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Reference Intervals for Some Serum Chemistry Parameters for the Ankole Long-Horned Cows

Richard M. Kabuusu
St. George's University, School of Veterinary Medicine,
Department of Pathobiology, Grenada

Abstract

The aim of this study was to establish reference intervals for routine serum chemistry parameters for the indigenous Ankole long-horned cows. 131 cows in western Uganda were randomly selected and clinically examined. Fecal and blood samples were obtained from cows and examined in the laboratory. Sample analyses on specimens negative for intestinal and blood parasites were performed using a photometric clinical chemistry analyzer. Homogeneity of observed values was determined using the Kolmogorov-Smirnov test and non-parametric data were transformed using natural logarithms. Outliers were detected using Grubb's test. Means and standard deviations were calculated using PH stat 2 for each serum chemistry parameter. Simple linear regression was used to determine the effect of the cow's age on each measured chemistry parameter. Reference intervals for AST, ALP, GGT, CK, Protein, Albumin, Globulin, Creatinine, Urea, Magnesium and Phosphate are reported.
Veterinarians and researchers working with Ankole long-horned cows should use these reference intervals when they make clinical, diagnostic or research decisions about them.

Keywords: reference intervals; chemistry; ankole cows.

1.0 Introduction

Reference intervals (RIs), also known as a reference ranges or reference limits for a particular test are a set of values that capture 95% of the healthy population (Lumsden et al., 1978). Determining the serum chemistry levels gives an indication of the extent to which a particular chemical exists in blood and thus give a veterinarian a 90% probability of determining which organ system is affected. Although it is recommended that each diagnostic or research laboratory should develop its own reference intervals specific by species, sex, age, breed, physiological status, physical activity and nutritional status (Lumsden, 1978; Solberg, 1987), it is almost practically impossible because of the costs and time involved. Therefore most laboratories depend on RIs provided by the manufacturer of the chemistry analyser, but these may not reflect true RIs for animals in a specific geographical location.

Many veterinary commercial and reference laboratories have constructed RIs for routine blood parameters, mostly for cats and dogs. On the other hand, RIs have been developed only to a small extent for production animals, and even to a lesser extent in developing countries. In Uganda, the adult ankole long horn cow is a very popular breed because it is a multi-purpose breed providing draught power, milk and meat (Fevre *et al.* 2001). It is also highly resistant to disease and harsh environmental conditions, grows fast, has a high birth weight, adequate weight gain and relatively high milk yield (Peterson, 2004; Fevre *et al.* 2001). Despite its socioeconomic importance and good attributes, not many studies have focused on this breed, and consequently there is limited information regarding its physiologic parameters including reference intervals. Thus, the purpose of this study was to construct a baseline serum chemistry profile in the form of reference intervals for the Ankole long-horn breed.

2.0 Materials and Methods

2.1 Study area and design

The study was conducted in Western Uganda. Prior to collection of blood samples, the cows were examined by a local veterinarian and found to be free of clinical disease. Fecal samples were obtained per rectum, whereas blood was obtained by venipuncture of the jugular (or coxygeal vein when animal was difficult to restrain physically) from 131 cows. Eligibility for inclusion in the study included being a long-horned ankole cow, aged between 6 months and 11 years, free from apparent clinical disease, and free from blood and intestinal parasites. Additionally, apparently healthy cows were further excluded from the study if they did not have a packed cell volume of at least 33%, a white blood cell (WBC) count of at least 6 X 10^3/µl, or a platelet count of at least 175 X 10^3/µl (Kabuusu *et al.* 2006).

2.2 Handling and analysis of samples

3 mls of blood were placed in plain tubes and 2 mls were placed in Ethylene Diamine Tetra-Acetate (EDTA) tubes (Becton- Dickinson, vacutainer system, USA). After, clot formation in the plain tubes, serum was separated from blood cells by centrifugation at 1500rpm. The serum was refrigerated and transported on ice and analysed within 3 days. Fecal samples were stored at 4^{o}C until analysed.

EDTA samples were used to determine PCV, WBC and platelet counts on the day of collection. Blood smear were stained with May-Grunwald-Giemsa and microscopically examined under oil immersion to detect intra-erythrocytic hemoparasites. Fecal samples were examined for parasitic eggs using the fecal flotation and sedimentation techniques.

The following chemical assays were performed on serum samples using a photometric clinical chemistry analyzer, Prochem-V (Synbiotics Corporation):- serum protein in g/dl; Albumin in g/dl; Globulin in g/dl; magnesium (Mg) in Mg/dl; Phosphate (Phos) in Mg/dl; Creatinine (Creat) in Mg/dl; Blood urea and nitrogen (BUN) in Mg/dl; Aspartate aminotransferase (AST) in U/L; Alkaline Phosphatase (ALP) in U/L; Gamma glutamyl transferase (GGT) and Creatinine kinase (CK) in U/L (Lumsden *et al.* 1980; Ceriotti, 2007; Kabuusu *et al.* 2006).

2.3 Statistical analysis

Data were stored and managed using Excel. Data was then evaluated for normality of distribution using Kolmogorov-Smirnov (KS) test at a level of significance, $\alpha = 0.05$ and at a critical value of 0.22 (Solberg, 1987; Romeu, 2007). Data, which showed non-guassian distribution, was transformed using natural logarithmic transformations (Poulsen *et al.* 1997). Outliers, detected using Grubb's t-test (Romeu, 2007), were eliminated and where possible replaced. The means (X), standard deviations (SD), standard errors of mean (SEM) for each measured chemistry parameter were determined. For each parameter, the upper limit of RI was defined as X + 2SD whereas the lower limit of the RI was defined as X- 2SD. Simple linear regression was used to determine whether these chemistry parameters were affected by age (Solberg, 1987; Kabuusu *et al.* 2006).

3.0 Results and Discussion

Only 33.6% (44/131) cows met the criteria for inclusion in the study. The number of assays performed (n) varied between 40- 44 and these depended on the availability of reagents. Hematological methods and results are only mentioned and not fully described or discussed because they are beyond the scope of this study.

It is understood that local veterinarians generally use their knowledge and experience to readily approximately the biological reference intervals for common blood parameters of this breed but, this report provides the first documented evidence of serum chemistry RIs for female animals for this particular breed in Uganda. The routine clinical serum chemistry RIs reported in Table 1 should be used to detect alterations in the physiological blood levels suggestive of possible pathology, to assess the health status of the ankole long-horned cattle, to monitor the effect of treatment and to determine prognosis in a diseased cow; all of which attributes should support conserve this prized breed.

Serum protein and globulins significantly increased with cow's age ($P<0.01$ and $r> 0.5$). This may be attributed to antibody production as cows become increasingly exposed to antigens with age. Piccione et al., (2010) has shown that age significantly alters protein values in ruminants. ALP and CK also increased significantly with age ($p<0.05$), but their r-values were ambiguous (0.3-0.49). Age and physical activity have been reported to affect ALP and CK respectively (grogor et al., 2004). Physical activity was not assessed in the cows but, it is known that these cows usually walk long distances in search of water and pastures under the communal grazing system (Grigor et al., 2004; Peterson *et al.* 2004), an observation which may explain the relatively high CK. On the contrary, GGT was not affected by age and/ or physical activity of the animal and this provides further support for its selection over ALP in detecting hepatic disease in large animals.

Wide reference intervals are recorded for ALP, BUN, Phos and Mg, and this might be due to inter-animal differences such as age and physiological status. The upper limits of reference intervals reported in this study are comparatively higher than those provided by the manufacturer of the system used. This may be a physiological adaptation of the breed or represent some persistently underlying pathophysiological alteration in the breed. Apparently healthy Ankole long horn cattle, which are also free of hemoparasites, have previously shown relatively high antibody titers for several tick borne diseases (Kabi *et al.* 2008).

Furthermore, the hydration status of the cows was not known at the time of sampling and could have caused a relative increase in the serum chemistry values of all measured parameters. Reference intervals may also be influenced by environment, nutrition and other management aspects.

The reference intervals established in this study should be used only if the same preanalytical conditions are applied in the methods section or when the effect of modifications such as using heparinized plasma instead of serum is insignificant. Even then, precaution such as incorporating the cow's clinical history, present medication, clinical signs, test results of any other investigations, such as parasitology, and the epidemiological aspects of the disease should be taken into account while utilizing this important decision- making tool. The reference intervals established here are only the first line of diagnosis and deviations from these RIs in ankole long-horned cattle should at least warrant further investigation.

Like all other reference intervals, the RIs established here are susceptible to certain limitations. One such limitation is that these RIs are calculated to capture only 95% (42/44) of the sampled healthy population of long-horned cows, indicating that that 5% (2/44) of the sampled healthy cows are not included in these reference intervals. This creates a grey zone as some serum chemistry values of healthy cows may fall outside of the reference interval or some chemistry values of sick animals may fall within the RI leading to undetected problems. Therefore caution should be taken when interpreting values that fall outside of the reference intervals.

4.0 Conclusion

Veterinarians and researchers are encouraged to use these reference intervals when they make clinical, diagnostic or research decisions, as well as forensic investigations about Ankole long-horned cows whenever the methods used here are available and accessible.

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Annexure

Table 1: Upper and lower limits for routine serum chemistry parameters

							Reference intervals	
Parameter	Units	n	X	SD	SEM	dsn	Lower	Upper
ALP*††	U/L	40	4.5	0.28	0.1	NP	44	181
GGT	U/L	43	23.2	8.5	1.3	G	6.5	39.9
AST	U/L	44	59.3	10.3	1.6	G	39.1	79.5
CK††	U/L	44	59.9	26.3	3.9	G	8.4	111.4
Protein†	g/dl	44	8.1	0.9	0.1	G	6.3	9.9
Albumin	g/dl	40	2.8	0.4	0.1	G	2.0	3.6
Globulin†	g/dl	44	4.3	1.1	0.2	G	2.1	6.5
Creatinine	mg/dl	42	1.0	0.4	0.1	G	0.2	1.8
BUN	mg/dl	43	9.8	4.2	0.6	G	1.6	18.0
Phos	mg/dl	44	5.7	1.9	0.3	G	2.0	9.4
Mg	mg/dl	42	1.1	0.4	0.1	G	0.3	2.9

n= number of tests run; X= mean; SD= standard deviation; SEM= standard error of mean, dsn= distribution; G= Gaussian distribution; NP= non-parametric; * values obtained after transformation of data using natural logarithms;
† Parameter was affected by age with $p< 0.01$, with $r = 0.61$ for rotein and $r= 0.67$for globulin
†† Parameters were affected age ($p<0.05$), but their $r = 0.33$ for ALP and $r= 0.37$ for CK.