Serum biochemical and haematological reference intervals for water buffalo (*Bubalus bubalis*) heifers

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Based on a review of the literature, reference intervals for water buffalo (*Bubalus bubalis*) serum biochemistry and haematology have not previously been published. The current study was done to establish reference intervals for water buffalo heifers. The International Federation of Clinical Chemistry stated that at least 120 values are necessary to obtain reliable estimates for reference intervals. A total number of 127 clinically healthy buffalo heifers (1–2 years old) were included in the study. Animals were examined at buffalo farms that belong to Assiut Governorate, Egypt. Three types of samples were collected: serum samples for biochemical analysis, whole blood samples for haematological analysis and faecal samples for parasitological examination. Animals that fitted the inclusion criteria were included in the study. Biochemical analysis included serum total proteins, albumin, total globulins, alpha, beta and gamma globulin levels, and aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, creatine phosphokinase and lactate dehydrogenase activity. In addition to the above, serum creatinine, urea, total bilirubin, direct bilirubin, indirect bilirubin, sodium, potassium, chloride, magnesium, calcium, phosphorus, copper, zinc, iron, triglycerides, high density lipoprotein, low density lipoprotein, very low density lipoprotein, glucose levels and 20 haematological variables were measured. The 95.0% reference intervals were calculated by removing the upper and lower 2.5% of the interval for each serum biochemical constituent to give the 2.5 and 97.5 percentiles. Confidence intervals were calculated for each reference limit. Reference intervals from the current study were compared with established values for cows. The current study is as far as could be determined the first that establishes reference intervals for the serum biochemical and haematological parameters in water buffalo heifers.

Introduction

Water buffaloes (*Bubalus bubalis*) are originally from Asia and they are mainly distributed in tropical and subtropical Asia. The buffaloes have been classified according to their appearance, wallowing habits and uses into river and swamp buffalo. The latter are used for draught power and are found in countries that include the Indian sub-continent and the Mediterranean countries. Buffaloes are used mainly as a source of meat and milk (Cockril 1977, 1980). Water buffaloes can compete very successfully with and even surpass cattle in their ability to adapt to hot climates and swamp lands (Webster & Wilson 1980). Water buffalo are therefore of special importance in milk and meat production in the Nile River valley in Egypt (GOVS 2005).

Animal health can be defined as the absence of disease determined by clinical examinations combined with various diagnostic tests (Bailey, Sarmandal & Grant 1989; Klinkhoff *et al*. 1988; Pattinson & Theron 1989; Theodossi *et al*. 1981). Serum biochemical and haematological reference values are used to establish normality and to diagnose disease and physiological alteration. Textbook reference intervals produced by European or United States veterinary laboratories (e.g. Kaneko, Harvey & Bruss 1997) are often based on animals living under good husbandry conditions in temperate climates, and the reference sample groups may differ from those of the developing countries. Potential differences may be attributed to genetic factors, the quality and quantity of nutrition, presence or absence of water, electrolyte losses in sweat, internal parasites and climatic conditions. This makes it difficult to depend on reference intervals from other countries to interpret results for animals living in Egypt or elsewhere in Africa.

Grasbeck *et al*. (1979) specified some factors that must be established when using reference intervals, such as the characterisation of the reference population with respect to age and sex. The inclusion and exclusion criteria that were used for selecting the reference sample group were based on physiological and environmental conditions. To the best of the authors' knowledge, reference intervals have not yet been established for water buffalo. Therefore, the current study was carried out to establish reference intervals for haematological and serum biochemical parameters in water buffalo heifers.

Materials and methods

Animals

Buffalo heifers (1–2 years old) were examined at various buffalo farms in Egypt, namely Land of Kheir, Valley of Sheeh and Bani Sanad, all belonging to Assiut Governorate.

Animals were examined carefully based on a number of inclusion criteria (Box 1). Only animals that met the inclusion criteria were included in the study. The management and feeding systems were approximately the same at all the farms and the buffalo heifers were kept together in a half-open shelter system. The food received by the buffaloes during the study was a mixture of silage, hay, roughage, concentrates and Egyptian clover (*Trifolium alexandrinum*). Water was supplied *ad libitum.*

In total, 286 heifers were examined and of these, 159 buffalo did not meet the inclusion criteria described in Box 1. The remaining 127 animals were included in the study. The buffaloes were identified by their ear tags.

The ear tag number of the individual animal was recorded on an examination sheet. A serial number was assigned to each individual animal. Tubes used for collection of blood and cups used for faecal samples were assigned the same serial number that was recorded on the examination sheet.

Samples

Samples were collected at 08:00 prior to feeding. Two blood samples were collected from the jugular vein. The first blood sample was collected in a plain Vacutainer tube (10 mL plain vacuum tubes, Biomedica Alex Co., Egypt), which was used for obtaining serum. The second blood sample was collected in Vacutainer tube (BD Vacutainer Tubes, Becton Dickinson, Rutherford, NJ) containing EDTA as an anticoagulant and used for haematological analysis. Faecal samples were collected from the rectum of all animals in clean, dry cups. Samples were transported on ice directly after collection to the research laboratory (Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Egypt) within 1 h – 2 h of collection of samples.

Samples were prepared and analysed by the research laboratory immediately upon arrival. Blood samples in plain tubes were centrifuged at 3000 rpm for 15 min and serum was harvested according to standard methods (Coles 1986) and then divided into two parts. The first part was kept in the refrigerator at 4 °C and used for serum protein electrophoresis and for lipid profile (analysed within 2 days). The second part was divided into four equal parts in Eppendorf tubes, stored at −20 °C and used for measuring serum biochemical constituents. Samples with haemolysis were excluded from the study. Serum samples were analysed within a maximum period of two weeks.

BOX 1: Inclusion criteria for the buffaloes in this study

Clinically healthy
Good body condition score
General attitude: Alert
No loss of skin elasticity
Normal mucous membrane: Pink
No diarrhoea in the previous 7 days
No urogenital abnormalities in the previous 7 days
No muscular abnormalities in the previous 7 days
No medication in the previous 7 days
Absence of skin lesions or alopecia
Absence of intestinal and blood parasites

Biochemical analysis

Serum proteins

Serum proteins were measured both electrophoretically and colorimetrically. Serum protein electrophoresis was carried out using a cellulose acetate electrophoresis kit (Biotec-Fischer GmbH, Germany) and by Electrophoresis Set (Filipo, Biotec-Fischer GmbH, Germany). Spectrophotometeric measurements of total serum proteins and albumin were done using commercial kits supplied by Spinreact (Spinreact, GIRONA, Spain) and using a UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea)

Serum lipid profile

Serum levels of total cholesterol, triglyceride, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and glucose were measured using commercial kits supplied by Spinreact (Spinreact, GIRONA, Spain) using UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea). Very low density lipoprotein (VLDL-C) was calculated mathematically by dividing triglycerides by five (McClatchey 2001).

Serum minerals and electrolytes

Serum calcium, magnesium and chloride levels were measured using commercial kits supplied by Spinreact (Spinreact, GIRONA, Spain). Serum phosphorus level was measured using the Emapol kit (Emapol, Gdansk, Poland). Serum iron and total iron binding capacity (TIBC) were measured using kits supplied by AMS International (AMS, UK Ltd). Unsaturated iron binding capacity (UIBC) was calculated mathematically by subtracting the serum levels of iron from serum total iron binding capacity as indicated in the commercial kit (UIBC=TIBC-Iron). Serum sodium and potassium levels were measured using kits supplied by Spectrum Diagnostics (Egyptian Co. for Biotechnology, Obour City Industrial Area, Cairo, Egypt). Serum zinc and copper were measured using kits supplied by Centronic GmbH (Wartenberg, Germany), using UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea).

Serum enzyme activities

Serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and creatine phosphokinase (CK) were measured using commercial kits supplied by Spinreact (Spinreact, GIRONA,

Spain), using UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea).

Other biochemical constituents

Serum blood urea nitrogen (BUN), creatinine, total bilirubin and direct bilirubin levels were measured using commercial kits supplied by Spinreact (Spinreact, GIRONA, Spain), using a UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea).

Haematological analysis

Blood film

An air-dried smear of fresh blood was prepared directly after blood collection, fixed and stained with Giemsa stain (Coles 1986) and examined for blood parasites and for differential leucocyte counts. Manual differential leucocyte counts were performed to calculate the relative and absolute counts for individual white blood cells (neutrophils, band cells, eosinophils, basophils, monocytes and lymphocytes).

Haematological analysis

Haematological analysis was performed by Medonic Vet. Hematology Analyzer (Medonic CA 620, Sweden) directly after the samples were received by the research laboratory and within 1 h – 2 h after samples were collected. Haematological variables measured were red blood cell count (RBCs), haemoglobin concentration (HGB), red blood cell distribution width (RDW), red blood cell distribution width absolute (RDWa), haematocrit (HCT), main corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Other parameters included the platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), large platelet concentration ratio (LPCR), and plateletcrit (PCT). Leucocyte variables measured were white blood cell count (WBCs) and differential white cell count.

Parasitological analysis

Parasitological analyses of faecal samples were done on the day of collection using sedimentation and floatation techniques according to Soulsby (1982). Animals harbouring parasites were excluded from the study. The parasitological findings were reported to the farms to enable them to treat the animals and take recommended control measures.

Data analysis

Data analysis was carried out according to International Federation of Clinical Chemistry's (IFCC) Approved Recommendations on the Theory of Reference Values (Solberg 1987). Statistical analysis was performed using Reference value advisor version 2.1 (Geffre *et al*. 2011); reference intervals were determined using the non-parametric method. Outliers were tested using Dixon-Reed's and Tukey's tests (Reed, Henry & Mason 1971). Data were tested for normal distribution according to the Anderson-Darling method (Anderson & Darling, 1954). The 95% reference intervals were calculated by removing the upper and lower 2.5% of the range for each serum biochemical and haematological parameters to give the 2.5 and 97.5 percentiles. The 90% confidence intervals (CI) were calculated for each reference limit to determine whether their precision was sufficient for clinical use.

Results

The results of the biochemical and haematological analyses are presented in Table 1 to Table 5.

TABLE 1: Reference intervals for serum proteins in buffalo heifers measured both by spectrophotometer and electrophoresis.

Method of analysis	Serum proteins	Reference value	s.d.	Reference interval	90% CI for lower reference limit	90% CI for upper reference limit	Kaneko, Harvey and Bruss (1997)	
							Reference interval	Mean ± s.d.
Spectrophotometer	Total proteins (g/L)	68.00	6.90	56.30–81.00	56.00–57.60	80.40–81.20	67.40–74.60	71.00 ± 1.80
	Albumin (g/L)	32.70	4.70	24.90–40.70	21.10–25.60	40.50–46.80	-	-
	Globulin (g/L)	35.20	6.50	23.40–50.00	20.80–25.10	46.70–52.90	-	-
	A/G ratio	0.960	0.27	0.56–1.67	0.45–0.61	1.44–1.96	-	-
Protein electrophoresis	Albumin (g/L)	36.10	4.90	26.40–45.70	25.20–27.70	44.50–47.00	30.30–35.50	32.90 ± 1.30
	Total globulins (g/L)	31.90	5.50	21.20–42.60	19.80–22.50	41.30–44.10	30.00–34.80	32.40 ± 2.40
	α-Globulins (g/L)	11.30	2.40	6.60–16.00	5.90–7.20	15.40–16.60	7.50–8.80	7.90 ± 0.20
	β- Globulins (g/L)	5.00	2.10	0.80–9.20	0.30–1.40	8.70–9.70	8.00–11.20	9.60 ± 0.80
	γ- Globulins (g/L)	15.60	4.90	5.80–25.30	4.60–7.10	24.1–28.10	16.90–22.50	19.70 ± 1.40

Note: Reference value, mean value; reference interval, 2.5 and 97.5 percentiles; reference interval, interval between and including two reference intervals.
Confidence intervals calculated for each reference limit as recommended by PetitClerc and Solberg (1987).

TABLE 2: Reference intervals for serum enzyme activities in buffalo heifers.

Serum Enzymes (U/L)	Reference value	s.d.	Reference interval	90% CI for lower reference limit	90% CI for upper reference limit	Kaneko, Harvey and Bruss (1997)	
						Reference interval	Mean ± s.d.
AST	42.62	13.93	22.29–68.71	19.25–23.92	67.08–69.56	78.00–132.00	105.00 ± 27.00
ALT	24.87	9.82	8.58–46.19	6.13–12.83	44.33–56.00	11.00–40.00	27.00 ± 14.00
GGT	11.35	3.79	3.91–18.78	2.94–4.86	17.82–19.75	6.10–17.40	15.70 ± 4.00
CK	98.27	98.51	14.66–309.80	12.22–17.77	305.00–311.00	4.80–12.10	7.40 ± 2.40
LDH	546.18	232.71	186.72–917.43	140.31–234.76	906.64–947.12	692.00–1445.00	1061.00 ± 222.00
ALP	182.19	68.27	80.33–364.81	68.66–96.12	350.17–385.64	0.00–488.00	194.00 ± 126.00

Note: Reference interval, 2.5 and 97.5 percentiles; reference interval, interval between and including, two reference intervals.
Confidence intervals calculated for each reference limit as recommended by PetitClerc and Solberg (1987).
AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; GGT, Gamma glutamultransferase; LDH, Lactate dehydrogenase; ALP, alkaline phosphatase; CK, creatine phosphokinase.

TABLE 3: Reference intervals for serum minerals and electrolytes in buffalo heifers.

Serum mineral and electrolytes	Unit	Reference value	s.d.	Reference interval	90% CI for lower reference limit	90% CI for upper reference limit	Kaneko, Harvey and Bruss (1997)	
							Reference interval	Mean ± s.d.
Calcium	mmol/L	2.57	0.28	2.03–3.12	1.96–2.10	3.04–3.19	2.43–3.10	2.78 ± 0.15
	mg/dL	10.29	1.11	8.11–12.46	7.83–8.39	12.17–12.74	9.7–12.4	11.08 ± 0.67
Phosphorus	mmol/L	2.12	0.24	1.42–2.54	1.42–1.68	2.47–2.58	1.81–2.10	-
	mg/dL	6.57	0.75	4.39–7.85	4.39–5.22	7.64–7.99	5.60–6.50	-
Magnesium	mmol/L	1.11	0.19	0.73–1.48	0.68–0.78	1.43–1.52	0.74–0.95	0.84 ± 0.10
	mg/dL	2.69	0.46	1.78–3.59	1.66–1.89	3.48–3.71	1.80–2.30	2.05 ± 0.25
Sodium	mmol/L	142.85	10.10	126.57–167.37	123.56–128.64	164.63–169.51	132.00–152.00	-
Chloride	mmol/L	92.99	6.72	79.83–106.16	78.13–81.54	104.45–107.86	97.00–111.00	-
Potassium	mmol/L	4.67	1.21	2.31–7.04	2.00–2.61	6.74–7.35	3.90–5.80	-
TIBC	µmol/L	37.04	5.72	25.84–48.25	24.39–27.29	46.79–49.69	41.20 ± 11.60	-
	µg/dL	206.95	31.94	144.36–269.55	136.26–152.46	261.45–277.64	230.00 ± 65.00	-
Iron	µmol/L	19.12	4.19	10.92–27.33	9.86–11.98	26.27–28.38	10.20–29.00	17.40 ± 5.19
	µg/dL	106.84	23.38	61.01–152.66	55.1–66.94	146.74–158.59	57.00–162.00	97.00 ± 29.00
UIBC	µmol/L	17.93	6.71	4.76–31.08	3.06–6.47	29.38–32.78	11.30–33.30	23.50 ± 6.40
	µg/dL	100.12	37.49	26.62–173.61	17.12–36.13	164.11–183.12	63.00–186.00	131.00 ± 36.00
Copper	µmol/L	15.31	3.16	9.11–21.35	8.32–9.92	20.69–22.29	9.13–25.20	-
	µg/dL	97.49	20.11	58–136	52.97–63.17	131.82–142.02	58.00–160.00	-
Zinc	µmol/L	15.58	3.75	8.23–22.93	7.28–9.18	21.98–23.88	-	-
	µg/dL	101.84	24.49	53.82–149.86	47.62–60.04	143.65–156.10	-	-

Note: Reference value, mean value; reference interval, 2.5 and 97.5 percentiles; reference interval, interval between and including, two reference intervals.
Confidence intervals calculated for each reference limit as recommended by PetitClerc and Solberg (1987).
TIBC, Total iron binding capacity; UIBC, Unsaturated iron binding capacity.

TABLE 4: Reference intervals for serum constituents in buffalo heifers.

Serum constituents	Unit	Reference value	s.d.	Reference interval	90% CI for lower reference limit	90% CI for upper reference limit	Kaneko, Harvey and Bruss (1997)	
							Reference interval	Mean ± s.d.
Total cholesterol	mmol/L	1.45	0.28	0.90–1.99	0.83–0.97	1.92–2.06	2.07–3.11	-
	mg/dL	55.87	10.69	34.92–76.82	32.22–37.63	74.11–79.53	80.00–120.00	-
Triglycerides	mmol/L	0.30	0.13	0.04–0.56	0.02–0.08	0.53–0.59	0.00–0.20	-
	mg/dL	26.87	11.50	4.02–49.72	1.41–7.24	46.49–52.32	0.00–14.00	-
HDL-C	mg/dL	32.41	10.12	12.58–52.25	10.01–15.14	49.68–54.82	-	-
LDL-C	mg/dL	18.08	6.83	4.69–31.47	2.96–6.42	29.74–33.21	-	-
VLDL-C	mg/dL	5.37	2.29	0.87–9.87	0.29–1.45	9.29–10.45	-	-
Glucose	mmol/L	3.55	0.81	1.97–5.13	1.76–2.17	4.93–5.33	2.50–4.16	3.19 ± 0.38
	mg/dL	63.96	14.55	35.45–92.47	31.76–39.14	88.79–96.16	45.00–75.00	57.40 ± 6.80
Total bilirubin	µmol/L	4.96	3.42	0.00–12.14	0.00–1.37	11.11–12.99	7.10–34.20	-
	mg/dL	0.29	0.20	0.00–0.71	0.00–0.08	0.65–0.76	1.00–2.00	-
Direct bilirubin	µmol/L	1.71	1.88	0.00–6.84	0.00–0.00	5.81–8.38	0.00–6.84	-
	mg/dL	0.10	0.11	0.00–0.40	0.00–0.00	0.34–0.49	0.00–0.40	-
Indirect bilirubin	µmol/L	3.25	2.91	0.00–10.94	0.00–0.00	10.6–12.14	3.42–34.2	-
	mg/dL	0.19	0.17	0.00–0.64	0.00–0.00	0.62–0.71	0.20–2.00	-
Creatinine	µmol/L	117.57	16.79	83.98–151.16	79.56–88.4	146.74–155.58	88.40–177	-
	mg/dL	1.33	0.19	0.95–1.71	0.90–1.00	1.66–1.76	1.00–2.00	-
BUN	mmol/L	14.42	3.42	7.73–21.12	6.86–8.59	21.98–23.46	-	-
	mg/dL	40.41	9.57	21.65–59.16	19.23–24.08	65.73–61.58	20.00–30.00	-

Note: Reference interval, 2.5 and 97.5 percentiles; reference interval, interval between and including, two reference intervals.
Confidence intervals calculated for each reference limit as recommended by PetitClerc and Solberg (1987).
HDL-C, High density lipoprotein; LDL-C, low density lipoprotein; VLDL-C, very low density lipoprotein; BUN, blood urea nitrogen.

Ethical considerations

This study was approved by the Animal Ethics Committee of Science and Technology Development Fund, Ministry of Scientific Research, Egypt (ID: 2947/2011-STDF).

Discussion

Haematological and serum biochemical reference intervals are essential for diagnosing diseases. The current study aimed to establish haematological and serum biochemical reference intervals for water buffalo heifers, and is considered to be the first study that established these values. Buffaloes used in the study were reared mainly under the same management system and their productive and reproductive status was regularly checked and recorded.

The IFCC sets out clear guidelines for the establishment of reference intervals whereby at least 120 values are necessary to obtain reliable reference intervals (Solberg 1987). This study used a carefully selected and relatively large reference population of 127 animals. The current study also defined the characteristics and distribution of the reference sample

TABLE 5: Reference intervals for haematological variables in buffalo heifers.

Haematological variables	Units	Reference value	s.d.	Reference interval	90% CI for lower reference limit	90% CI for upper reference limit	Feldman, Zinkl and Jain (2000)
T. RBCs count	($\times10^{12}$/L)	8.52	1.08	6.41–10.64	6.13–6.68	10.37–10.91	5.00–10.00
HGB	(g/L)	133.10	19.10	95.7–170.5	90.80–100.50	165.70–175.30	80.00–150.00
HCT	(%)	40.17	5.06	30.25–50.08	28.96–31.53	48.79–51.36	24.00–46.00
MCV	(fl)	47.26	3.57	40.26–54.24	39.36–41.17	53.34–55.15	40.00–60.00
MCH	(pg)	15.66	1.47	12.77–18.55	12.39–13.14	18.17–18.92	11.00–17.00
MCHC	(%)	33.15	1.94	29.36–36.95	28.87–29.85	36.46–37.44	30.00–36.00
RDW	(%)	21.18	2.03	17.19–25.16	17.50–18.30	24.80–26.70	-
RDWa	(fl)	32.94	2.93	27.02–38.68	26.46–27.94	37.94–39.42	-
PLT	($\times10^{9}$/L)	186.33	64.04	60.82–311.85	44.58–77.05	295.61–328.08	-
MPV	(fl)	6.46	0.40	5.66–7.25	5.56–5.77	7.15–7.36	-
PDW	(%)	9.92	0.60	8.73–11.09	8.58–8.89	10.95–11.25	-
PCT	(%)	0.12	0.04	0.04–0.19	0.03–0.05	0.018–0.21	-
LPCR	(%)	8.03	2.53	3.08–12.98	2.11–4.05	12.02–13.96	-
T.WBCs count	($\times10^{9}$/L)	12.15	2.44	7.35–16.94	6.74–7.98	16.32–17.56	4.00–12.00
Lymphocyte count	($\times10^{9}$/L)	7.67	1.67	4.79–11.05	4.13–5.11	10.15–12.28	2.50–7.50
Neutrophil count	($\times10^{9}$/L)	3.74	1.50	1.39–6.78	1.15–1.70	6.67–7.74	0.60–4.00
Band cell count	($\times10^{9}$/L)	0.13	0.11	0.00–0.48	0.00–0.00	0.45–0.75	0.00–0.12
Eosinophil count	($\times10^{9}$/L)	0.20	0.21	0.00–0.87	0.00–0.00	0.53–1.22	0.00–0.24
Monocyte count	($\times10^{9}$/L)	0.43	0.24	0.00–0.90	0.00–0.10	0.83–0.96	0.25–0.84
Basophil count	($\times10^{9}$/L)	0.00	0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.20
Lymphocytes	(%)	63.56	8.10	48.2–75.80	38.00–49.00	75.0–79.00	45.00–75.00
Neutrophils	(%)	30.39	9.23	15.2–48.0	14.00–17.00	47.0–54.00	15.00–45.00
Band cell	(%)	1.08	1.16	0.00–4.00	0.00–0.00	3.00–5.00	0.00–2.00
Eosinophils	(%)	1.70	1.60	0.00–5.80	0.00–0.00	4.00–9.00	0.00–20.00
Monocytes	(%)	3.53	1.93	0.00–8.00	0.00–1.00	7.00–9.00	2.00–7.00
Basophils	(%)	0.00	0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–2.00

Note: Reference interval, 2.5 and 97.5 percentiles; reference interval, interval between and including two reference intervals.
Confidence intervals calculated for each reference limit as recommended by PetitClerc and Solberg (1987).
T.RBCs count, Total red blood cells count; HGB, haemoglobin concentration; RDW, red blood cells distribution width; RDWa; red blood cells distribution width absolute; HCT, haematocrit; MCV, femitoliter; (fl), main corpuscular volume; MCH, pictogram; (pg), mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelets count; MPV, mean platelets volume; PDW, platelets distribution width; LPCR, large platelets concentration ratio; PCT, plateletcrit; T.WBCs, total white blood cells count.

population and calculated reference intervals as 0.025 and 0.975 fractiles with 90% confidence intervals for the limits.

During the study, body temperature was measured for all heifers, and the following reference values were observed: Mean value (38.35 °C), reference interval (37.7 °C – 39.01 °C), 90% CI for lower reference limit (37.61 °C – 37.79 °C), and 90% CI for upper reference limit (38.92 °C – 39.10 °C). These reference intervals for body temperature agree with those of the Food and Agriculture Organization (FAO 1994), and are also in accordance with values reported by Radostits *et al.* (2006).

No significant differences were observed between serum albumin measured by electrophoresis and by colorimetric methods (Table 1). Furthermore, the difference between globulins calculated by colorimetric methods when compared with globulins measured by electrophoresis was insignificant (Table 1). The insignificant difference between serum albumin and globulins measured by spectrophotometeric and electrophoretic methods (Table 1) indicated that the latter is a precise method for measuring serum albumin and globulins. In addition, the electrophoretic method has the advantage of measuring globulin fractions. The largest proportion of globulins was in the form of γ-globulins (15.6 g/L ± 4.9 g/L), followed by α-globulins (11.3 g/L ± 2.4 g/L) and then *β*-globulins (5.0 g/L ± 2.1 g/L).

Data from the present study were compared with established reference intervals for serum biochemical (Kaneko, Harvey & Bruss 1997) and haematological (Feldman, Zinkl & Jain 2000) constituents of cows. Intervals for total proteins and their fractions from the present study were wider than those established for cows by Kaneko *et al*. (1997). The minimum reference value was lower and the maximum reference value for buffalo heifers was higher than the established reference values for cows for all serum total proteins and their fractions (Table 1).

The reference interval for serum AST (22.29 U/L – 68.71 U/L) in buffalo heifers was lower than the reference interval for cows (78 U/L – 132 U/L). On the other hand, reference intervals for serum ALT (8.58 U/L – 46.19 U/L) and GGT (3.91 U/L – 18.78 U/L) in buffalo heifers were slightly wider than the reference intervals for cows, which were 11 U/L – 40 U/L and 6.1 U/L – 17.4 U/L for ALT and GGT respectively (Table 2). However, reference intervals for serum CK, LDH and ALP in buffalo heifers were totally different from those established for cows by Kaneko *et al*. (1997). Differences between results of the present study and established reference intervals for cows may be attributed to variation in species and age of the animals.

Minerals are essential nutrients with a significant role in reproduction, because their excess or deficiency has a

detrimental effect on the performance of livestock. Trace elements including copper, zinc and iron, certain macro-elements like calcium, magnesium and phosphorus, and electrolytes like sodium and chloride have been found to be essential for normal livestock growth (Underwood 1981). Reference intervals for minerals established in the present study reflected their serum levels in buffalo heifers (Table 3), which were somewhat different from the previously reported intervals for cows (Kaneko *et al*. 1997).

Large species differences in lipoprotein profiles and the percentage of total cholesterol and triglycerides carried by each lipoprotein class have been recorded in different animals. Whereas in humans and pigs most of the cholesterol is transported as LDL, in cattle cholesterol is equally divided between LDL and HDL, while in sheep and horses the majority of the cholesterol circulates as HDL (Latimer, Mahaffey & Prasse 2003). The present study revealed that serum HDL-C levels were higher than LDL-C levels (Table 5). Mean values of total cholesterol, HDL-C, LDL-C and VLDL-C established in the present study were lower than findings of previous studies on non-pregnant buffaloes (Abd Ellah 2011; Tajik & Nazifi 2011). The current study revealed lower levels of serum cholesterol and lipoproteins, especially HDL-C, in Egyptian buffalo heifers when compared with those reported by Tajik and Nazifi (2011), which may be attributed to variation in the breed and age of the animals investigated.

At present, the complete blood cell count can be performed using an automated haematology analyser, which can increase the throughput of the test. Recently, new indices related to erythrocytes (RDW, RDWa) and to platelets (PCT, MPV, PDW, LPCR) have been provided by haematology analysers (Lombarts, Koevoet & Leijnse 1986). The current study is unique in that it has provided reference values for these new indices in buffalo heifers. Reference limits for haematological parameters developed in the present study (Table 5) differed slightly from those developed by Feldman, Zinkl and Jain (2000) for cows (Table 5) and from those reported by Ciaramella *et al*. (2005). Under different physiological conditions, variations in haematological values can be due to a variety of factors such as environmental temperature, muscular activity, quality of nutrition and water balance (Ciaramella *et al*. 2005).

Conclusion

It is difficult to depend on reference intervals from other countries to interpret results for animals living in Egypt and probably elsewhere in Africa. Furthermore, the buffaloes used in the present study were heifers aged 1–2 years and their data may not be used for interpreting data from adult or young buffalo under different physiological conditions. It is recommended that data from the present study be used for interpreting blood tests from female water buffaloes aged from 1–2 years only.

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Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

Authors' contributions

M.R.A. (Assiut University) was the project leader, and was responsible for clinical examination of animals, collection, analysis of samples, and statistical analysis of data, wrote the manuscript and sent for publishing. M.I.H. (Assiut University) was responsible for clinical examination of animals, collection and analysis of samples. D.R.I. and H.Z.R. (Assiut University) were responsible for clinical examination of animals and collection of samples

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