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Reference Intervals for Rectal Temperature in Water Buffalo (*Bubalus bubalis*) Heifers

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ABSTRACT

To the best of the authors knowledge, reference intervals for rectal temperature in water buffalo (*Bubalus bubalis*) has not been previously published. This study was undertaken to establish this reference interval for water buffalo heifers. Based on the statement of the International Federation of Clinical Chemistry, at least 120 values are necessary to obtain reliable estimates for reference intervals. A total number of 127 healthy buffalo heifers (1–2 years old) were selected based on a set of inclusion criteria. The health status of heifers was confirmed based on clinical examinations and laboratory analyses. Animals were examined at buffalo farms that belong to Assiut Governorate, Egypt. Rectal temperature was measured using a clinical thermometer. Three types of samples were collected: serum samples for biochemical analysis, whole blood samples for haematological analysis and faecal samples for parasitological examination. The 95% reference intervals were calculated by removing the upper and lower 2.5% of the interval to give the 2.5 and 97.5 percentiles. Confidence intervals were calculated for the reference limit. The current study was able to establish reference intervals for rectal temperature in water buffalo heifers.

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Introduction

Water buffaloes (*Bubalus bubalis*) are mainly distributed in many countries. They are considered an important source for milk and meat production in the Nile River valley in Egypt (GOVS, 2005). They can surpass cattle in their ability to adapt to hot climates and swamp lands (Webster and Wilson, 1980).

Animal health can be defined as the absence of disease, which is established by clinical examinations in association with different laboratory tests (Theodossi *et al.* 1981; Klinkhoff *et al*. 1988; Bailey *et al*., 1989; Pattinson and Theron, 1989). Textbook reference intervals published by European or United States veterinary laboratories (Kaneko *et al*., 1997) are often based on animals (other than buffaloes) raised under good husbandry conditions in moderate climates, in which the reference sample groups may differ from those of the developing countries. Possible differences may be attributed to genetic factors, nutrition quality and quantity, availability of water, electrolyte balance, parasitic infestations, and climatic circumstances. This will result in differences in reference intervals between countries, and it will be difficult to use intervals produced in one country to interpret results in another one.

Some factors were specified by Grasbeck *et al.* (1979), which must be established when using reference intervals, such as the categorization of the reference population with respect to age and sex. In addition, a clear set of inclusion and exclusion criteria should be used for selecting the reference sample group that based mainly on physiological and environmental conditions (Abd Ellah *et al*., 2014a). To the best of the authors' knowledge, reference intervals for rectal temperature have not yet been established for water buffalo heifers, which constitute the aim of this study.

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Materials and methods

Animals

Buffalo heifers (1–2 years old) were examined at buffalo farms belong to Assiut Governorate, Egypt. During the period from July 2011 to June 2012.

Animals were selected for inclusion in the study based on inclusion criteria (Table 1). Animals were examined during the same period of the year in all farms. The management and feeding systems were approximately the same 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 concentrates, roughage, silage, hay, and Egyptian clover (Trifolium alexandrinum). Water was supplied *ad libitum*.

Examination of animals and collection of samples were carried out around 08:00 a.m., prior to feeding. An examination sheet numbered serially and included the ear tag number was used to record the clinical findings and data of the indi-

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vidual 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.

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

Clinical Examination

Animals were inspected for presence of any abnormal clinical signs. Rectal temperature was measured from the rectum of each individual animal using a clinical thermometer according to Rosenberger (1979).

Table 1. Inclusion criteria for the buffaloes in this study

Inclusion criteria
Buffalo heifers
1-2 years old
Clinically healthy
Normal laboratory findings
General body condition is good
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

Samples

Two blood samples were collected from the jugular vein. The first blood sample was collected in a plain Vacutainer tube (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 and for preparation of blood film for blood parasites.

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 1–2 h of samples collection.

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, serum was harvested according to standard methods (Coles, 1986) and used for measuring serum biochemical constituents. Samples with haemolysis were excluded from the study. Serum samples were stored at -20 °C and analysed within a maximum period of two weeks.

Biochemical analysis

Serum biochemical analyses included total proteins and fractions, lipid profile, serum enzymes, bilirubin, urea and creatinine, minerals and electrolytes were measured using commercial kits supplied by Spinreact (Spinreact, GIRONA, Spain) and by means of UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea).

Blood film

An air-dried smear of whole blood was prepared, fixed, and stained with Giemsa stain (Coles 1986) and examined for blood parasites, and used also for differential leucocyte counts.

Haematological analysis

Haematological analysis was performed by Medonic Vet. Hematology Analyzer (Medonic CA 620, Sweden) directly after receiving the samples by the research laboratory.

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.

Data analysis

Data analysis was carried out according to the International Federation of Clinical Chemistry (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 *et al*., 1971). Data were tested for normal distribution according to the Anderson-Darling method (Anderson and 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

A total of 286 buffalo heifers were examined, out of them, 159 buffalo did not fit with the inclusion criteria described in Table 1 and excluded from the study. The remaining 127 animals were included in the study.

The results of the biochemical and haematological analyses for the animals included in this study were previously published (Abd Ellah *et al*., 2014b).

Reference intervals for rectal temperature was presented in Table 2.

Discussion

Internal body temperature is an important clinical measurement that used to assess the health status of animals. The current study aimed to establish rectal temperature reference intervals for water buffalo heifers. To the best of the authors knowledge this study is the first that established these values.

The IFCC established out clear regulations for the estab-

Table 2. Reference intervals for rectal temperature in buffalo heifers.

	Reference value	SD	Reference interval	90% CI for lower reference limit	90% CI for upper reference limit
Rectal temperature (°C)	38.4	0.3	37.80-39.00	37.60- 37.80	38.90-39.10

Reference value: Mean; SD: Standard Deviation; reference interval: 2.5 and 97.5 percentiles. Confidence intervals calculated for each reference limit as recommended by PetitClerc and Solberg (1987).

lishment of reference intervals whereby at least 120 values are required to get trustworthy reference intervals (Solberg, 1987). This study used a carefully selected and relatively large reference population of 127 healthy buffalo heifers. The current study also defined the characteristics and distribution of the reference sample population and calculated reference intervals as 0.025 and 0.975 fractiles with 90% confidence intervals for the limits.

Rectal temperature is good indicator of body temperature (Omeran *et al*., 2011). It is determined by the balance between loss and gain of heat (Perissinotto *et al*., 2007). Studies (Koga *et al*., 2004; Aggarwal and Singh, 2008; Singh *et al*., 2010) reported that the rectal temperature change much more in buffaloes than cattle under increased surrounding temperature. The collection of data for the present study was lasted for one year to ensure that the established reference intervals are consistent with different environmental changes in atmospheric temperatures at different seasons.

As shown in Table 2, the reference intervals for buffalo heifers were as follow: Mean value (38.40 °C), reference interval (37.8–39.00 °C), 90% CI for lower reference limit (37.60–37.80 °C), and 90% CI for upper reference limit (38.90–39.10 °C). The obtained mean reference value (38.40 °C) for body temperature is slightly lower that that reported by Kumar *et al*. (2017), who found that rectal temperature was 38.67±0.09°C in Murrah buffaloes. However, mean reference value from this study is similar to that reported by Yadav *et al*. (2016), who observed that mean value for rectal temperature in adult buffalo was 38.41°C. The slight difference between the recorded rectal temperature in the examined animals and previous studies may be attributed to variations in number and age of the examined buffaloes. In addition to different geographical locations.

Based on the literature reviews, there were no single study on rectal temperature reference intervals in buffalo heifers, the current study can be used as guide for normal rectal temperature in buffalo heifers (1-2 Years old) in Egypt.

Conclusion

This study established rectal temperature reference intervals for buffalo heifers in Egypt. 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.

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Conflict of interest

The authors declare that they have no conflict of interest.

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