,1977; Jain, 1986; Christopher et al. 1990; Robertson et al.1998).

The occurrence of 'Heinz bodies' in the peripheral circulation of any species studied till today or in human including the bat*Megadermalyralyra* may be due to oxidative damage which effect the heme portion of hemoglobin creating methemoglobin (MetHb) which is incapable of carrying oxygen. It is formed by oxidation of the iron in hemoglobin from a ferrous (Fe^{2++}) to a ferric (Fe^{3++}) state in methemoglobin (Weiss et al.1990; Duncan et al.1994).Furthermore,the presence of significant amounts of Heinz bodies in the blood stream must be evidence of some injury to the erythrocyte as well as ill functioning of spleen which acts as a red-cell "bouncer" of deformed cell from the blood stream.In fact the Heinz bodies should be phagocytosed by the spleen in a process called "pitting", or the entire RBC may be culled when spleen fails to respond, but sometimes these Heinz bodies circulate in the blood stream as noted in the present study (Duncan et al. 1994).

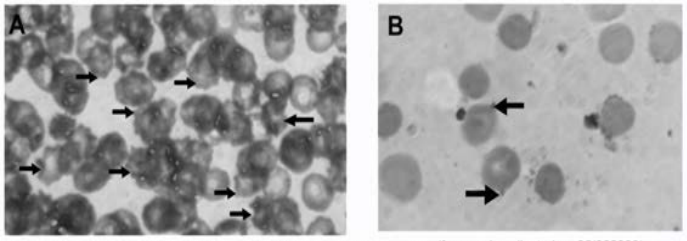
Production of large numbers of Heinz body may result in intravascular hemolytic anemia due to concurrent RBC membrane damage (Robertson et al.1998; Bain, 1999) but in our studies the percentage of their formation was insignificant.Other direct and indirect processes that may contribute to the removal of HzB – containing RBC's include membrane skeletal protein cross-linking, lipid peroxidation, glutathione depletion, antibody binding and cation imbalance (Christopher et al.1990) and hence their non occurrence in *Megadermalyralyra*is justifivable. Similarly the protrusion of Heinz bodies suggest that extrusion of Heinz bodies may be a means of cell healing which were more frequent in *Megadermalyralyra*(Rifkind, 1965; Christopher et al.1990; Bain,1999; Baker,2007).

The nonoccurrence of Heinz bodiesfrom the remaining months of the annual cycle from both the sexesof*Megaderma*may be justified to the presence of metabolic antioxidant pathways that protect against ongoing oxidative processes and hence the production of Heinz bodies.The hexose-monophosphatepathway produces reduced glutathione, a free radical scavenger that binds to reactive oxygen species before they can harm the cell and reduces disulfide bonds induced by oxidant stress. The methemoglobinreductase pathway reduces metHb to OxyHb, restoring the RBC's oxygen carrying capacity and hence the formation of Heinz bodies(Duncan et al.1994). As red blood cells are anucleate and lack the ability to regenerate enzymes, these protective mechanisms are exhausted as the cell ages. Older RBCs are therefore most susceptible to oxidative damage.

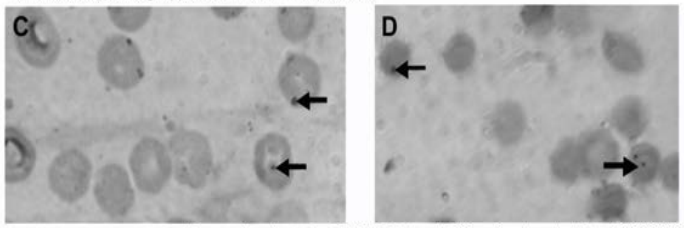
Formation of such Heinz bodies in *Megadermalyralyra* impairs RBC deformability by altering intracellular fluidity and this decrease in malleability leads to increased entrapment in narrow splenic sinusoids as blood is filtered through the spleen as stated by Williams et al.1972; Hasegawa et al.1993; Tarigo-Martinie and Koimer,2002. Thus the occurrence of Heinz bodies in *Megadermalyralyra*maybe correlated to systemic diseases, hyperthyroidism.

CONCLUSION

From the foregoing it is concluded that eventhough formation of Heinz bodies do not cause acute hemolysis but may shorten RBC survival,membrane hyperpermeability of erythrocyte, premature splenic entrapment and ultimately osmotic destruction of red blood cells, similarly cellular lesions may cause chronic or accelerated RBC destruction.



The blood smear in Panel A (from female collected on 02/09/2009) and B (from male collected on 25/062009) 1 to 2 um. darker and distinct protruding from the edge of abnormal erythrocytes" Spherocytes"(arrow). Heinz bodies appear slightly paler than the normal blood cells.



The blood smear in Panel C (from male collected on 25/06/2009)and D (from female collected on 16/08/2009) single smooth ,rounded inclusion with a pale ring of cytoplasm (arrow).

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