Sudan University of Science and Technology

College of Graduate Studies

Clinicopathological Study on Abu Rigaiba Syndrome (Botulism) in Sheep in River Nile State

دراسة إمراضية متلازمة أبو رقيبة (مرض التسمم الوشيقى) في الضان في ولاية نهر النيل

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DEDICATION

TO MY

FATHER

MOTHER,

DAUGHTERS,

SISTERS & BROTHERS,

ACKNOWLEDGEMENT

First and foremost praise Allah, who provided me with the strength to start and finish this research successfully.

I would like to express my sincere thanks and appreciation to my supervisor, Professor *Amel Omer Bakhiet* for her excellent supervision, professional guidance, leadership, assistance and encouragement.

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ABSTRACT

The present work was conducted to investigate the clinical signs and pathological lesions of sheep botulism in River Nile at Al Butana area. Many outbreaks of botulism were detected in sheep in River Nile State. In 10406 sheep flocks, 879 were affected and 797 were died with morbidity rate 8.4% and mortality rate 7.6%. Mortality among infected sheep was 90.6%. The main symptoms were flaccid tetraparesis leading to tetraplegia that was often accompanied by twisted neck, tongue paralysis, continuous tail movement, and loss of appitite, profuse salivation, and nasal discharge and muscle tremors then complete recumbency occured. Ambulatory sheep had uncoordinated and swaying gaits with hypometria and weakness, mainly of the hind limbs. Then recumbent ambulatory sheep with twisted neck eventually died. The recumbent sheep were necropsied. The gross lesions were conestion in all organs, congestion and edema of lungs, pale and fatty liver, fragile muscles congested and inflamed intestine. Accumulation of gases in large intstines and enlarged lymph nodes were observed. On histopathology sections, severe conestion, edema and emphysema were observed in lungs, percarditis, and fatty change in hepatic cells was seen. No significant changes were seen in brain tissues. Samples of the liver, gut and rumen content were collected from the sheep and examined for botulism using the mouse serum neutralization test. Marked osteophagia was observed when the sheep had access to bones in the pasture, and the farmer mentioned that osteophagia was common among sheep of the many survived flocks. Sample from the plant, *Aristida annulata (Alghabash)*; the most abundant forage available for the sheep, was collected and authenticated at the herbarium of Aromatic and Medicinal Plant Institute, National Center for Research, Khartoum Sudan. It contained 2800 mg/kg of Ca and 450 mg/kg of P. One soil sample contained

58.12 mg/kg of Ca and 2.02 mg/kg of P. Epidemiological data and the history of the outbreak were collected on visits to the flocks being affected. During the first visit, 9 affected animals were examined clinically. The serum samples of seven sheep were examined for urea and creatinine concentrations, and serum activities of creatinine kinase (CPK), aspartate-aminotransferase (AST), and glutamyltransferase (GGT). Two recumbent sheep were euthanized and necropsied. Samples from multiple organs and the central and peripheral nervous tissue were collected and processed for histological examination. Samples of the CNS were examined. It is concluded that in these outbreaks, botulism was severely occurred due to loss of green fodders and animals licking bones of dead animals. Drastic economical losses happened due to this syndrome and periodical vaccination is needed to avoid this botulism.

المستخلص

هدف هذا البحث للتحقيق في العلامات السريرية والآفات المرضية من التسمم الغذائي الأغنام في نهر النيل بمنطقة البطانة. تم الكشف عن العديد من حالات تفشي التسمم الغذائي في الأغنام في ولاية نهر النيل. في 10406من قطعان الأغنام، تأثرت 879 ونفق 797 مع معدل الإصابة 8.4٪ ومعدل وفيات 7.6٪. كان معدل الوفيات بين الأغنام المصابة 90.6٪. كانت الأعراض الرئيسية خزل رباعي رخو مما يؤدي إلى الشلل الرباعي الذي كثيرا ما يكون مصحوبا بالرقبة الملتوية وشلل اللسان وحركة مستمرة في الذيل واللعاب الغزير ورشح الأنف والهزات في العضلات ثم يحدث الاستلقاء الكامل. الضان المتنقل مشيته غير متسقة ومتمايله وخاصة من أطرافه الخلفية. وعند التشريح العياني كانت الآفات الواضحة هي الاحتقان في جميع أعضاء الجسم واهمها ذمة الرئة، شحوب و التغيير الدهني في الكبد وضعف العضلات والأمعاء محتقنة وملتهبة. وقد لوحظت تراكم الغازات فيها وتضخم الغدد الليمفاوية. على الانسجة المرضة لوحظ الاحتقان وذمة وانتفاخ في الرئتين و التهاب غشاء التامور في القلب و التهاب في عضلة القلب وشوهدت التغييرات الدهنية في الخلايا الكبدية. تم جمع عينات من محتوى الكبد والأمعاء والكرش من الأغنام وفحصها للتسمم باستخدام اختبار القئران لتحييد المصل. وأكدت الدراسة ان لحس العظام كان ملحوظاً عندما كان الأغنام في المرعي للحصول على الغذاء وذكر الرعاة أن أكل العظام شائع بين الضان في قطعان قيد الحياة. اخذت عينة من نبات الغباش(Alghabash) وهو العلف الأكثر وفرة والمتاح للخراف، تم جمعها والتعرف عليها في المعشبة في معهد النباتات الطبية العطرية بالمركز القومي للبحوث، الخرطوم السودان. وبالتحليل اتضح انها تحتوي على 2800 ملغ / كغ من الكالسيوم و 450 ملغم / كغم من الفسفورP . أخذت عينة من التربة و وجدت انها تحتوي 58.12 ملغم / كغم من الكالسيوم و2.02 ملغ / كغ من الفسفور.اجريت دراسة الوبائية وتاريخ اندلاع المرض في زيارات القطعان المتضررة. خلال الزيارة الأولى، تم فحص 9 الحيوانات المصابة سريريا. تم فحص عينات مصل سبعة من الأغنام لتركيزات اليوريا والكرياتينين، والأنشطة مصل الكرياتينين كيناز(CPK) ، اسبارتاتي-الألانين(AST) ، و glutamyltransferase (GGT). تم التشريح لشاتين راقدتين و تم جمع عينات من أجهزة متعددة في الجسم والنسيج العصبي المركزي والمحيطي ومعالجتها للفحص النسيجي. تم فحص عينات من الجهاز العصبي المركزي. وخلصت الدراسة إلى أنه في هذه المراعي حدث التسمم الشديد بسبب فقدان الأعلاف الخضراء وان الحيوانات تلعق عظام الحيوانات النافقة. وقعت خسائر اقتصادية جذرية بسبب هذه المتلازمة وهناك حاجة إلى التطعيم الدوري لتفادي التسمم الوشيقي.

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CHAPTER ONE

INTRODUCTION

Abu Rigaiba (ARS) is a serious syndrome affecting mainly sheep leading to a high morbidity and mortality. It occurs usually in the pasture during dry season. Affected animal shows: loss of appetite (off food), continuous tail movement, tremors, lateral deviation of the neck in its middle or upper part, semi paralyzed tongue protruded out side and normal body temperature.

Death may occur within (24-72) hours in per acute form. In acute form may extend to 7 days.

Animal Resources Directorate (ARD), River Nile State shows that morbidity rate range from 10-90% of the affected flocks. And it is a high fatal disease that reaches up to 100% mortality rate.

The syndrome appeared in 1971 in Shandi area, and it had been reported as unknown disease and it recorded high mortalities in year 1982 up to year 1985 (the drought years). Then the syndrome continues to appear in inter-mitten dry seasons in ELhammad area. In 1973 the so called Abu Regiaba disease came out and covered many parts in Sudan mainly River Nile, Eastern Nile Province and Northern Kordofan.

ARD dealt seriously with the diseases since 1998 and many attempts had been done to investigate the real cause depending serosurveillance, plant, water and soil analysis and the diagnosis revealed that the syndrome might be hypomagnesaemia, hypocalcaemia, phosphorus deficiency, enterotoxaemia (*Clostridium perfringens*), nitrate toxicity and /or some toxic plants. Some features and cardinal signs resemble intoxication of Botulism in South Africa and Australia, accordingly the suspection point to *Clostridium botulinum* as one of possible causes of Abu Regiba. In Abu Regiba pathological changes

can be investigated, but no definite diagnosis or effective treatment was proved. Thousands of sheep died, highly costed treatment was done but no one put forward a good idea to solve the problem. Recently some efforts have been starting by some veterinarians. This is a contribution as integrated work to investigate some studies on the disease. The study is a trial to focus the proplem and find out some aspects of the disease.

The objectives of this study were:

(1) To investigate a clear concept of the disease.

(2) To study pathological picture of the disease and use it as a diagnostic tool as possible.

CHAPTER TWO
LITERATURE REVIEW

2-1. General background

Sudden death (no premonitory clinical signs) is one of the most common presentations of disease in ruminant livestock, partly due to the extensive nature of the husbandry and subsequent lack of observation but also due to the number of causes of rapid death that can occur (Irwin, 2011).

A starting point to investigations into all ruminant disease is to understand the area, the husbandry practices that apply, and the pasture types that are available. For instance, in a summer dominant rainfall area with subsequent summer growing pasture species plant poisonings do tend to occur well into winter when stock are hungry, protein and energy deficient, and poisonous plants (Irwin ,2011).

Irwin, (2011) suggested that the use of pathology is very important in verifying a tentative diagnosis based on clinical signs and history but when investigating death in livestock it is rarely economically feasible to go on a "fishing expedition" for all possible causes if there is no initial suspicion on the cause of death.

A variety of syndromes occur in sheep, goats, and beef and dairy cattle due to a lack of utilizable minerals such as magnesium (Mg). Non ruminant species are not afflicted by this problem. The symptoms may vary from hyperaesthesia, aggressiveness, incoordination, tetany, and convulsions to flaccid paralysis. Although the pathogenesis of all the hypomagnesemic diseases is probably the same, they are given common names indicating the conditions under which they occur: grass tetany, grass staggers, wheat pasture poisoning, lactation tetany, transport tetany, winter tetany, and milk tetany of

calves (Robert , 1997). In River Nile (Nahre ElNeil) State the common name indicating the condition is Abu Rigaiba.

Many attempts had been done to investigate the real cause depending Serosurveillance, plant, water and soil analysis and the diagnosis revealed that the syndrome might be due to hypomagnesaemia, hypocalcaemia, phosphorus deficiency, enterotoxaemia (*Clostridium perfringens*), nitrate toxicity abd /or some toxic plants and one of them may acts as predisposing factor to the syndrome (Robert , 1997).

Then further investigation showed that it is ecologically tied disease. As many observations insure that movement of livestock out side the pasture area to the revering land leads to disappearance of the syndrome (Robert , 1997).

2.2. Magnesium (Mg)

Magnesium (Mg) is abundant in most common feedstuffs relative to apparent requirements by animals. It is widely distributed among plant and animal tissues (70% of total body Mg present in bones). Magnesium has many diverse physiological functions. It is the second most plentiful cation (after K) of intracellular fluids. It plays a key role as an essential ion in many fundamental enzymatic reactions in intermediary metabolism and also as an "activator" of enzymes, in particular those involving phosphate compounds such as ATPases, kinases and phosphatases, require Mg^{++} for activation (Dalley et al., 1997a). Magnesium is vitally involved in the metabolism of carbohydrates and lipids as a catalyst of a wide array of enzymes which require this element for optimal activity.

Mg^{++} is involved with several physiological and biochemical processes including synthesis of RNA, DNA or protein and stabilization of membranes (Dalley et al., 1997b). Recently, it has been shown that Mg^{++} plays an important role in the regulation of membrane channels (Leonhard-Marek et al, 1998a,) as well as excitation-contraction coupling in skeletal muscle.

The dietary Mg requirements of livestock vary with the species and breed of animals, age and rate of growth or production, and with biological availability in the diet. Many dietary factors influence Mg absorption, and therefore Mg requirements, and include K, Ca, P, Al, Fe, Na, protein, fat, organic acids, carbohydrate type, ionophores, Mg status, and frequency of feeding (Dalley et al.,1997b, Leonhard-Marek et al, 1998b). Conditions in the rumen, such as high pH, which adversely affect Mg absorption, will therefore raise dietary requirements (Sykes and Russel, 1991).There are some other factors affecting magnesium absorption in the rumen, both potassium and rapidly degradable protein have a negative effect on magnesium absorption, as has a high rumen pH. The coincidence, therefore, of high dietary intake of potassium and degradable protein in rapidly growing spring herbage means that conditions for magnesium absorption are critical at this stage (Leonhard-Marek et al, 1998a, Leonhard-Marek et al, 1998b, Terashima et al., 1982, Wachirapakorn et al., 1996a, Wachirapakorn et al., 1996b).

Feeding high levels of potassium generally has depressed blood serum magnesium in ruminants as a result of reduced magnesium absorption (http://www.saltinstitute.org/). The main effect of potassium is on preintestinal magnesium absorption. Magnesium is transported across the ruminal mucosa by an active sodium-linked process. Dietary or salivary sodium deficiency decreases the sodium: potassium ratio in rumen fluid, which results in depressed magnesium absorption. The imbalance between protein and carbohydrate in the rumen may lead to a deficiency of absorbable energy from the rumen. If volatile fatty acids and carbon dioxide production are depressed due to a lack of carbohydrate, blood flow to the rumen wall may be decreased resulting in low magnesium absorption. (http://www.saltinstitute.org/)

2.3. Hypocalcaemia

(Milk Fever/ Lambing Sickness)

The condition is attributable to a reduction in calcium in the body fluids and the severity of the clinical syndrome parallels the degree of hypocalcaemia. Hypocalcaemia is seen more frequently in the older ewe and commonly occurs following stress, e.g. change of feed, change of weather, transportation etc. It has been described in ewes brought off bill or upland pasture and moved on to low ground grazing. Because of this association with stress it is common to observe a number of animals affected at any one time. In the early stages of the disease the affected animal has a staggering gait and appears weak on its legs; frequently the animal trembles, this feature being most marked in the shoulder muscles. The animal becomes progressively more restless, may breath rapidly and eventually assumes sternal recumbency. Death may take place rapidly and certainly within 24 hours of onset (Belonje et al., 1982; Peet et al., 1985; Pickard et al., 1988; Sykes and Russel, 1991; Tindall, 1986).

2.4. Phosphorus deficiency

Deficiency may be diagnosed by observing symptoms in affected animals and by considering the country they are grazing. Symptoms will be more pronounced and therefore more easily observed in animals with a high demand for phosphorus such as lactating breeders and young growing stock. The symptoms observed are depraved appetite**,** peg leg, stiff gait and arched back and poor growth rates (Tindall, 1986; Corlett and Care, 1988; Davies, 1985).

2.5. Enterotoxaemia

Overeating disease also known as "Enterotoxaemia" or "pulpy kidney" disease. It is a highly significant and costly disease in the sheep industry. This

disease is a major killer of lambs from shortly after birth throughout the entire feeding period. Proper preventative practices are strongly recommended to sheep producers to avoid loss of sheep to this disease. Such practices would include proper feeding, management, and immunization.

Enterotoxaemia is caused by the bacterium *Clostridium perfringens*, Types C and D. Under certain conditions, the bacteria produces toxins that will show signs that will frequently cause animal death. *Clostridium perfringens* is found universally in the soil and manure. It is also present in certain amounts in the intestinal tract of the sheep and most mammals. The bacteria normally inhabit the lower digestive tract and cause no harm to the animal. It thrives on sugars and starches, which are usually digested in the upper portion of the tract. However, when a lamb overeats, not all the starches and sugars are utilized and therefore are passed on. Then the *Clostridium perfringens* grow and trigger excessive bacterial growth. This excessive growth allows the bacteria to produce lethal amounts of toxin that are absorbed into the animals system. This occurs when the lamb takes in more than 3/4 pound per head per day. It can also occur if you allow your feeding lambs to rapidly engorge themselves, or if you feed your lambs during irregular feeding times. Allowing nursing lambs' access to large amounts of milk to consume can also cause an increase in the bacteria's growth.

Type C: Is thought to be related to milk consumption rather than grain consumption.

Type D: Is usually caused by overgrazing or excess grain consumption.

2.5.1. The Signs:

Death is usually the first sign of the disease. Enterotoxaemia progresses very rapidly, with death occurring within two hours after onset of clinical signs.

Some animals will show some symptoms of colic, muscle tremors, or convulsions. However, the animal may rise between seizures, but soon will

collapse again. Some other nervous system signs are frothing of the mouth, grinding of the teeth, and oscillations of the eyes (www.ag.ansc.purdue.edu/sheep).

2.6. Nitrate poisoning

Nitrate poisoning is a condition which may affect ruminants consuming certain forages or water that contain an excessive amount of nitrate.

Causes of Nitrate Poisoning

Under normal conditions, nitrate ingested by ruminant livestock like cattle, sheep and goats is converted to ammonia in the rumen by bacteria. The steps of conversion in this process are as follows:

------Bacterial Protein------

Nitrate (NO3) —› Nitrite (NO2) ——› Ammonia (NH3) ——› Amino Acid ——› Protein

Nitrate is converted to nitrite faster than nitrite is converted to ammonia. Consequently, when higher than normal amounts of nitrate are consumed, an accumulation of nitrite may occur in the rumen. Nitrite then will be absorbed into the bloodstream and will cause haemoglobin to be converted to methaemoglobin, which is unable to transport oxygen. Thus, when an animal dies from nitrate poisoning, it is due to a lack of oxygen.

Nitrates are less toxic to ruminants than nitrites. Fortunately, nitrites do not occur in feed or water in nearly as high concentrations as nitrates.

The occurrence of nitrate poisoning is difficult to predict because nitrate levels can change rapidly in plants and the toxicity of nitrate varies greatly among livestock of various age, health status, and diets.

Symptoms of Nitrate Problems

Symptoms of acute nitrate poisoning in animals are related to the lack of oxygen in the tissues. These include muscular weakness, incoordination,

accelerated heart rate, difficult or rapid breathing, cyanosis, coma, and death (Dan Undersander et al., 2001).

2.7. Toxic Plants

Fall is the time of year where we see a greater incidence of sheep exposure to toxic plants and chemicals. Unfortunately in most cases the first signs of toxicity are sudden death and depending on the plant or compound death rates can be catastrophic. The most important aspect of treating toxicity is simply to prevent it. This requires a knowledge of potentially toxic plants and chemicals and situations which may lead to toxicity. When we encounter toxicity the causes is either ignorance on the part of the shepherd not realizing that a plant is toxic or accidental exposure where a gate is left open and sheep gain access to areas of the farm that they are not supposed to (Goelz, 2002).

2.8. The genus *Clostridium*

The genus *Clostridium* consists of over 100 species, ranking second in size next to Streptomyces (Dong *et al.,* 2010; Sabina and Nicodemus, 2014). Many *Clostridium* species are known to cause disease to human beings. These include neurotoxigenic clostridia (*C. botulinum* and *C. tetani*) (Weingart *et al.*, 2010), clostridia involved in gas gangrene and necrotizing infections (*C. perfringens, C.sordellii* and *C. septicum*) (Hatheway, 1990; Aldape *et al.*, 2006), and the enteropathogenic *C. difficile* (Twine *et al.,* 2009). *Clostridium chauvoei*, which has a strong phylogenetic relationship with *C. septicum*, a human pathogen, has a long history of veterinary importance. It was believed for a very long time that this species was exclusively a veterinary pathogen, only associated with blackleg in ruminants (Useh *et al*., 2006a). Recent reports of the disease in human beings have placed the pathogen on the list of

very important lethal disease agents of human beings (Nagano *et al.*, 2008; Weatherhead and Tweardy, 2011). Indeed zoonotic strains of the *Clostridium* spp pose a great threat to community health in endemic areas. The aforementioned notwithstanding, genetic engineering of pathogenic strains is beginning to suggest that members of the genus could also serve therapeutic purposes. In tumor managements, the use of viral vehicles in gene therapy could be successful or not, depending on the delivery systems employed. There are many tumors in which this procedure has not been successful. Thus, there is intensive on-going research in so many expert laboratories to develop alternative management approaches (Minton, 2003; Sabina and Nicodemus, 2014). On the other hand, some *Clostridium* species are of great industrial importance. For example, *C. thermocellum* can produce ethanol from lignocellulosic waste at high temperature, while *C.acetobutylicum* and *C. beijerinckii* produce solvents (acetone, butanol, and ethanol) by utilizing a variety of substrates from monosaccharides to polysaccharides (Ezeji *et al.*, 2007; Otte *et al.*, 2009; Ezeji *et al*., 2010; Jia *et al*., 2011; Sabina and Nicodemus, 2014).

In sheep, the disease is mainly due to food or water contamination (Thiogane et al., 1984; Kriek and Odentaal, 2004; Van der Lugt et al., 1995, 1996; Swift et al., 2000; Van der Burgt et al., 2007; Lobato et al., 2008); in Australia, however, the disease has been associated with the ingestion of decaying rabbit carcasses (Rose and Edgar, 1930; Bennetts, 1928). It has also been observed that sheep from some areas of South Africa suffer severely from osteophagia; their bones are light and brittle, botulism is fairly common amongst them, and toxigenic C. botulinum was isolated from their carcasses (Robinson, 1929). Few outbreaks of botulism are reported in goats. Of two outbreaks in Angora goats in South Africa, one was caused by the ingestion of milled lucerne containing rodent car-casses and the other by the ingestion of poultry litter

(Smith and Sherman, 2009). Another outbreak of botulism caused by C. botulinum type D in sheep and goats was reported in South Africa (Van der Lugt et al., 1995), but the source of infection was not determined, although it was thought to have been the water. In Senegal, one outbreak of botulism due to C. botulinum type D that affected goats, sheep, horses, and cattle was caused by the presence of a dead small mam-mal in the drinking water (Thiogane et al., 1984). There have been no outbreaks of botulism in goats that were asso-ciated with osteophagia, and this form of allotrophagia has not been reported in this species.

CHAPTER THREE
MATERIAL AND METHODS

3.1. The study area:

The study area lies within Shendi locality and extends from Abudelig area in Khartoum State and northerly easterly and joint El Neil to Kabouchya town in Nahre El Neil. The area is rich with under ground water and pastures resources. There are many water pools and yards. The area is inhibited with Fadenya, Nafaab, Kemilab, and Manasier. They perform a transhumance life system. They keep sheep and goat mainly.

3.1. Study design and samples

Serosurveillance using sera, plants, soil, water analysis.

Socioeconomic surveillance was done and questionnaire was prepared. Experimental induced syndrome was done to rats. Experimental studies on different plants available in the area and their toxic possibility.

Reconnaissance

Primary visit will be done for the study areas to investigate the problem; informal interviews will be done with livestock owners and herders in order to study the size and elasticity of the problem.

This survey was conducted during the period from July to October 2014. A questionnaire (Appendix) was designed and distributed to nomads

3.2. The questionnaire

Questionnaires were distributed (Appendix). The questionnaire included observed clinical signs, age, number of affected flocks etc.

The questionaire was also aimed to gather knowledge about the appearance and disappearance of the disease investigated.

3.3. Methodology:

Epidemiological data and the history of the outbreak were collected on visits to the flocks located in the River Nile State. During the first visit, 11 affected animals were examined clinically. The serum samples of seven sheep were examined for urea and creatinine concentrations, and serum activities of creatinine kinase (CPK), aspartate-aminotransferase (AST), andglutamyltransferase (GGT) (Schmid and von Forstner, 1986). Two recumbent sheep were euthanized and necropsied. Samples from multiple organs and the central and peripheral nervous tissue were collected and processed for histological examination. Samples of the CNS were also collected. Samples of approximately 100 g of the liver, small intestine (jejunum) and rumen contents were collected from the two necropsied sheep and examined for botulinum toxins by the mouse serum neutralization test (Smith, 1977).

A sample of the plant *Alghabash*, which the nomads associated with the disease and which was the most abundant forage available for the sheep, was sent to the AMPI National Research Center Khartoum Sudan for protein and mineral concentration measurements.

3.4. Blood samples

Blood samples were collected for haematology and serum biochemical analysis

Blood samples collection

Blood samples were collected directly from the jugular vein of the animals using 5 ml syringes.

Blood samples were transferred immediately into two containers; the first ones were plain vacutianer tubes and they were allowed to clot, the clotted blood samples were centrifuged and sera were separated and sorted at -20°C until analyzed.

The other one was kept in vacutianer containing heparin as anti coagulant, these samples were immediately used to evaluate the haematological parameters.

3.5. Haematological methods

Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell (RBCs), white blood cell (WBCs) and differential (WBC) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and corpuscular haemoglobin concentration (MCHC).

3.5.1 Red blood cells count (RBCs)

Red blood corpuscles were counted in an improved Neubauer haemocytometer (Neubauer-improved -Germany) using hymen's solution as diluents. Consisting of 0.5g mercuric chloride 0.5g sodium sulphate and 1.0g sodium chloride, made up to 200 ml with distilled water as described by Jain (1986).

3.5.2 Haemoglobin concentration (Hb)

Haemoglobin was determined by acid haematein method (Sahli technique) using Sahli haemoglobinometer.

Sahli method depends up on conversion of haemoglobin to acid haematein. The red cells are lacked in dilute hydrochloric acid (HCL) to form acid haematein which is brown in colour and is matched with standards colour.

3.5.3 Packed cell volume (PCV)

Blood samples were drawn into heparinized capillary tubes sealed at one end by cristeasl and centrifuged at 1200 rpm for five minutes using microhaematocrit centrifuge (Hettich-ZENTRIFUGEN –Germany). The PCV% was read in microhaematocrit reader.

3.6. Biochemical methods

Serum samples Table (1) were subjected to biochemical tests to assess the effect of treatment, if any, on the liver and kidneys functions, and effect on minerals level.

The following blood biochemical parameters were tested using standard methods: Total proteins, albumin, bilirubin, Aspartate aminotransferase enzyme (AST), Alanine aminotransferase enzyme (ALT), Urea, Creatinine.

Analyses were conducted using A15-BioSystem (Barcelona, Spain) biochemistry analyzer, random access full automated machine (150 samples /hr, and designed to estimate 60 parameters).

Table 1: Location and numbers of blood samples of affected sheep

Location	Number	Location	Number
Beer Elshare	1	Wad Elebeid	7
Umm dirwa	1	Trmade Eltahir	14
Elgehade	3	Wad Elsawad	7
Elhawad	1	Umm hatab	4
Shirasha	1	Deim Elgarray	2
elhomrani	1	Donki Elahamda	1

3.7. Biochemical methods

3.7.1. Plasma total protein:

Plasma proteins are synthesized in the liver, lymph nodes, spleen and the bone marrow. Hypoproteinaemia can be caused by diseases and disorders such as loss of blood, severe burns, acute hepatitis and nephrotic syndrome.

hyperproteinaemia observed in cases of severe dehydration and illnesses such as myeloma.

Assay principle

The most suitable method used for routine analysis of samples in the current investigation is the Biuret Colorimetric Endpoint Method described by Doumas *et al*., (1981). In this method the divalent copper reacts with the peptide bonds of protein under alkaline conditions to form the characteristic pink to purple biuret complex. Total protein was measured using a commercial kit (Vitro Scient-Egypt). The intensity of colour is determined by measuring the increase in absorbance at 530 nm using spectrophotometer (Jenway 6305 U. V./vis. Spectrophotometer, U.K.).

3.7.2. Blood urea

Urea is the major end product of protein nitrogen metabolism. It is synthesized in the liver and excreted mostly by the kidney and minimal amount excreted in sweat and degraded in the intestine by bacterial action. Determination of urea is a screening test for renal function; pre renal elevations are seen in shock, inadequate renal perfusion, and diminished blood volume. Renal causes of elevations are chronic nephritis, tubular necrosis, and nephrosclerosis. The main cause of post renal elevations is urinary tract obstruction.

Assay principle

Urea was measured using commercial kit (Vitro Scient-Egypt) according to enzymatic colorimetric method described firstly by Marshal (1913) who added urease enzyme and measured the liberated ammonia with titration with an acid, this method has been used and modified and later modified by Fawcett

(1960). In this modified method urea is hydrolyzed by urease to form ammonium and carbonate, the ammonium ions react with the salicylate and hypochlorite to form a green coloured indophenol. The optical density of the developing colour was measured at 578 nm using Spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

3.7.3. Blood creatinine

Creatinine is a waste product formed by dehydration of creatine. Most of the body creatine is found in muscle tissue where it presents as creatine phosphate and serves as energy storage reservoir for conversion to adenosine triphosphate. As in urea, determination of creatinine is a screening test for renal function but urea levels are affected by factors such as diet, degree of hydration and protein metabolism on the other hand serum creatinine is constant and unaffected by these factors.

Assay principle

Creatinine was analyzed using commercial kit (Vitro Scient-Egypt).Vitro creatinine reagent is based on modified Jaffe reaction which was firstly described by Jaffe (1886) in a method involved precipitation of protein and still widely used today. In this reaction creatinine forms a yellow-red complex with alkaline picrate. The optical density of the developing colour was measured at 578 nm using Spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

3.7.4. Aspartate aminotransferase (AST)

AST belongs to the group of transaminase enzymes which catalyze the conversion of amino acid to α-Keto acid via the transfer of amino acid group and reverse process. AST is commonly found in tissues presented in both cytoplasm and mitochondria and significant activities seen in cardiac and

skeletal muscles, liver, kidney, gastric mucosa and adipose tissue. Elevated AST levels found in liver disease associated with some degree of hepatic necrosis, muscular myopathies, damage of internal organs and myocardial infarction.

Assay principle

AST was analyzed in accordance with kinetic ultraviolet method. Karmen et al, (1955) described the first kinetic method using a couple reaction of MDH and NADH in 1960 this method modified by Henry et al. AST analyzed using commercial kit (Vitro Scient-Egypt). Vitro reagent is based on the recommendation of the IFCC (IFCC, 1986) where the amino group is enzymatically transferred by AST present in the specimen from aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate. Oxaloacetate is reduced to malate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD. The rate of oxidation of the coenzyme NADH is proportional to the AST activity in the specimen and it was determined by measuring the decreases in absorbance at 340 nm using Spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

3.8. Pathology

Tissue samples

Carry out post-mortem examination to identify gross lesions, collect specimens of vital organs, including liver, gall bladder, pancreas, intestine, heart , kidneys, spleen and any other organ, fix in 10% neutral buffer formalin and process for histopathological examination

CHAPTER FOUR

RESULTS

4.1. Questionaire data and survey

Buttana area Sudan is a wide rangeland. Most of it lies in River Nile State at eastern of Shendi Town. The survey coveres this area and included these area: Beer Elshain, Elsitier, Elhomra, UmmEshair, Ummhatab, Wad Elhammad, Beer Backery, Anbasa, Beer Elyazee, Shirusha, Elhomrany, Umm dirwa, Elgchade, Wad Elebied, Timade haj Eltahir and other wells of easrern Shendi.

Muddy, sandy and stony lands cover ElButtana. The rangeland divided into two types: one for grazing but the second for agriculture. Grzing vegtaion and forage plants vary from mixture of grasses like Aristida spp., safel, turba, Indigolfers hochstettern, Euphoria scordifolia, Corchorm kabedtoor, Tribubus, Samadeb, Paricum spp., Corchorus depressus, Casriteria spp., Acacia tortitis and Acacia chrenbergiuana.

The vegetation and forage plant become dried and decayed after the end of rainy season. It was also noticed low quality and quantity of variable forages especially during the dry season and would not supply the maintenance requirements of animals.

Deficiency of essential nutrient sourses in sheep feed usually results in animals began to feed on devour carcasses of animals, bones of mices and snakes, feces and rumen contents of dead animals and feces of birds (pica).

The disease arises after rain fall between December and July (dry season) when sheep flocks suffer from malnutrition and mineral deficiencies.

Two sources of drink water are available in Butana. First one ground water the availability of which depends on geological formation called (Donki). The second sourse is suface water and called (surface wells).

Animals drink water each 1-3 days in dry season. The disease occurs in all animal species in the areas; sheep, cattle, goats, donkeys and hens. All ages of sheep are infected except suckling lambs but severely affected pregnant and lactating ewes and male sheep.

The outbreak occurred sporadically affecting few animals within the flock but high incidence occurred during the dry season.

4.2. Symptoms

The disease presented with albasia (unability to move) and tremors. Symptoms appeared within 2-3 days but some died after few hours.

Initial observations were tremors, flaccid paralysis, continous movement of tail, loss of appitite, profuse salivation, nasal discharge, recumbency, lateral deviation of neck, semi paralyized tongue, curved back, putrefactive and bad mouth smell, increased respiratory rate and pulse (Figs 1-2).

4.3. Mortality and morbidity rate

Table (1) shows the total number of sheep flocks was10406; infected animals were 879 with morbidity rate 8.4% and mortality rate was 7.6 (dead animals were 797).

4.4. Pathological lesions

Grossly all animal organs were congested. Lungs showed edema, congestion and emphysema, pale and fatty change in liver, fragile muscles, percarditis, and congested intestine filled with gases, enlarged lymph nodes. Marked

osteophagia was observed when the animals had access to bones from carcasses present in the pastures. Clinical signs were flaccid tetraparesis (Fig. 1A) leading to tetraplegia (Fig. 1B and C) often accompanied by twisted neck (torticollis) (Fig. 1B), tongue paralysis and muscle tremors. At the time of the visit, 11 sheep were affected. All were in recumbence. Two of the recumbent sheep and four of the ambulatory sheep showed twisted neck (Fig. 1B and D). One of the ambulatory sheep was affected for approximately 20 days; the main sign was severe twisted neck, in which the neck could not be straightened onto the median plane (Fig. 1D). Ambulatory sheep were uncoordinated and had swaying gaits with hypometria and weakness. When examined, they showed limb weakness with easy flexion of the hooks and fetlocks. During clinical examination, decreased tongue tonus was observed in the recumbent sheep, with the tongue staying out of the mouth after exposition (Fig. 2A and B). Despite the recumbence, the sheep were still eating and ruminating and showed normal sensation and consciousness. When the animals were forced to stand up, there was severe weakness with flexion of the four limbs and poor limb tone (Fig. 1A). Sometimes they stood by themselves but fell down easily. Two of the recumbent animals had severe muscle tremors due to weakness when forced to stand; they were reluctant to move and had arched backs. They also bellowed and grunted, suggesting pain (Fig. 2C). Abdominal-type respiration was observed in a recumbent sheep and head tremors in another. Another recumbent sheep occasionally kept its head on the ground (Fig. 1C).

4.5. Histopathological lesions

Severe conestion, edema and emphysema were observed in lungs, percarditis, and fatty change in hepatic cells was seen. No other significant histologic

lesions were observed. No significant changes were seen in the central nervous system.

4.6. Biochemical analysis

Serum concentrations of urea and creatinine and serum activities of AST and GGT were within normal values. Serum activities of CK were within normal levels, but the recumbent sheep had elevated levels up to 1231 U/L. Some of the smples taken to detect the toxin were positive for type C toxin

4.7. Plant

Aristida annulata (Alghabash); the most abundant forage available for the sheep, was collected and authenticated at the herbarium of Aromatic and Medicinal Plant Institute, National Center for Research, Khartoum Sudan. It contained6.1 total protein, 2500 mg/kg of Ca, 340 mg/kg of P, 2200 mg/kg of Mg, 22,300 mg/kg of K, 123.13 mg/kg of Fe, 66.52 mg/kg of Zn, 250.12 mg/kg of Mn, and 1.81 mg/kg of Cu.

4.8. Soil

One soil sample contained 60.54 mg/kg of Ca and 1.91 mg/kg of P, 68.71 mg/kg of K, and 1.02 mg/kg of Mg.

After the presumptive diagnosis of botulism, the flock was vaccinated against botulism. Many casesappeared within 10 days after vaccination, but later no more affected animals were observed.

(A)

(B)

.

(C)

(D)

Fig. 1. Sheep showing signs of botulism: (A) severe weakness evidenced by flexion of the limbs; (B) permanent recumbence and twisted neck; (C) recumbent sheep with the head touching the ground; and (D) severe twisted neck

(A)

(B) **(C)**

Fig. 2. Sheep showing signs of botulism: (A) sheep with the tongue remaining out of the mouth after exposition; and (B,C) this sheep was recumbent but it was forced to stand up

Table 2: N umber of affected sheep and their locations

Location	Total animals	Risk animals	Dead animals
Umm hatab	305	66	62
Umm Eshara	150	10	10
Wad Elhammad	335	20	15
Temade HajEltahir	1594	156	143
Elgehade	2173	136	117
Anbasa	160	52	33
Elhomra	20	9	9
Ummdirwa	3695	130	120
Elhomrani	159	4	3
Beer Elshane	265	5	2
Elsitier	150	7	7
Beer Backory	100	20	20
Wad Elebeid	735	18	13
Shirasha	134	5	3
Wad Elsawad	500	240	240
Total	**10475**	**878**	**797**

CHAPTER FOUR
DISCUSSION

In this study the clinical signs of flaccid paralysis and the absence of histologic lesions in the central nervous system are agreed with the findings of Riet-Correa *et al.*, (2012) and are important features for the diagnosis of the disease. Observation of clinical signs of botulism and the absence of gross and histological lesions are very important to establish a presumptive diagnosis of the disease. Osteophagia was seen in some os affected sheep and this agreed with the results of Kriek and Odentaal, (2004); Riet-Correa *et al.*, (2012). Radostits *et al.*, (2007) recorded pica in sheep, and it is associated with dietary deficiency of protein or net energy. In the present study, the low phosphorus concentrations in Alghabash and also in the soil suggest that the osteophagia was probably due to phosphorus deficiency (Riet-Correa *et al.*, 2012). In the present study the clinical signs reported in sheep affected by spontaneous were similar to goats studied by (Van der Lugt *et al.*, 1995; Lobato *et al.*, 2008) and similar to the experimental botulism in cattle (Santos *et al.*, 1993). In these results of survey of outbreak some animals were found dead or died suddenly, without exhibiting clinical signs; others had a short clinical manifestation period of 2–12 h, during which they showed restlessness, reluctance to move, a stiff gait, muscle tremors, foaming at the mouth, pupillary dilation, and paresis and/or paralysis in the terminal stages of the disease. These are agreed with the study done by reported by Van der Lugt *et al.*, (1995), In the outbreak reported here, despite the fact that the main sign was the flaccid paralysis leading to recumbence and tongue paralysis, as mentioned in cattle as reported by Van der Lugt *et al.*, (1995), other signs not commonly observed in cattle were also important. Twisted neck, mentioned as cervical torticollis or

wryneck, is a rare sign in large animals and is commonly associated with mild cervical spinal cord lesions, and muscle or bone lesions of the neck (Mayhew, 2009; Riet-Correa *et al.*, 2012). In botulism, twisted neck is probably due to weakness of cervical muscles. Muscle tremors due to weakness seem to also be a common sign of botulism in sheep. This sign is infrequently seen in cattle, which are generally found in recumbence (Lisboa *et al.*, 1996), but is a common sign in horses (Mayhew, 2009; Riet-Correa *et al.*, 2012). Another difference is that in cattle with botulism due to osteophagia, gait alterations evolve rapidly to recumbence, and nearly all animals are found to be recumbent. In a study with 32 naturally affected cows, all were in permanent recumbence at the first examination (Lisboa *et al.*, 1996). In contrast, in the outbreak reported here, some sheep were recumbent and some others were ambulatory with gait alterations and twisted neck. The duration of the clinical signs in the affected animals, from 7 to 30 days, was more prolonged than those observed in outbreaks due to feedstuffs contamination in goats that were from less than 24 h to 5 days (Van der Lugt *et al.*, 1995; Lobato *et al.*, 2008; Riet-Correa *et al.*, 2012). In experimental induction of botulism in goats using subcutaneous doses of type C toxin ranging from 15.6 to 500 LD/kg bw, goats given 250 to 500 LD/kg bw died at 42–46 h post inoc-ulation; goats given 31.3, 65 or 125 LD/kg bw developed subacute disease, and one goat receiving 15.6 LD/kg bw developed a chronic form of the disease, indicating that the clinical response in goats is dose dependent (Santos *et al.*, 1993, Riet-Correa *et al.*, 2012). In another experiment, the oral administration of type C toxin demonstrated a cumulative effect of the toxin (Fjolstad, 1973). In cattle, the clinical manifestation periods of botulism have been reported as hyperacute (with death in less than 24 h), acute (24–48 h), sub-acute (48 h to 7 days), and chronic (more than 7 days) (Dutra et al., 2005). In the present outbreak, data related to morbidity (8.26%) and case fatality (9.7%) were very similar to

those observed in botulism due to osteophagia in cattle in Brazil (morbidity of 3.2 ± 3.6% with a maximum of 12.5%, and case fatality of 100%) (Lisboa *et al.*, 1996 and Riet-Correa *et al.*, 2012). In outbreaks of botulism due to water or food contamination in ruminants, mortality is frequently higher than 30%, sometimes nearly 100% (Van der Lugt *et al.*, 1995; Dutra *et al.*, 2001, 2005; Lobato *et al.*, 2008).

It can be concluded that also in goats, outbreaks of botulism caused by water or feed-stuffs contamination can be devastating. In contrast, in this outbreak of botulism in grazing animals due to phospho-rus deficiency and osteophagia, the disease occurred over a more prolonged period of time and with lower mortality.

CONCLUSION AND RECOMMENDATIONS

Conclusion:

Dry season increases the rate of botulism in sheep. Due to deficient food animals tends to lick up the bones of carcasses in rangeland during the dry season and become infected. These bones are contaminated with botulinum toxin sheep get infected. The diagnosis depends on clinical signs and symptoms.

Recommendations

1. More research is needed to determine the extent of phosphorus deficiency in the region, and it is proba-bly necessary to vaccinate livestock against types C and D botulism.
2. More care should be done to compensate the deficiency in food supplements
3. Vaccination should be regular and periodically done.

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Appendicies

APPENDIX (1)

جامعة السودان للعلوم والتكنلوجيا

كلية الدراسات العليا

أستبيان

دراسة امراضية متلازمة أبو رقيبة في الضان ولاية نهر النيل

السيد:.....

المعلومات في هذا الإستبيان سوف تستخدم في دراسة امراضية متلازمة أبو رقيبة في الضان ولاية نهر النيل

.

هذه المعلومات سوف تستخدم بغرض الدراسة والبحث فقط.

أشكر لكم تعاونكم وإستجابتكم

الباحث: عبد الحليم جعفر محجوب

In the Name of Allah the Most Gracious the Most Merciful

Questionnaire

Special Data on Research Examination of Abu Regaiba Disease

Date: ……..Owner: …….

Patient name and / or Tag Number:

Date of Birth:

Species:……Breed:……Age:……..Weight:……..

Other Characters:

Duration of Present problem (days):

Presenting complain:

Feed:……Feed in take:……..

Nutritional Status:

Vaccination:……Housing:……

Previous illness…

Epidemiology data, Morbidity…….Mortality……Risk…… and other data

General body condition

Lateral body shape…

Posterior body shape

Gait

Skin

Eyes……Ears…….

Nose……Mouse/ Tongue……..

Lymph nodes

Sclera and Vessel

Nervous system

Vascular System: Veins……..Jugular vein…….

Respiratory System…….

i) Breathing sounds……..
ii) Percussion of chest……

Rumen exam: i) Contents…….

ii) Other observation……

Digestive System…

Duration of Pregnancy……..

Offspring birth……

Faeces…….Urine……

Environmental

Other remarks:

Other Observations ……

…….

……..

Note: If possible attach Photo of PatientViews of Environmetal closed to the disease, also other related documents available should be attached be attached here

Date:…….

Signature:……..

APPENDIX (2)

Different symptoms of Abu Regaiba syndrome

Cattle showing twisted neck