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Clinical, Haematological, Serum Biochemical and Cytogenetic Study in Cows with Primary Ketosis

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ABSTRACT

Twenty four Anatolian Black cows with primary ketosis (PK) and 10 clinically healthy cows (considered as control) were used in the study. The clinical, haematological, serum biochemical and cytogenetical parameters of all the animals were measured. Primary clinical signs included diminished appetite, decreased milk production, loss of weight, firm faeces and depression. Although no significant differences were seen with regard to haematological findings between PK and the control groups, significance increases ($P<0.05$) in aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT) and urea concentrations were observed in PK group compared to the control group. Glucose concentration was significantly low and reversely correlated to ketone bodies in urine in the PK group, but it was within normal limits in the control group. GTL-banded karyotypes of the animals were obtained using the standard karyotype of *Bos taurus*. Chromosomal complements were 2n = 60 in Anatolian Black cattle of normal and diseased groups. In the light of these molecular cytogenetic data, it was detected that all the autosomal chromosomes were acrocentric and gonosomal chromosomes were submetacentric. The results of the study showed that no morphological differences occurred in chromosomes in cattle suffering from PK.

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INTRODUCTION

Primary ketosis (PK) in cattle typically occurs in early lactation. Clinical signs include diminished appetite, decreased milk production, loss of weight, hypoglycemia, and hyperketonemia. Susceptibility to ketosis is probably due to the combination of appetite limitation and a high degree of precedence given to the demand of the mammary gland for nutrients, in particular glucose (Baird, 1982; LaManna, 2009).

Non communicable diseases, especially metabolic diseases, are assumed to be a major cause of morbidity and mortality in animals. Their chronic nature requires life-long medical attention, expensive supportive and symptomatic therapy and specialized care. Furthermore, dynamic development of cytogenetical studies has led to discovery of many cases of abnormalities in breeding animals associated with the number and structure of autosomes or sex chromosomes. The aim of the present study was thus to conduct a preliminary cytogenetic survey for the Anatolian Black cattle suffering from primary ketosis (PK).

MATERIALS AND METHODS

Totally 24 Anatolian Black cows diagnosed as showing PK dominantly nervous signs were subjected to clinical, haematological, metabolic profile, and cytogenetic examination. Ten clinically healthy cows were used as control group. Diagnosis of PK was based on clinical and haemato-biochemical examination, as described by Baird (1982) and Kelly (1984). Blood samples were analysed for haematology (WBCs, RBCs and PCV) and serum biochemistry (ALT, AST, GGT, total proteins, albumin, glucose, cholesterol and urea) using commercial kits according to the manufacturers' instructions. Urine samples from all examined cows were collected for the detection of ketone bodies, blood and haemoglobin.

Cytogenetical examinations were carried out using blood sample collection from the external jugular vein

under sterile conditions into a heparinized tube (Vacutainer). Lymphocytes do not normally undergo subsequent cell divisions. In the presence of a mitogen, lymphocytes were stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor was added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes were microscopically observed and evaluated for abnormalities, as described below:

Test procedure

Lymphocytes from huffy layer were cultured in RPMI 1640 (4 ml) medium (developed by Roswell Park Memorial Institute, hence the acronym RPMI) added with L-glutamine (0.1 ml), foetal calf serum (1 ml), antibiotics (0.1 ml penicillin 250 U/ml and streptomycin 250 mg/ml) and pokeweed and incubated at 39°C. At the 70^{th} h, colchicine solution (25%) was added and incubated for 2 h. The cell suspension was treated with 6 ml of hypotonic potassium chloride solution (0.075 M KCl), incubated at 39ºC for 12 min and fixed with methanol:acetic acid (3:1). Samples were vortexed at1000 rpm and supernatant was separated. The plates were restrained in trypsin solution (0.04gr trypsin in 80 ml PBS) for 3 min and stained with 10% Leishman solution (30 ml gurr and 10 ml Leishman stock solution composed of 0.1g Leishman stain added to 50 ml mathanol) for 3 min. Karyotype analysis was based on the evaluation of 25 metaphase plates directly under the microscope by the use of immersion lens identified using the GTL-band pattern (GTL banding refers to the chromosome banding technique in which G-bands are acquired through treatment with Trypsin, followed by staining with Leishman's stain to produce differential staining of metaphase chromosome). To elaborate the karyotypes, chromosomes were classified and numbered according to the recommendations of Di-Berardino (2001). Metaphases were examined under epifluorescence (Olympus Bx50 with COHU CCD Camera) photomicroscope with 50W Hg-lamp, and microscope slides were examined with immertion. Suitable metaphase plates were identified with Mac Os 8.6 programme and Macktype 5.6 image analyzer programme. All the plates were cleaned in xylol for 5 min and covered by 24x60 lamel including entellan according to International System for Chromosome Nomenclature of Domestic Bovines (ISCNDB 2000) (Cribiu *et al*., 2001).

Statistical analysis

Mean values (± SE) for various haematological and serum biochemical parameters for animals of the two groups were computed. The data were analyzed statistically, using student's T-test.

RESULTS

No differences were observed with regard to clinical (except for mild weakness, reduced milk yield, and inappetence) between the PK and the control groups. Similarly, haematological parameters (WBCs, RBCs and PCV) did not differ between the two groups. In biochemical examination, significant increases in AST and GGT were observed in PK group. The average levels of glycemia showed that control group cows with no ketosis (keton bodies were not seen in their urine samples) had statistically higher glycemia than ketogenic cows ($P<0.05$). However, the average concentration of cholesterolemia, as presented on Table 1 was significantly lower in PK group compared to control group, while reverse was true for serum urea concentrations (Table 1). There was non significant difference in the average serum total proteins and albumin concentrations (Table 1) between cows with ketosis and non ketogenic cows in the present study.

Cytogenetical analysis of 24 Anatolian Black cattle based on the evaluation of metaphase plates of the blood lymphocytes demonstrated the presence of 60 chromosomes in all analyzed cells. Morphology and karyotype positions of gonadosomal and autosomal chromosomes for each cattle were noted. Karyotype position of X chromosome on all of the animals were submetacentric, while those of autosomes were acrocentric for clinically healthy cows (Fig. 1) and those with primary ketosis (Fig. 2), and there was no cytogenetic difference between cows of the two groups.

Table 1: Some haematological, serum biochemiacal and urine parameters of control cows and those with primary ketosis (mean ± SE)

Parameters	Control	PK
WBCs ($x10^6$/L)	7.46 ± 0.40	7.09 ± 0.32
RBCs ($x10^{12}$/L)	6.58 ±1.22	6.22 ± 1.00
PCV (L/L)	32.42 ± 0.80	34.0 ± 0.12
Hemoglobin in urine	-	-
Blood in urine	-	-
Ketone bodies in urine	-	+++
Glucose in urine	-	-
ALT (IU/L)	21.24 ± 2.12	23.18 ± 2.40
AST (IU/L)	38.34 ± 2.40^a	56.26 ± 3.80^b
GGT (IU/L)	18.22 ± 0.44^a	22.12 ± 1.00^b
Total protein (g/L)	65.28 ± 4.22	68.12 ± 3.24
Albumin (g/L)	32.64 ± 3.22	31.03 ± 2.66
Glucose (mmol/L)	5.44 ± 3.42^a	2.32 ± 1.44^b
Total cholesterol (mmol/L)	5.42 ± 1.64^a	2.16 ± 0.14^b
Urea (mmol/L)	4.18 ± 1.08^a	6.12 ± 1.10^b

a,b: Different superscripts in the same row indicate significant difference ($P<0.05$).

DISCUSSION

Although no significant clinical and haematological findings were obtained, there were remarkable blood biochemical findings as shown in Table 1. Remarkable hypoglycemia in diseased cows observed in this study may be correlated to ketosis, as reported by Radostits *et al.* (2007). Primary ketosis, occurs when high producing cows simply cannot eat enough carbohydrate to satisfy their glucose needs, or where the feed available is deficient in carbohydrate (Baird, 1982; Foster, 1988). This imbalance leads to mobilization of the body fat reserves in the form of fatty acids that are oxidized in the liver into Acetyl Coenzyme A. The latter is utilized in the Krebs' citric acid cycle when there is sufficient glucose, otherwise it is catabolized into ketone bodies when there is hypoglycemia.

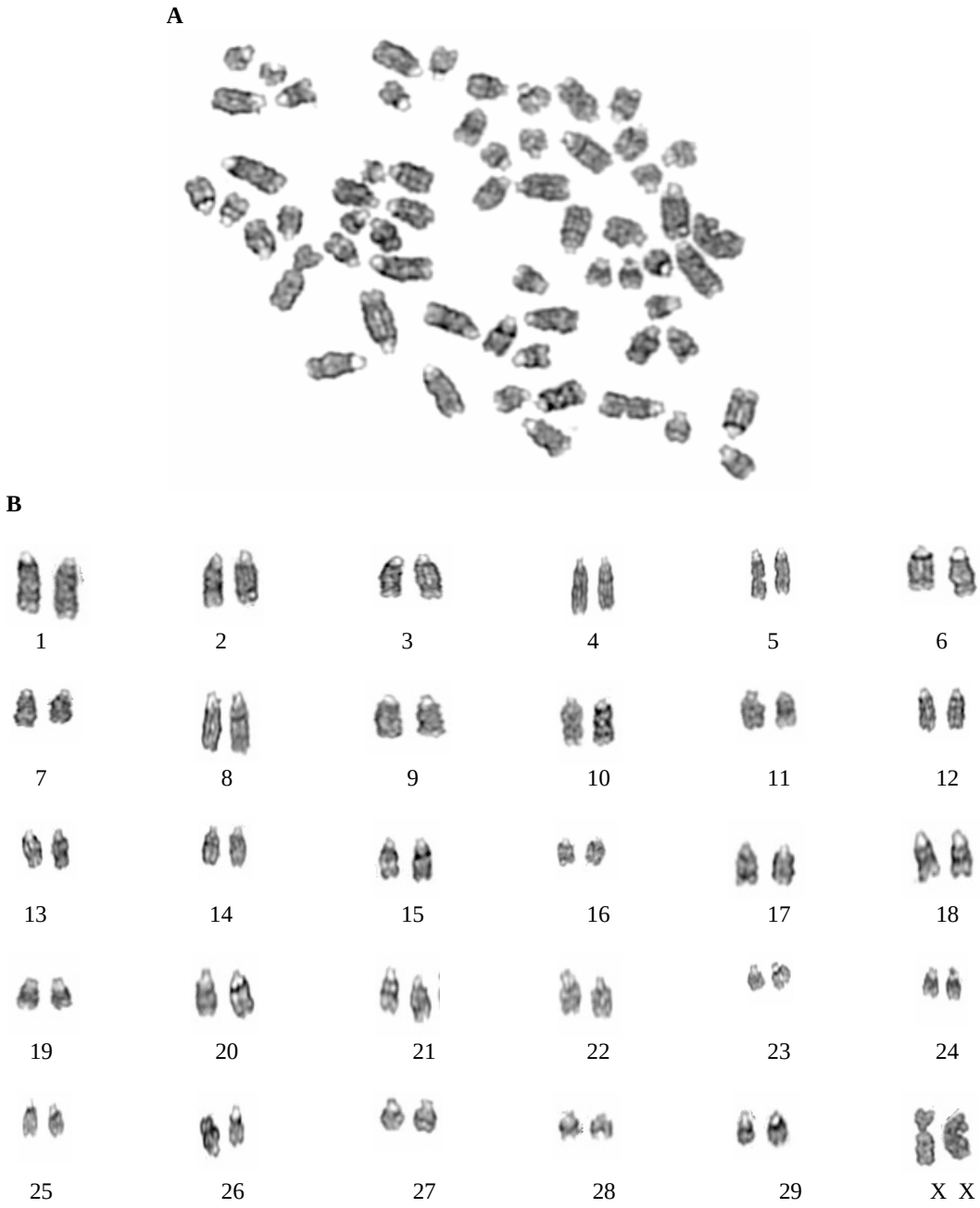

Fig. 1: A. Metaphase plate. B. GTL-banded composite karyotype of control Anatolian Black cattle.

The avarage values of ALT and GGT in PK group were with in normal limits (23.18 ± 2.40 and 22.12 ± 1.00 IU/L, respectively) and no significant difference was found between the PK group and control group. This implies that the liver function was not affected in the cows with ketosis in this study. Some authors declared that chronic cases of ketosis can lead to lipidosis which deteriorates the integrity of the hepatocytes (liver cells). Therefore, a strongly positive correlation was found between AST and the state of ketosis in dairy cows, as well as a significant effect of the plasma concentration of acetoacetate on the concentrations of glutamate dehydrogenase and sorbitol dehydrogenase (Ropstad *et al*., 1989). Similar blood chemical findings were also observed in this study.

The blood cholesterol and urea concentrations in PK group were 2.16 ± 0.14 and 6.12 ± 1.10 nmol/L, respectively. Our results showed negative relationship between cholesterolemia and ketosis, as Sevinc *et al.* (1998) reported that cholesterolemia can be used to testify an energy imbalance and the integrity of the liver. Uremia was observed in the present study in PK group compared to control group. Higher urea levels in PK group may be due to possible liver damage along with higher AST levels. Urea production rises by 67% during pregnancy and falls by 36% following parturition and lactation (Ramin *et al.*,

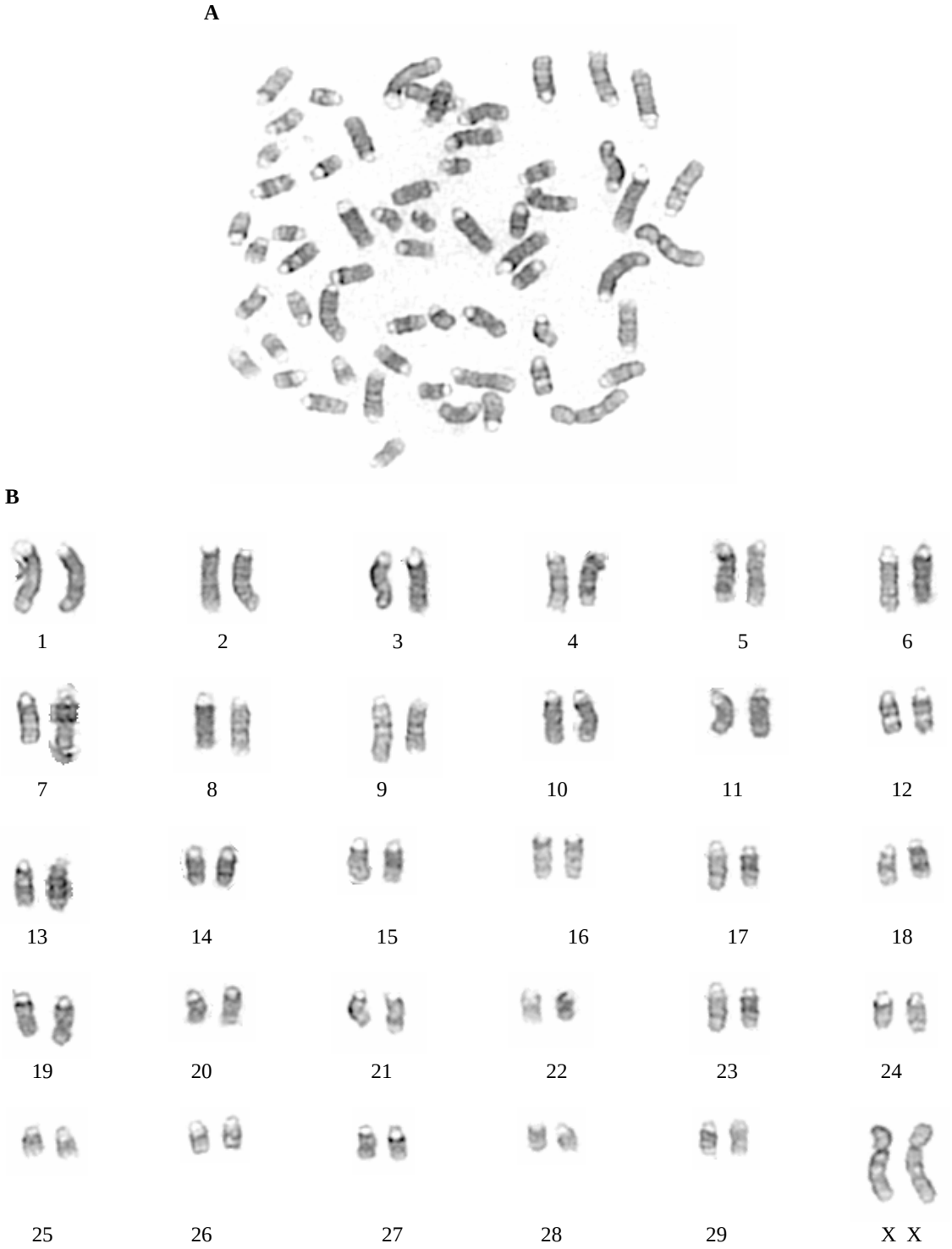

Fig. 2: A. Metaphase plate. B. GTL-banded composite karyotype of Anatolian Black cattle with primary ketosis.

2007). Diarrhea, renal failure and pregnancy toxemia can also cause clinical uremia (Radostits *et al.,* 2007; Ramin *et al.*, 2007).

Balanced translocations have been used to localize genes responsible for a variety of conditions. Translocations are likely to mediate disease processes by disrupting expression of genes in the vicinity of the breakpoints. The first disease for which genetic cause was identified by mapping of a balanced chromosomal translocation breakpoint was chronic granulomatous disease (Di Bernardino 2001). Subsequently, genes responsible for a variety of conditions, such as obesity, cleft palate, blepharophimosis syndrome, DiGeorge syndrome, Duchene muscular dystrophy and congenital cataracts have been identified using this strategy (Holder *et al.,* 2000).

Elitok *et al.* (2001) reported no success in treatment of approximately 30% of primary ketosis cases by

classical medical therapy. Our thinking at the beginning of the study was that it might probably be due to chromosomal instability disorders, and we decided to conduct this study. Over the past few years, it was observed that chromosome dysfunctions or abnormalities are an effective etiology of some metabolic disorders such as diabetes mellitus (Kim *et al.*, 2006) and osteoporosis (Yang *et al*., 2005) and in cattle with chronic enzootic haematuria increased numbers of chromosomal aberrations were seen (Lioi *et al.*, 2004). According to our knowledge, this is the first detailed study on chromosome morphology in both healthy Anatolian Black cattle and those with primary ketosis. Karyotype positions of X chromosome of all the animals (100%) were submetacentric, while those of autosomes were acrocentric for clinically healthy and cattle with primary ketosis. Similar karyotypes were observed in Anatolian Black cattle previously (Poyraz *et al.*, 1995), where microscope slides were stained by Giemsa and metaphases were examined under epifluorescence, while photograph examination was not computer based as in the present study. Although, no chromosomal defects were observed in the study, the importance of cytogenetic analysis for the establishment of screening protocols for the assessment of metabolic disorders as a risk factor needs further investigations.

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