Glycoconj J (2007) 24:491–495
DOI 10.1007/s10719-007-9041-4

Erythrocyte surface sialic acid levels of clinically healthy mongrel and exotic (alsatian and terrier) breeds of dogs

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Received: 16 March 2007 / Accepted: 11 April 2007 / Published online: 16 May 2007

Abstract The erythrocyte surface sialic acid concentration of clinically healthy mongrel and exotic (Alsatian *i.e.* German shepherd and Terrier) breeds of dogs was analyzed in order to determine their role in the genetic resistance of these breeds of dogs to diseases that cause anaemia. The mean erythrocyte surface sialic acid (ESA) concentration was 57.08±1.67, 34.50±2.30 and 20.20±3.54 mg/dl for Mongrel, Alsatian (German shepherd) and Terrier breeds of dogs, respectively, on acid hydrolysis. The mean values of ESA obtained following enzymic hydrolysis of haemoglobin-free erythrocyte membranes using *Clostridium chauvoei* (Jakari strain) sialidase were 49.08±0.41, 30.97±1.82 and 18.64±0.75 mg/dl for Mongrel, Alsatian (German shepherd) and Terrier dogs respectively. When *Trypanosoma vivax* sialidase was used the ESA values obtained were 50.81± 0.37, 41.70± 0.94 and 19.65+0.65 mg/dl for Mongrel, Alsatian (German shepherd) and Terrier breeds of dogs respectively. This represents a statistically significant difference ($P<0.001$) between the mean ESA concentration of all the breeds of dogs investigated in this study. The higher mean ESA concentration in Mongrel dogs, compared to the exotic breeds may be responsible for their resistance to disease conditions, whose aetiologic agents produce neuraminidase and also cause anaemia.

Keywords Sialic acid · Mongrel · Exotic dogs

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Introduction

Sialic (neuraminic) acids are terminal structures of glycoproteins found in human and animal erythrocytes, thereby imparting their high surface negative charge [8, 16, 24]. The enzyme neuraminidase, usually produced by infectious agents can easily cleave-off the surface sialic acid and thereby lead to accelerated aging of erythrocytes, and results in the reduction of erythrocyte lifespan and anaemia due to erythrophagocytosis [9, 14, 19]. The erythrophagocytosis occurs as a result of the recognition and binding of the exposed galactose (galactosyl) residues on the erythrocyte surfaces (following enzymic hydrolysis in clinical disease) to galactose—specific receptors (lectins) on the kupffer cells (hepatic macrophages) and subsequently, removal of such desialylated erythrocytes from peripheral circulation [9, 22].

The Ndama breed of cattle has been reported to contain higher sialic acids on its erythrocyte surfaces, compared to the Zebu breed and this property confers on it a genetic resistance to trypanosomiasis, a phenomenon referred to as trypanotolerance [10]. Recent studies also indicate that some indigenous Nigerian poultry species, for instance the duck, with high sialic acid concentration on their erythrocyte surfaces are resistant to Newcastle disease virus infection [15, 22].

Studies on canine anaemia and chemotherapy in Nigeria suggest that canine trypanosomal anaemia is more devastating in exotic, than mongrel dogs [3]. Whether this is

related to the concentration of erythrocyte surface sialic acids of these breeds has yet to be investigated. Here we report, for the first time, the erythrocyte sialic acid levels of clinically healthy Mongrel, Alsatian (German shepherd) and Terrier breeds of dogs.

Materials and methods

Isotonic buffer (310 ideal milliosmolar, Imosm) and hypotonic buffer (20 Imosm) for washing and lysing red blood cells (RBC) were prepared as described previously [6].

Experimental animals

A total of 300 clinically healthy dogs, comprising Mongrel, Alsatian (German shepherd) and Terrier breeds (n=100 for each breed) belonging to clients of the Small Animal Unit of the Ahmadu Bello University Veterinary Teaching Hospital (ABUVTH), Zaria, Nigeria were used for the experiment. Before the commencement of sample collection, a complete physical examination of the animals was done [17].

Faecal and blood samples were also collected and examined for the presence of gastrointestinal and haemoparasites [18, 20] and only animals adjudged to be clinically healthy were used for the experiment. Haematological parameters (Packed Cell Volume, PCV; haemoglobin concentration, HB; total leucocyte counts, WBC and total erythrocyte counts, RBC) were deermined [12].

Blood collection and determination of haematological parameters and total protein (TP) concentration

Exactly 2 ml of blood from clinically healthy Mongrel, Alsatian (German shepherd) and Terrier breeds of dogs (n= 100 for each breed) were collected into vacutainers containing either 10 mg of EDTA for the determination of haematological parameters (PCV, Hb, WBC and RBC) and TP or 0.3 ml acid citrate dextrose (ACD) for the preparation of haemoglobin free erythrocyte membranes (ghosts). For each dog sampled, blood was collected using both EDTA and ACD. The haematological parameters (PCV, Hb, WBC and RBC) and TP concentration were determined as described previously [11].

Preparation of haemoglobin-free erythrocyte membranes

The blood collected in ACD was centrifuged at 1,000 g for 5 min at 4°C (Beckman, J-21 B) to separate the red blood cells (RBC) from the buffy coat and plasma. Thereafter, the buffy coat and plasma were decanted and the sediment containing RBC was washed several times with isotonic buffer (5 ml) by centrifuging at 20,000 g for 5 min at 4°C (Beckman, J-21 B) and decanting until the RBCs were adjudged to be clean. Hypotonic buffer (5 ml) was added to lyse the RBC. This was followed by thorough washing as stated above. Subsequently, clean haemoglobin-free erythrocyte membranes (ghosts) were prepared using the method described by Dodge *et al.* [6].

Isolation and partial purification of neuraminidases from Clostridium chauvoei (Jakari strain) and Trypanosoma vivax

Lyophilized *Clostridium chauvoei* (Jakari strain) obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria was cultured using the method described [7] and the enzyme sialidase was isolated as described [23]. The sialidase was partially purified using ammonium sulphate fractionation, diethyl amino ethyl (DEAE) cellulose chromatography, hydroxyapatite chromatography and phenyl sepharose chromatography [23]. Similarly, *Trypanosoma vivax* which was already characterized was obtained from the National Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria and inoculated into a donor goat and parasitaemia was observed daily until peak parasitaemia was attained on day 11 post-infection. Blood (20 ml) was then collected from the donor goat through jugular venipuncture and *Trypanosoma vivax* was isolated by differential elution of the trypanosomes on DEAE cellulose using phosphate buffered saline glucose (PBSG) pH 8.0 [13]. Sialidase was isolated from the trypanosomes and partially purified [4].

Determination of total sialic acid concentration on the haemoglobin-free erythrocyte membranes of mongrel and exotic dogs

This was carried out using both acid hydrolysis (with 1 N H_2SO_4) and enzymic hydrolysis with partially

Table 1 Mean values of haematological parameters of Mongrel, Alsatian (German shepherd) and Terrier breeds of dogs

Breed of dogs	PCV (%)	Hb (gdl^{-1})	WBC ($10^9/l$)	RBC ($10^9/l$)
Mongrel	48.73±9.32	16.24±3.11	8.57±2.87	8.11±1.56
Alsatian (German shepherd)	53.67±2.52	17.90±0.85	15.33±3.06	8.93+0.40
Terrier	54.33±0.383	18.12±1.28	17.27±0.55	9.06±0.64

Table 2 Mean values of total protein (gdl^{-1}) and erythrocyte surface sialic acid (ESA) concentration ($mgdl^{-1}$)

Breed of Dog	TP (gdl^{-1})	ESA (gdl^{-1})		
		Acid hydrolysis	Enzymatic hydrolysis	
			C. chauvoei Sialidase	*T. vivax* Sialidase
Mongrel	7.85±1.44	57.08±1.67	49.08±0.41	50.81±0.37
Alsatian (German shepherd)	6.07±0.23	37.50±2.30	30.97±1.82	41.70±0.94
Terrier	5.67±0.76	20.20±3.54	18.64±0.75	19.64±0.63

purified *Clostridium chauvoei* (Jakari strain) and *Trypanosoma vivax* sialidase [4, 23]. The free sialic acid liberated was assayed using the thiobarbituric acid assay method [2].

Statistical analysis

The data obtained from the experiment was analyzed using analysis of variance (ANOVA, Duncan multiple range test) and the means ± standard deviations (SD) were compared, so that values of $P<0.001$ were considered to be statistically significant. Correlation and regression statistics were computed between mean packed cell volume and mean erythrocyte surface sialic acid (ESA) concentrations [5].

Results

Haematological parameters

Table 1 shows the results of haematological parameters (PCV, HB, WBC and RBC) of the dogs investigated. There was a statistically significant difference ($P<0.001$) between the mean PCV, HB, WBC and RBC of all the breeds of dogs investigated. Mean PCV, HB, WBC and RBC were higher in the Terrier, followed by Alsatian (German shepherd), while Mongrel dogs had the least mean haematological values (PCV, HB, WBC and RBC).

Total protein (TP) concentration

Mean TP concentration was higher in the Mongrel followed by Alsatian, with Terrier breed having the least mean TP concentration. This represents a statistically significant difference ($P<0.001$) between the TP concentration of all the breeds of dogs investigated in the study.

Erythrocyte surface sialic acid (ESA) concentration

The Mongrel dogs showed a higher sialic acid concentration on their erythrocytes, compared to the exotics (Alsatian *i.e.* German shepherd and Terrier breeds of dogs) (Table 2). Both on acid and enzymic hydrolysis of the haemoglobin-free erythrocyte membranes (ghosts) from the breeds of dogs examined, there was a statistically significant difference ($P<0.001$) between the erythrocyte surface sialic acid concentration of all the breeds of dogs examined in this study. Also, there was a negative correlation ($r=-0.68$, $P<0.001$) between mean erythrocyte surface sialic acid (ESA) concentration and packed cell volume (PCV).

Discussion

This study shows that Mongrel dogs have higher concentration of sialic acid on their erythrocytes, compared to the exotic breeds (Alsatian *i.e.* German shepherd and Terrier) which were investigated in the present study. There was a statistically significant difference ($P<0.001$) between the erythrocyte surface sialic acid concentration of all the breeds of dogs investigated.

Mongrel dogs are known to be less vulnerable to canine trypanosomal anaemia, compared to the exotic breeds of dogs (Alsatian *i.e.* German shepherd and Terrier). The response of the erythrocyte bound sialic acid to acid hydrolysis (using 1 N H_2SO_4) and enzymic hydrolysis using sialidase from *Clostridium chauvoei* (Jakari strain)

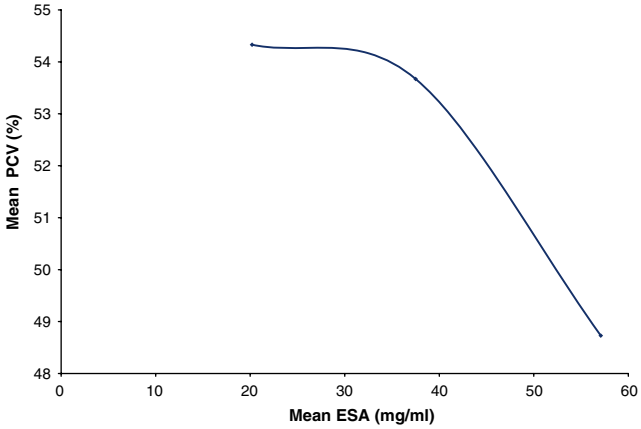

Fig. 1 The correlation between packed cell volume (PCV) and erythrocyte surface sialic acid (ESA) concentration of clinically healthy mongrel and exotic breeds of dogs (acid hydrolysis)

and *Trypanosoma vivax in vitro* may be a reflection of what obtains in clinical trypanosomiasis. A similar finding was observed by Esievo *et al.* [10] who also reported higher levels of sialic acids on the erythrocytes of Ndama cattle which are known to be trypanotolerant, compared to Zebu cattle which are trypanosusceptible. In a recent study [1] it was shown that young Mongrel dogs (puppies) demonstrated tolerance to *Trypanosoma congolense* during experimental infection. In the study which lasted up to 8 weeks, there was no statistically significant difference ($P>0.05$) between the daily mean packed cell volume (PCV) of the healthy (controls) and the infected animals up to week 7 of infection. There was also no significant variation in the daily mean weights of both the infected and control animals. It is possible to insinuate, from the findings in the present study, that the high sialic acid complement of erythrocytes of Mongrel dogs may be a factor responsible for the ability of the breed to withstand trypanosomiasis, compared to Alsatian (German shepherd) and Terrier breeds which are highly susceptible and characterized by a devastating anaemia during clinical trypanosomiasis.

In the PCV analysis, Terrier breed had the highest PCV, compared to Mongrel and Alsatian breeds. The difference between the mean PCV values of Mongrel on the one hand and the exotic breeds on the other was statistically significant ($P<0.001$). There was no statistically significant difference ($P>0.001$) between mean PCV values of Alsatian (German shepherd) and Terrier breeds. The correlation between PCV values and erythrocyte surface sialic acid concentration was negative ($r=-0.68$), so that dogs with high mean PCV values had low ESA concentrations. Terrier breed which had the highest mean PCV value was found to have the lowest mean ESA concentration. This implies that even in the presence of an infectious agent, for instance *Trypanosoma vivax* or *Trypanosoma congolense*, which cause lyses of RBC or cleave sialic acid to cause anaemia, this breed (Terrier) can still retain adequate levels of red blood cells (erythrocytes) to guarantee its survival. However, the Mongrel breed had the lowest mean PCV value in the present study and highest sialic acid concentration on its erythrocytes, so that the few erythrocytes in this breed are better protected from the menace of infectious agents that cleave sialic acid from erythrocyte surfaces or even cause mechanical damage to erythrocytes, leading to anaemia, compared to Alsatian and Terrier breeds. This implies that a higher infection with trypanosomes, for instance, may be required to produce clinical anaemia in Mongrel dogs, compared to exotic Alsatian and Terrier breeds (Fig. 1).

Hydrolysis of haemoglobin-free erythrocyte membranes (ghosts) using *Trypanosoma vivax* sialidase was higher in all the breeds of dogs examined, compared to the enzyme from *Clostridium chauvoei* (Jakari strain). It is known that dogs are resistant to *Clostridium chauvoei* [21], as they are not natural hosts of the disease caused by the infectious agent, and this may explain in part why the hydrolysis of ghost cells from dogs by its sialidase was less, compared to the enzyme from trypanosomes that are known to infect dogs.

The mean TP concentration of Mongrel dogs was higher than that of the exotics (Alsatian and Terrier breeds). This does not indicate that the nutritive status of the Mongrel, with regard to protein intake was higher than the exotics. Instead, it may be a reflection of the water deprivation status of Mongrel dogs, since Mongrels are usually free range and allowed to scavenge for food and water on their own. They may be fed and given drinking water only once a day or not at all by their owners. In the circumstance, blood samples collected from such Mongrels may reflect an obviously high total plasma protein concentration, indicating possible water deprivation, as was the case in the present study.

This study has provided baseline information on the erythrocyte surface sialic acid complements of clinically healthy Mongrel and exotic (Alsatian and Terrier) breeds of dogs and the possible role of the sialic acid of these breeds in disease resistance. Further work is under way to determine the sialic acid types on the erythrocyte surfaces of these breeds of dogs which can be exploited in selective breeding.

Acknowledgement The authors of this manuscript are grateful to the National Veterinary Research Institute (NVRI), Vom, Nigeria for providing lyophilized *Clostridium chauvoei* (Jakari strain) and the National Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria for providing *Trypanosoma vivax* which were used for the experiment.

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