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## Interrelationships between thirteen parameters of blood in apparently healthy buffalo-calves (Pakistan)

**C**orrélation entre 13 paramètres sanguins chez de jeunes buffles apparemment sains au Pakistan – On a procédé à une numération des globules rouges et une glycémie dans le sang de 178 jeunes buffles de race Nili-Ravi, des deux sexes et de tous âges, apparemment sains, examinés aux deux saisons extrêmes (été/hiver). Ces deux facteurs ont montré une corrélation positive ( $r = 0,6989$ ) ainsi qu'entre les taux d'hémoglobine et de cholestérol ( $r = 0,5705$ ). Au contraire, les facteurs suivants étaient inversement proportionnels deux à deux :

- l'azote uréique sanguin et l'index ictérique ( $r = -0,5114$ ) ;
- l'azote uréique sanguin et le taux de sédimentation globulaire ( $r = -0,6433$ ) ;
- le taux d'ions sodium et le temps de coagulation ( $r = -0,6329$ ) ;
- le taux d'ions potassium et l'index ictérique ( $r = -0,5158$ ).

Ces résultats sont basés sur des valeurs significatives de "r", lequel est calculé à partir des moyennes des 13 paramètres étudiés sur 16 lots (4 classes d'âge par sexe et 2 saisons). L'ampleur de ces corrélations est mise en évidence au moyen d'équations de régression. *Mots clés* : Buffle – Hématologie – Pakistan.

### INTRODUCTION

For all practical purposes, interrelationships between different parameters of buffalo blood remain more or less untapped. However, in spite of a few individual variations, THIMMAIAH *et al* (7) mention a negative correlation between iron content and erythrocyte sedimentation rate, but a positive correlation between iron content, packed cell volume and haemoglobin contents, specially in their oldest age group of 2,168.6 days average age. It may be noted that this observation had no statistical backing.

An attempt is made to ascertain relationships, if any, between 13 different parameters of blood among the growing buffalo-calves of either sex sampled in the two extremes of weather.

### MATERIALS AND METHODS

Apparently healthy Nili-Ravi buffalo-calves of either sex, 178 in all, were selected randomly from the stocks maintained at the Livestock Production Research Institute: Bahadar Nagar and Qadir Abad stations. Of these, 93 samples were obtained in the first week of January (winter) and 85 during the first week of July (summer). Their body weights varied from 25 to 520 kg, while their ages ranged from 6 to 1,095 days (6).

About 5 ml of blood was drawn before the morning feed by the usual jugular puncture method. It was taken directly in two dry clean test tubes; one of which contained few oxalate crystals. Clear straw coloured serum, when separated, was carefully transferred into screw capped bottles and stored at  $-15$  to  $-20$  °C, until used. Centrifugation for a few minutes at about 1,500 rpm facilitated serum separation in few difficult cases. Samples showing slightest sign of haemolysis were all discarded.

Red blood cell (RBC) count was done with the help of a hemocytometer. The blood was diluted 200 times in Toisson's fluid and five small squares of the counting chamber were scanned (5). The count was expressed in millions per cubic millimeter ( $\text{mm}^3$ ) of blood.

Total leukocytic count (TLC) was also obtained with the help of a hemocytometer. A 1:20 dilution was made in 3 p.100 aqueous acetic acid. The count was spread over four large squares (5); which was expressed in thousands per  $\text{mm}^3$  of blood.

Erythrocyte sedimentation rate (ESR) was determined by the Westergen sedimentation tubes after BENJAMIN (1). Readings in millimeters (mm) were recorded after the first hour.

Packed cell volume (PCV) was obtained in percentage by the microhematocrit method after BUSH (2).

Slide method was used after KOLMER *et al.* (5) to

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M. A. Majeed, I. R. Khan, M. A. Toor, A. Ahmad

**TABLE I** The primary data comprising of group means obtained from a variable number of buffalo-calves sampled in the two extremes of weather under the four age groups and the two sexes. In each case the number of observations (n) is shown in parenthesis.

Parameters	Summer							
	Sucklers		Calves		Yearling		Young stock	
	♂	♀	♂	♀	♂	♀	♂	♀
RBC	6.19 (8)	6.74 (12)	6.86 (11)	7.85 (11)	7.09 (9)	6.44 (10)	5.97 (10)	5.46 (9)
ESR	29.00 (8)	28.33 (12)	84.36 (11)	102.75 (12)	97.00 (9)	71.40 (10)	69.90 (10)	52.34 (9)
TLC	16.30 (12)	15.94 (12)	14.32 (11)	12.63 (11)	13.98 (9)	12.51 (10)	13.19 (9)	10.38 (8)
PCV	37.07 (8)	37.92 (12)	34.96 (11)	37.00 (11)	32.89 (9)	34.00 (10)	28.60 (10)	33.67 (9)
Cgn	4.57 (8)	4.92 (12)	5.43 (13)	4.23 (11)	5.70 (10)	5.50 (10)	7.30 (10)	5.60 (10)
Hb	10.95 (8)	12.46 (12)	10.20 (11)	10.50 (12)	12.09 (9)	11.81 (10)	10.60 (10)	15.53 (9)
TSP	7.22 (8)	6.77 (12)	7.10 (13)	7.21 (10)	7.56 (8)	7.81 (10)	8.09 (10)	7.88 (10)
BUN	22.50 (8)	29.42 (12)	9.24 (13)	11.80 (10)	5.70 (10)	15.50 (10)	40.20 (10)	21.30 (10)
Chl	183.25 (8)	208.67 (12)	333.00 (13)	256.80 (10)	115.78 (9)	183.30 (10)	396.80 (10)	874.00 (10)
Na	340.88 (8)	343.33 (9)	342.08 (13)	341.83 (12)	340.88 (8)	342.89 (9)	334.80 (10)	343.11 (9)
K	15.83 (8)	15.95 (10)	15.61 (13)	15.64 (12)	15.58 (8)	15.53 (8)	15.69 (10)	15.53 (8)
Glu	171.75 (4)	106.34 (12)	57.17 (12)	93.82 (11)	67.63 (8)	73.50 (10)	103.50 (10)	126.40 (10)
Ict	22.00 (8)	20.92 (12)	17.37 (11)	17.89 (9)	16.58 (7)	26.23 (9)	9.10 (10)	13.30 (10)
Parameters	Winter							
	Sucklers		Calves		Yearlings		Young stock	
	♂	♀	♂	♀	♂	♀	♂	♀
RBC	7.27 (18)	6.46 (10)	6.30 (10)	5.92 (18)	4.69 (10)	5.26 (10)	7.16 (7)	6.41 (10)
ESR	13.28 (18)	17.90 (10)	12.30 (10)	31.44 (18)	43.20 (10)	71.40 (10)	49.75 (8)	61.80 (10)
TLC	12.54 (18)	12.79 (10)	13.04 (10)	11.81 (18)	13.50 (10)	12.23 (10)	13.00 (7)	12.78 (10)
PCV	33.92 (18)	34.70 (10)	40.00 (13)	38.75 (18)	31.45 (10)	34.30 (10)	38.43 (7)	32.55 (10)
Cgn	4.00 (17)	3.08 (10)	7.80 (10)	3.62 (18)	4.25 (9)	3.76 (10)	3.14 (7)	5.70 (10)
Hb	10.29 (18)	10.59 (10)	12.78 (10)	11.65 (18)	8.68 (10)	10.28 (10)	12.59 (7)	11.70 (10)
TSP	7.15 (18)	7.28 (10)	7.31 (10)	7.47 (17)	7.18 (10)	7.13 (10)	8.02 (7)	7.61 (10)
BUN	36.05 (10)	34.90 (9)	111.30 (10)	75.02 (18)	31.12 (7)	16.40 (8)	22.50 (4)	19.87 (9)
Chl	163.50 (16)	174.80 (10)	158.13 (8)	188.65 (17)	179.50 (10)	121.23 (9)	170.34 (6)	128.75 (8)
Na	344.78 (18)	342.00 (10)	336.90 (10)	340.00 (18)	341.90 (10)	339.70 (10)	344.14 (7)	340.60 (10)
K	16.41 (18)	15.83 (10)	16.25 (10)	16.07 (18)	16.55 (10)	16.28 (10)	16.00 (7)	16.71 (10)
Glu	105.13 (17)	120.72 (9)	126.15 (8)	89.39 (18)	50.60 (10)	103.49 (9)	25.93 (5)	102.74 (9)
Ict	11.17 (16)	11.79 (8)	8.69 (9)	10.24 (17)	12.40 (8)	11.69 (10)	11.86 (7)	10.94 (10)

record coagulation (Cgn) time of fresh whole blood, in minutes.

Direct reading on proteinometer (Bausch & Lomb) gave grams (g) of total proteins (TSP) in 100 ml (dl) of serum.

Units of icterus (Ict) index were estimated by the comparison of optical densities as described by KOLMER *et al.* (5).

Sodium (Na) and potassium (K) ions were ascertained by a flame photometer after COLES (4). These values were expressed in milligram (mg) per cent which multiplied by factors 0.435 and 0.256 can respectively be converted into m Eq/l.

HAURY's (\*) kits were employed for the estimation of haemoglobin (Hb) in g per dl of blood ; blood urea nitrogen (BUN) in mg per dl of blood ; and cholesterol (Chl) in mg per dl of serum.

Using the Ingleheim (\*\*) kit, mg of glucose (Glu) per dl of blood was obtained by the orthotoluidin colorimetric method after BUSH (2).

The whole data was first tabulated in 16 lots, separating the two seasons : summer and winter ; four age groups : sucklers, calves, yearlings and young stock ; and the two sexes : males and females. The values of "r" were calculated following CHATFIELD (3) from the arithmetic means of these lots. From this array of means (Table I), it may be seen that the number of observations, *i.e.* n, varies from 4 to 18 in different parameters. The magnitude of these relationships were further ascertained by prediction equations, which were shown as regression lines.

## RESULTS AND DISCUSSION

Correlation coefficients or the "r" values were calculated between the 13 parameters of blood studied

among 178 buffalo-calves. The 16 group means obtained from 4 to 18 observations from an equally variable number of calves studied in the two extremes of weather, under the four age groups and the two sexes formed the primary data (Table I). This transformation was made necessary by mere biophysical compulsions because equal number of observations on all the 13 parameters studied were not forthcoming simultaneously. Furthermore, the effects of age, sex, as well as season, which were found to influence respectively 10, 4 and 8 of these parameters significantly (6), were thus minimized.

Significant values of "r" (Table II) suggest positive correlation between red cell count and serum glucose ( $r = 0.6989$ ), as well as, haemoglobin and cholesterol ( $r = 0.5705$ ). On the other hand, negative correlations were observed in between coagulation time and sodium ( $r = -0.6329$ ) ; blood urea nitrogen and icterus index ( $r = -0.5114$ ) ; erythrocyte sedimentation rate and blood urea nitrogen ( $r = -0.6433$ ) ; and potassium and icterus index ( $r = -0.5158$ ).

The magnitude of these relationships was also ascertained in terms of regression coefficients. Prediction equations and regression lines (Fig. 1) depict the overall trends. These statistics revealed that :

- Each mg rise in blood urea nitrogen lowered :
  - the erythrocyte sedimentation rate by 0.696 mm ;
  - the icterus index by 0.097 units.
- Each mg rise in potassium reduced :
  - the icterus index by 6.622 units.
- Each mg rise in sodium quickened :
  - the coagulation time by 0.336 minutes.
- Each mg rise in glucose raised :
  - the red cell count by 0.016 million.
- Each g rise in haemoglobin raised :
  - 68.2 units cholesterol.

As stated earlier, the authors were unable to find any account of interrelationships between the blood parameters under discussion in buffalo. Occasionally one comes across sporadic information on one of these blood values but then the parameter with which it is correlated is not included in the present study. Comparison of these findings with any previous study is therefore impossible.

According to BUSH (2) millions of red blood cells per  $\mu$ l of blood, when of normal size, approximately equal to :

- packed cell volume, in percentage, divided by 6,
- haemoglobin, in g p. 100, divided by 2.

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M. A. Majeed, I. R. Khan, M. A. Toor, A. Ahmad

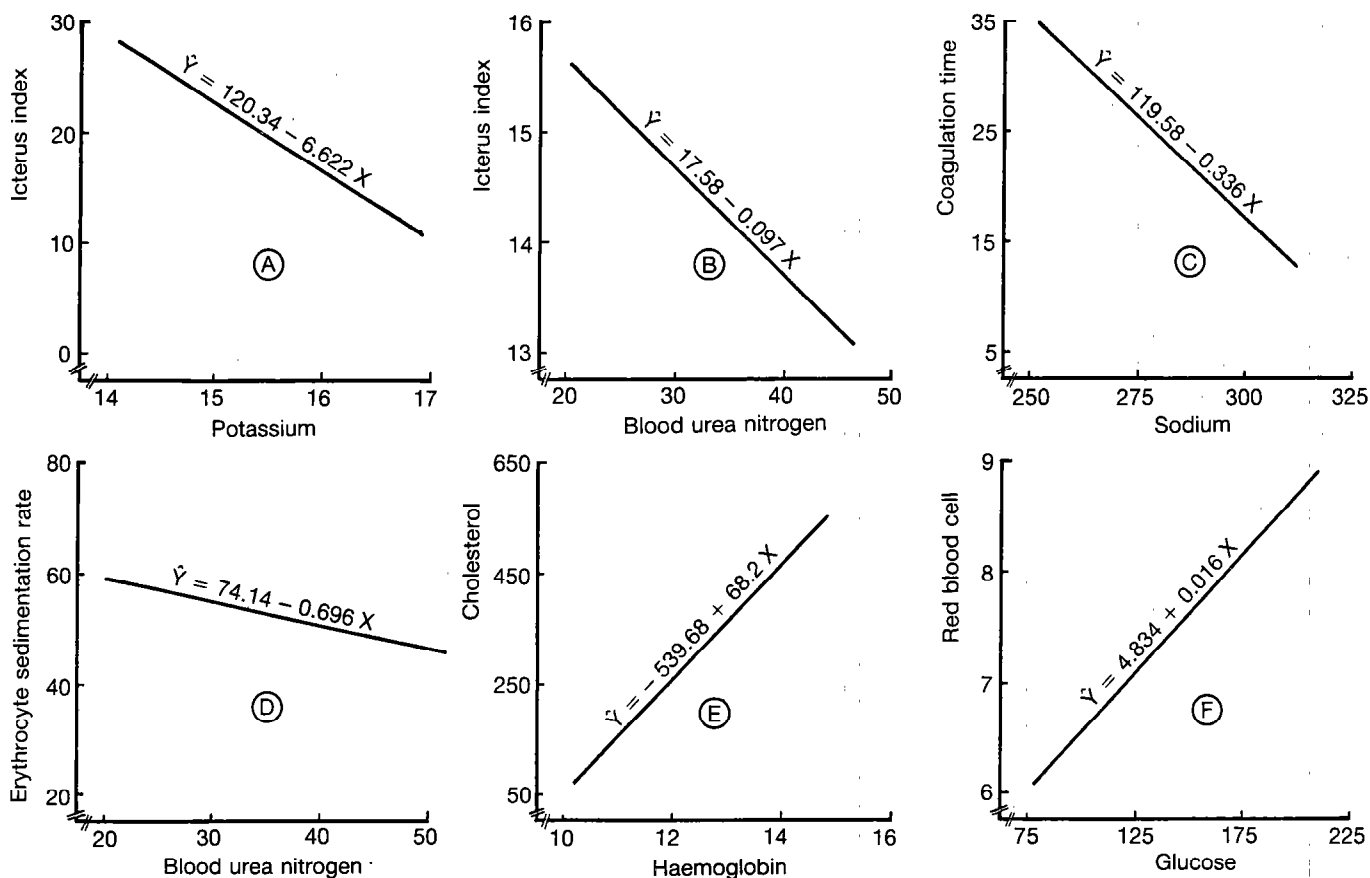


Fig. 1 : Showing significant regression lines and prediction equations : A. Icterus index on potassium ; B. Icterus index on blood urea nitrogen ; C. Coagulation time on sodium ; D. Erythrocyte sedimentation rate on blood urea nitrogen ; E. Cholesterol on haemoglobin ; F. Red blood cells on glucose.

TABLE II Correlation coefficients or values of 'r' between 13 parameters of blood.

Parameters studied	RBC count (RBC)	Erythrocyte sed. rate (ESR)	Total leuk. count (TLC)	Packed cell volume (PCV)	Coagulation time (Cgn)	Haemoglobin (Hb)
Ict	0.2654	0.2871	0.4794	0.1089	- 0.0508	0.0337
Glu	0.6989**	- 0.4206	0.1402	0.1286	0.2125	0.1968
K	- 0.3347	- 0.4014	- 0.0740	- 0.0451	- 0.1723	0.3488
Na	0.3151	- 0.0866	- 0.0048	0.1603	- 0.6329**	0.1089
Chl	- 0.2416	0.1168	- 0.4188	- 0.2134	0.2584	0.5705*
BUN	- 0.1971	- 0.6433**	- 0.1453	0.4656	0.3236	0.1441
TSP	0.0637	0.2448	- 0.4721	- 0.3058	0.2574	0.4093
Hb	0.2140	- 0.0891	- 0.3053	0.3052	0.2926	
Cgn	- 0.0754	0.1427	0.0610	- 0.2018		
PCV	0.3347	- 0.3716	0.1736			
TLC	0.2065	- 0.0931				
ESR	0.2222					

TABLE II (Continuation).

Parameters studied	Total serum protein (TSP)	Blood urea nitrogen (BUN)	Cholesterol (Chl)	Sodium (Na)	Potassium (K)	Glucose (Glu)	Icterus index (Ict)
Ict	- 0.2005	- 0.5114*	- 0.0447	0.4095	- 0.5158*	0.0398	
Glu	- 0.2220	0.2663	0.1897	- 0.2815	- 0.0132		
K	- 0.3151	0.2838	- 0.4671	- 0.2719			
Na	- 0.2247	- 0.4855	0.0345				
Chl	0.3605	- 0.1135					
BUN	- 0.0375						

Significant at 1 (\*\*\*) and 5 (\*) p. 100 levels, respectively. Minus (-) sign when prefixed indicates negative correlation.

The overall averages of MAJEED *et al.* (6) seem to fit well into these relationships because they found that 6.4 million RBC count per mm<sup>3</sup> of blood approximately equalled (a) 35/6 p. 100 PCV, and (b) 11.33/2 g p. 100 haemoglobin. These three parameters are interdependent yet health or nutritional status of an individual affect these values one way or the other (2). Even in apparently healthy growing buffalo-calves factors, e.g., age, season and sex have been shown to affect 10,8 and 4 of these 13 blood values significantly (6). The net result of these rather complex inter-relationships therefore cannot be grasped until and unless a larger data from populations of different geographical,

physiological and genetic backgrounds are analysed and the whole data subjected to statistical testing.

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MAJEED (M. A.), KHAN (I. R.), TOOR (M. A.), AHMAD (A.). Interrelationships between 13 parameters of blood in apparently healthy buffalo-calves (Pakistan). *Rev. Elev. Méd. vét. Pays trop.*, 1986, 39 (3-4) : 409-414.

In the blood of 178 Nili-Ravi apparently healthy buffalo-calves of growing ages and either sex, examined in the two extremes of weather, red cell count and serum glucose were found positively correlated with each other ( $r = 0.6989$ ) and so were haemoglobin and cholesterol ( $r = 0.5705$ ). Blood urea nitrogen and icterus index ( $r = -0.5114$ ); blood urea nitrogen and erythrocyte sedimentation rate ( $r = -0.6433$ ); sodium ions and coagulation time ( $r = -0.6329$ ); as well as potassium ions and icterus index ( $r = -0.5158$ ) were all inversely proportional to each other.

These conclusions are based on significant values of "r" as calculated from 16 group means of the 13 parameters studied (4 classes of age per sex and per selected season). Magnitude of these relationships is also demonstrated by the regression lines. *Key words* : Buffalo-calf - Hematology - Pakistan.

MAJEED (M. A.), KHAN (I. R.), TOOR (M. A.), AHMAD (A.). Correlación entre 13 parámetros sanguíneos en jóvenes búfalos aparentemente sanos en Paquistán. *Rev. Elev. Méd. vét. Pays trop.*, 1986, 39 (3-4) : 409-414.

Se examinaron en verano y en invierno 178 jóvenes búfalos de raza Nili-Ravi, machos y hembras de todas las edades. Existe una correlación positiva ( $r = 0,6989$ ) entre el recuento de glóbulos y la glicemia, y entre las tasas de hemoglobina y de colesterol ( $r = 0,5705$ ). En cambio, los factores siguientes son inversamente proporcionales dos a dos :

- el nitrógeno sanguíneo y el índice de bilis ( $r = -0,5114$ ) ;
- el nitrógeno sanguíneo y el índice de sedimentación globular ( $r = -0,6433$ ) ;
- la proporción de iones sodio y el tiempo de coagulación ( $r = -0,6329$ ) ;
- la proporción de iones potasio y el índice de bilis ( $r = -0,5158$ ).

Se basan estos resultados en valores significativos de "r", el cual se calcula a partir de los términos medios de los 13 parámetros obtenidos a partir de 16 grupos (4 clases de edad por sexo y 2 estaciones). Las rectas de regresión demuestran la importancia de estas interrelaciones. *Palabras claves* : Búfalo - Hematología - Paquistán.

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