A study of some biochemical variables for different stages of pregnancy and lactation in cows

N. K. Zidan
College of Veterinary Medicine/ University of Fallujah

Abstract

The present study was undertaken to evaluate the effects of change in stage of pregnancy and lactation on blood metabolites and ions in cows. The animals (n = 38) were divided into three groups: the first group included lactating cows (n=12); the second group included pregnant cows (n =11); the third group included control cows (not pregnant and not lactating) (n=15). Serum samples were obtained and analyzed for glucose, urea, triglyceride and cholesterol and measured electrolytes were sodium, potassium, calcium and magnesium. Serum Ca^{++} and Mg^{++} concentration were determined by using commercial kits with spectrophotometer. Sodium (Na^{+}) and potassium (K^{+}) values were obtained with the use of a flame photometer. The results presented showed the least of glucose level in lactating and pregnant cows compared with control cows. Cholesterol level recorded higher level in pregnant and intermediary in lactating cows, while triglyceride level has Suffered a decline lower than control stage. The urea concentration did not differ significantly between the three stages. Serum calcium was lower in lactating and pregnant cows compared with control cow. The magnesium level did not differ significantly between the three stages. Sodium level recorded a highly level in lactating and intermediary in pregnant, the level of Na^{+} higher than the normal value. In contrast Na^{+}, potassium level was least than normal value in lactating and pregnant stages. The results which confirmed in this paper show that the blood serum biochemical parameters considered in this paper were affected by the different stages of cows.

Key words: biochemical variables, pregnancy, lactation, cows.
Email: wissamatea@yahoo.com

دراسة بعض المتغيرات الكيموحيوية لمراحل مختلفة من الحمل والرضاعة في الأبقار

نور خالد زيدان
كلية الطب البيطري/ جامعة الفلوجة

الخلاصة

أجريت الدراسة الحالية لتقييم تأثير التغير في مجاميع مختلفة من الأبقار الحوامل والحلابة على العمليات الأيضية للدم وأيونات المختلفة في الدم. تم تقسيم الحيوانات (ن = 38) إلى ثلاث مجاميع: المجموعة الأولى تضمنت الأبقار الحلابة (ن = 12)، واشتملت المجموعة الثانية الأبقار الحوامل (ن = 11) والثالثة مجموعة السيطرة (غير الحوامل وغير حلابة) (ن = 15). تم الحصول على عينات من مصل الدم وتحليلها لقياس سكر الدم والكولسترول ودهن ثلاثي واليوريا، كما تم حساب تركيز الالكتروليتات المختلفة (الصوديوم والبوتاسيوم والكالسيوم والمغنسيوم). بينت النتائج انخفاض مستوى السكر في الأبقار الحلاب والحوامل مقارنة مع مجموعة السيطرة. سجلت مستويات الكولستيرول ارتفاع في الأبقار الحوامل ومستوى متوسط في الحلابات، بينما سجل مستوى الدهون الثلاثية انخفاض في المجاميع المدروسة مقارنة مع مجموعة السيطرة. لم يظهر تركيز اليوريا أي اختلاف واضح في المراحل المختلفة. انخفض مستوى الكالسيوم في مجموعتي الحوامل والحلابة مقارنه مع مجموعة السيطرة، لم تظهر مستويات المغنسيوم أي اختلاف في المجاميع الثلاثة المختلفة. سجلت مستويات

الصوديوم أعلى تركيز في الأبقار الحلابة وتركيز متوسط في الحوامل، كان مستوى الصوديوم في هذه المجاميع أعلى من المستوى الطبيعي. على عكس الصوديوم انخفض تركيز البوتاسيوم اقل من القيم الطبيعية في مجموعتي الحوامل والحلابة. بينت نتائج البحث أن المتغيرات الكيموحيوية للدم قد تغيرت في اغلب المجاميع المدروسة.

الكلمات المفتاحية: المتغيرات الكيموحيوية، الحمل، الرضاعة، الأبقار.

Introduction

Hematological and biochemical variables are most widely used medical decision making tool. Hematological and biochemical analyses of blood are very useful to get an insight in metabolic and health status of animal (1). There are numerous studies on the effects of different phases of the reproduction cycle on biochemical parameters in domestic animal species. In sheep and cow they were carried out, among others, in relation to oestrus cycle, pregnancy and lactation (2, 3). Pregnancy is one of the physiological conditions leading to remarkable and dramatic change in biochemical variables in all animal species. Preparation dairy cows are at high risk of metabolic and reproductive disorders and oxidative stress is considered to be involved in these events(4). Pregnancy and lactation are physiological statuses considered to modify metabolism in animals (5), during pregnancy the concentration of number of blood constituent are significantly altered in cattle (6). Blood biochemical parameters including glucose, triglycerides, cholesterol, urea and electrolytes are important indicators of the metabolic activity in pregnant and lactating animals (7). Early lactation in dairy cows resulted in negative energy balance, high mobilization of lipids from bodily fat reserves as well as hypoglycemia (8). During lactation, electrolytes Na^+, K^+, Cl^- and Ca^{++} are lost in milk, which puts an extra burden upon mechanisms regulating electrolyte balance. From data available on goats, it can be calculated that the amount of Na^+ secreted via milk is equal to that lost in urine (9). The sodium is the most important cation in extracellular fluid, where it is responsible for maintenance of osmotic pressure. Together with chlorine (Cl) collaborates in metabolism of water and regulation of acid-base balance in the organism (10). In ruminants the potassium is absorbed from rumen and small intestine and excreted over the kidney and with feces. The majority of calcium (99%) in organism is stored in bones and teeth. Calcium is important for activation of numerous enzymes and hormones (11), therefore; this study was aimed at examine biochemical parameters in healthy cows during different period of pregnancy and lactation.

Materials and Methods

The study was carried out In total 38 cows, they belong to different physiological stages of which (12 cows) were lactating, (11 cows) were pregnant and (15 cows) were control (not pregnant and not lactating). The cows varied in age from 3 to 7 years. Collection of Samples: Blood samples (10 ml) were collected aseptically by jugular vein puncture using plastic disposable syringes. 2 ml of blood was kept in a tube containing EDTA (Ethelyne Diamine Tetra Acetic acid) as an anticoagulant and after centrifugation, using for biochemical parameter. The rest of the blood sample was left without anticoagulant then centrifuged and used in biochemical test (electrolytes), samples were harvested and immediately frozen at -20°C for subsequent analysis. Biochemical parameter: The plasma glucose concentration was determined by the enzymatic method using a kit (Randox Laboratories-London). Serum urea concentration was determined using a kit (SPINREACT, S.A. Spain). Serum cholesterol level was determined using a kit (SPINREACT, S.A. Spain). Serum triglyceride concentration was determined using the enzymatic method (Liner Chemical). Serum Ca and Mg concentration were determined by using commercial kits with spectrophotometer (12). Sodium (Na^+) and potassium (K^+) values were obtained with the use of a flame photometer (13).

Results and Discussion

The knowledge about normal values of biochemical variables in blood serum and other physiological variables is important for assessment of damage of organs and tissues in different diseases and for assessment of development from the welfare aspect (14). The biochemical and hematological parameter of the experimental cows had a profound influence on their blood profile as seen from table (1). The lactating cows recorded the least glucose level of (43.6±2.5 mg/ dl), pregnant cows recorded (53.2±1.7 mg/ dl) compared with control cows recorded the level of glucose as (76.6±3.4 mg/dl) of blood. These results similar with study in (15) that declare adaptation of glucose metabolism in early lactation leads to increased gluconeogenesis in the liver to direct glucose into the mammary gland for lactose synthesis. Other study showed the hypoglycemia are more common obvious biochemical features of pregnancy because during pregnancy, fetuses have a large glucose demand that is satisfied by the mother. If the fetal demand and the mother supply become imbalanced due to fasting of the mother or the increased nutritional demands of the rapidly developing fetal placental unit, females suffer from negative energy balance and resulting in severe hypoglycemia (16). The physiological state of the animal such as parturition, pregnancy and lactation had a profound influence on total plasma cholesterol levels (17). Such was the case with the experimental cows with highly significant difference between the stages such as control, lactating and pregnant. The cholesterol level was highest as (222.7 ± 8.46 mg/dl) in pregnant stage and in lactating intermediary as (179.17 ± 3.66 mg/ dl), while in the cows were control the cholesterol level was least as (167.91 ± 6.46 mg/dl). Triglyceride levels were lower during the lactating stage as (25.2±4.3 mg/dl) and intermediary in pregnant cows as (31.4±6.3 mg/dl) compared with control cows as (39.4±5.7 mg/dl). It was in close agreement with study declare that lipid mobilisation characterized by highly concentrated free fatty acids in blood starts in a high degree of pregnancy, reaching its maximum in early lactation. Free fatty acids are reesterified and accumulated in the form of triacylglycerols in the liver. As a result, lipid mobilisation intense ketogenosis and lipogenesis in the liver and consequently lower concentrations of glucose (18). The urea concentration did not differ significantly between the three stages, it recorded level as (38.5±7.2 mg/dl), the concentration of urea in blood depends from nutrition, diagnostically is important also at diseases of kidneys (19). The results presented in tables (1) show that the blood serum biochemical parameters considered in this report were affected by the different stages of cows.

Table (1) the parameter in different stages (groups)

Blood constituents	Physiological stages		
	Lactating (12)	pregnant (11)	Control (15)
Glucose mg/dl	43.6±2.5	53.2± 1.7	76.6±3.4
cholesterol mg/dl	179.17± 3.66	222.7± 8.48	167.9±6.46
Triglyseried mg/dl	25.2± 4.3	31.4± 6.3	39.4±5.7
Urea mg/dl	38.4±7.1	38.6±6.9	38.5±7.2
Ca^{++} mg/dl	7.33± 0.73	7.6± 0.83	8.28±0.59
Mg^{++} mg/dl	1.99±0.02	1.91±0.03	1.98±0.02
Na^{+} mmol/l	189±9.9	148.5±8.4	136± 0.04
K^{+} mmol/l	3.28±0.17	3.55±0.52	3.75± 0.15

The result of the analysis of Ca^{++}, Mg^{++}, Na^{+} and K^{+} are shown in table (1). Serum calcium was lower in lactating cows than in pregnant cows, in both stages the level of calcium is less from control group. This result was similar with study (17) that declare Serum calcium was higher in late pregnant cows than in early lactating cows. The decrease in Ca^{++} level in pregnant may also be associated with aemodilution which has been reported in cows (20). However, the study (21) reported increased Ca^{++} level

during late pregnancy in cows and attributed this to increase of intestinal absorption of Ca^{++} and bone resorption because of hormonal changes. The magnesium level did not differ significantly between the three stages. This result was similar with observation in (22) that observed the same level of Mg^{++} in different stages in cow. Sodium and potassium concentrations in serum also differed between control, lactating and pregnant cows at a highly level in lactating and intermediary in pregnant, this level of Na^{+} higher than the normal value. In contrast Sodium and potassium level was least than normal value in lactating and pregnant stages. Hypokalemia may result from depletion of body K^{+} store or from loss of K^{+} in large quantities to milk (23).

Conclusions:

1. Serum calcium was lower in lactating cows than in pregnant cows, in both stages the level of calcium is less from control group.
2. Sodium and potassium concentrations in serum differed between control, lactating and pregnant cows at a highly level in lactating and intermediary in pregnant, this level of Na^{+} higher than the normal value.
3. Hypokalemia may result from depletion of body K^{+} store or from loss of K^{+} in large quantities to milk.
4. The physiological state of the animal such as parturition, pregnancy and lactation had a profound influence on total plasma cholesterol levels.

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Serological survey of Brucellosis in some areas of Baghdad city

S. S. Salman, I. A. Al-Samarrae and A. K. Mahmood
College of Veterinary Medicine/ University of Baghdad

Abstract

A serological survey for brucellosis was conducted in some farm animals, farmers and veterinarians in Baghdad city during the period from October, 2012 to April, 2013. A total of 140 serum samples were taken from farm animals 24 cows, 14 sheep and 102 goats and 26 human serum samples were randomly collected from different ages and sexes in different area in Baghdad (Abo-Graib, Al-Radwania, Al-Gehad and Al-yosefia). Serological tests (Rose Bengal and tube agglutination test) were done on these serum samples and the prevalence in farm animals was 30% (12.5% in cattle, 28.57% in sheep and 34.31% in goats) by rose Bengal test and 19.28% (8.33% in cattle, 21.42% in sheep and 21.56% in goats) by tube agglutination test. The prevalence of brucellosis in human was 26.92% by rose Bengal test and 19.33% by tube agglutination test. The highest titers of antibodies were recorded between 1/80-1/640 in goats, while in human the titers were between 1/160-1/320. The high prevalence of brucellosis in human and animals indicates that the disease is endemic in this area and control programs should be implemented to reduce or eradicate brucellosis.

Keywords: **Survey, Brucellosis, Farmers, RBPT.**
Email: Bababoyak2@yahoo.com

مسح مصلي لداء البروسيلات في بعض مناطق بغداد

سفيان صالح سلمان، إكرام عباس السامرائي وعلاء كامل محمود
كلية الطب البيطري/ جامعة بغداد

الخلاصة

اجري مسح مصلي لداء البروسيلات في بعض الحيوانات والمزارعين والأطباء البيطريين في مدينة بغداد خلال الفترة من تشرين الثاني 2012 وحتى نيسان 2013. جمعت بشكل عشوائي 140 عينة مصل من حيوانات المزرعة 24 أبقار و14 أغنام و102 ماعز و26 عينة مصل من أناس وبأعمار وأجناس مختلفة وفي مناطق مختلفة من بغداد (أبو غريب والرضوانية والجهاد واليوسفية). أجريت الاختبارات المصلية (اختبار الروزبنجال واختبار التلازن الأنبوبي) على هذه العينات وكانت نسبة الإصابة بالحيوانات اعتمادا على اختبار الروزبنجال هي 30% (12.5% في الأبقار و28.57% في الأغنام و34.31% في الماعز) أما باختبار التلازن الأنبوبي فكانت نسبة الإصابة 19.28% (8.33% في الأبقار و21.42% في الأغنام و21.56% في الماعز). كانت نسبة الإصابة في الإنسان 26.92% باختبار الروزبنجال و19.33% باختبار التلازن الأنبوبي. كانت النسب العالية للمعيار الحجمي للاجسام المضادة بالماعز وتراوحت بين 80/1 و640/1 أما في الإنسان فكان المعيار الحجمي للأجسام المضادة يتراوح بين 160/1 و320/1. ان النسبة العالية للإصابة بداء البروسيلات في الإنسان والحيوانات تشير إلى ان المرض مستوطن في هذه المناطق ويجب تطبيق برامج السيطرة للحد من انتشار هذا المرض.

الكلمات المفتاحية: مسح، داء البروسيلات، مزارعيين، اختبار الروزبنجال.

Introduction

Brucellosis is an important zoonotic and endemic disease in human and various animal species, although several control and eradication programs have been established, the disease continues to produce a large economic losses especially in cattle and small ruminants (1, 2). The major economic importance of brucellosis includes loss of production, abortion, preventive programs and restriction in internal trade in animals and their products (3). Brucellosis is prevalent in some middle-eastern countries such as Iran, Iraq, Saudi Arabia, Egypt and Syria (4). Farmers, veterinarians and others involved in animal handling are at a higher risk of direct infection and individuals who ingest unpasteurized dairy products especially from area of endemic infection are at risk of food-borne brucellosis (5). Diagnosis of clinical brucellosis in humans and animals is made by the use of an appropriate serological tests such as Rose Bengal plate test (RBPT), tube agglutination test (TAT), ELIZA test, coombs test and complement fixation test (CFT) (6, 7). In this study, we aimed at determining the seroprevalence of brucellosis in some areas of Baghdad city due to the importance of this disease in both human and farm animals.

Materials and Methods

A total of 140 blood samples were taken from farm animals 24 cows, 14 sheep and 102 goats and 26 human blood samples from farmers and veterinarians. All samples were randomly collected from animals of different ages and sexes in different area in Baghdad city (Abo-Graib, Al-Radwania, Al-gehad and Al-yosefia) during the period from October, 2012 to April, 2013. Serum then separated from each blood sample and kept at -20c until serological test were performed. Rose Bengal plate test (RBPT) was done on all serum samples according to (8) by using antigen prepared from *B. abortus* (Omega diagnostic company). All positive serum samples to RBPT were tested with tube agglutination test (TAT) according to (9) by using antigen supplied by (Snbiotic Corporation, France).

Results

Prevalence of brucellosis in farm animals was 30% by RBPT 12.5% in cattle, 28.57% in sheep and 34.31% in goats and it was 19.28% by TAT 8.33% in cattle, 21.42% in sheep and 21.56% in goats as in table (1).

Table (1) Prevalence of brucellosis in farm animals by RBPT and TAT

Animal species	Total no. of animals	RBPT	TAT
Cattle	24	3 12.5%	2 8.33%
Sheep	14	4 28.57%	3 21.42%
Goat	102	35 34.31%	22 21.56%
Total	140	42 30%	27 19.28%

There was a fluctuation in antibodies titers recorded in farm animals and humans by using TAT and a high titers were found in cattle between 1/160 to 1/320, in sheep between 1/320 to 1/640 while in goats was the highest titers between1/80 to 1/640, but in human the titers were 1/160-1/320 Table (2).

Table (2) Titers of brucella antibodies in farm animals and human by TAT

Species	No. of serum samples	No. of positive	Titers				
			1/40	1/80	1/160	1/320	1/640
Cattle	24	2	-	-	1	1	-
Sheep	14	3	-	1	-	1	1
Goat	102	22	3	4	6	4	5
Human	26	5	-	-	4	1	-

The prevalence of brucellosis in human by RBPT was 26. 92% while it was 19.23% by TAT as in table (3).

Table (3) Prevalence of brucellosis in human by RBPT and TAT

No. of samples examined	Tests	No. of positive samples	Percentage%
26	RBPT	7	26.92%
	TAT	5	19.23%

Discussion

In general, the susceptibility to brucellosis depends on various factors such as immune status, routes of infection, size of the inoculums and the species of brucella (8). Also there is a positive association among population density, grazing, strategy and disease prevalence (10). In addition, the seroprevelance of brucellosis is characterized by considerable geographical variability, these factors may reflex the variations in the prevalence between animals and also in humans between our results and results recorded by (11) 7.9% and (12) 23.3% in Baghdad province, also (13) who referred that the infection in rams was higher than ewes 12.1%, 11.7% respectively, while (14) mentioned that morbidity rate in rams was 65,6%. On the other hand, (15) recorded 1, 4% morbidity rate in rams and 1.68% in ewes. Our results in sheep disagreed with (16) who recorded morbidity rate by RBPT 10.31%. This may be due to breeding age (17) or increasing animal exposure to the bacteria (10) Also, sexually mature animals are more prone to infection than sexually immature animals of both sexes due to sex hormones and erythritol present in males and in females allountic fluids stimulate the growth and multiplication of brucella organism and tend to increase concentration with age and sexual maturity (15). Due to the factors mentioned above our results disagree with (18) who found the seroprevelance rates in cattle, sheep and goats was 0.58%, 6.26% and 7.24% respectively and (15) who found morbidity rate 2.5% by RBPT in sheep and (19) who found that prevalence rate by RBPT in cattle, sheep and goats 1.8%, 3.5% and 2.4% respectively and (20) found brucella prevalence in cattle, sheep and goats were 5.3%, 7.6% and 15% respectively. Our results disagree with (21) in Iraq who found high morbidity rates in cattle by RBPT and TAT which was 54% and 32% respectively, Also disagreed with (16) who found that the infection rate in sheep by TAT was 49, 35% which was higher than our results and also disagreed with his findings about the antibodies titer, which was in his study between 1/40 to 1/160. The differences between results of both tests in this study may be due to that animals were in incubation period of disease or after incubation or during the chronic stage of the disease which the serum agglutinating tend to wane, Also IgG1 produced in some sera has the ability to block agglutination by other immunoglobulin, Particularly IgM, therefore IgG1 fail to agglutinate while IgM is far most efficient (22). The appearance of low antibodies titers may be due to decline antibodies level after recovery from the disease and the agglutination occurred due to residual immunoglobulin's especially IgG which persist for several months or for one year (23). Our results in humans agreed with (24) who found the prevalence of brucellosis in human in Iran was 19.1% and disagreed with (25) that indicated a 4,8% prevalence of brucellosis in Ethiopia, it also disagreed with (5) who found the prevalence of brucellosis in human in Turkey was 13.2%. The detection of a higher rate of sero-positive serum from farmers comparing with other studies indicates that exposure to brucellosis is more common and people involved in this study consume dairy products such as butter, white cheese and cream made of raw or insufficiently heated milk or through direct contact with infected animals and their aborted fetus and discharges. It's concluded that brucellosis is prevalent in human and farm animals in Baghdad city due to the lack of control and vaccination programs for animals and the consuming of contaminated animal products for humans, so preventive

and control programs should be implemented to protect animals and humans from brucellosis.

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Histopathological study in liver and spleen of mice infected with *Brucella melitensis*

S. M. Hamad*, A. Y. Abd-Al-Kareem* and N. Abdulla**
*Collage of Medicine\ University of Anbar
**Collage of Dentistry\ University of Anbar

Abstract

Brucellosis is a chronic infectious disease caused by *Brucella spp*.,a Gram-negative facultative intracellular pathogen that affects humans and animals, leading to significant impact on public health and animal industry. The mouse is the animal model more commonly used to study chronic infection caused by *Brucella*. This model is most frequently used to investigate specific pathogenic factors of *Brucella spp*. This work was done to study the histopathological in liver and spleen in mice infected with *Brucella melitensis*. A total of 20 female mice (8 week of age) a 10 mice control and 10 injected with 10^5cfu (colony forming unit) of *Brucella melitensis* by injection intraperitonial per animal. Samples (liver and spleen) were collected at 6 weeks period of infection and kept in 10% formalin study which revealed congestion, granulomatous , fibrosis in liver and increased number of lymphohistiocytic cells and increased amount of red pulp in spleen. This study concluded that the histopathological in liver and spleen caused by *Brucella melitensis* in mice are similar to those observed in humans with brucellosis, which indicated that the mice are suitable model for histopathological studies of human being diseases especially brucellosis.

Key word: *Brucella,* liver, spleen.

دراسة التغيرات النسيجية المرضية في كبد وطحال الفئران المصابة ببكتريا *Brucella militensis*

سميعة مجبل حمد*، عبير يوسف عبد الكريم* ونجم الدين عبد الله**
***كلية الطب/ جامعة الأنبار**
****كلية طب الأسنان/ جامعة الأنبار**

الخلاصة

مرض الحمى المالطية Brucellosis من الأمراض المزمنة والمشتركة بين الإنسان والحيوان التي تسببه بكتريا سالبة لصبغة كرام وهي *Brucella sp.*. تعتبر الفئران من اكثر الحيوانات المختبرية استخداما في هذا الحقل للبحث عن المسببات المرضية لهذه البكتريا. تناول البحث دراسة تأثير الإصابة ببكتريا البروسيلا من جنس*Brucella militensis* ودرجة ضراوتها على أنسجة كلا من الكبد والطحال في الفئران. تضمنت الدراسة استخدام 20 من الفئران الإناث بعمر ثمانية أسابيع، 10 منها استخدمت كمجموعة سيطرة و10 أخرى تمت معاملتها ببكتريا البروسيلا حيث تم حقن 10^5 من عزلة نقية للبروسيلا في البريتون. جمعت العينات بعد مرور ست أسابيع من الحقن بعدها شرحت الحيوانات وجمعت الأعضاء (الكبد والطحال) والاحتفاظ بها في 10٪ من الفورمالين لغرض الدراسة النسيجية. أظهرت النتائج وجود تغيرات واضحة في أنسجة كل من الكبد والطحال تمثلت بظهور احتقان وأورام حبيبية وتليف في الكبد مع زيادة عدد خلايا النسيج اللمفي وزيادة نسبة اللب الأحمر في الطحال مع تجمع للخلايا الالتهابية. وخلصت هذه الدراسة إلى أن التغيرات النسيجية في كبد وطحال الفئران المصابة ببكتريا *Brucella militensis* مماثلة لتلك التي لوحظت في البشر مع الحمى المالطية. وفي السنوات

الأخيرة الماضية قد استخدمت النماذج الحيوانية، وخاصة الفئران، على نطاق واسع للحصول على معلومات قيمه تتعلق بامراضية هذه البكتريا في الجسم الحي.

الكلمات المفتاحية: البروسلا، الكبد، الطحال.

Introduction

Brucellae are gram-negative coccobacilli that are considered facultative intracellular pathogens capable to survive and replicate in phagocytic and nonphagocytic cells, establishing a chronic infection in both humans and animals (1). Infection causes a chronic disease termed brucellosis, the most important worldwide zoonosis (2). The genus consists of several species, differing from each other on the basis of the specific host they invade. Bacteria that are pathogenic to a variety of livestock animals and humans. Many species of wild animals including mice are susceptible to brucellosis and may serve as natural reservoirs of brucellosis for domestic animals and human beings (3, 4). The potential role of wild rodents as *Brucella* reservoirs was also reported by many authors (5, 6). Relevant aspects of *Brucella* pathogenesis have been intensively investigated in both cellular and animal models. The mice are the animal model most extensively used to study chronic infection caused by *Brucella spp* (7). The *Brucella* organism's predilection for organs rich in reticuloendothelial cells (spleen, liver, bone marrow, lymph nodes) and its intracellular location are responsible for the chronicity of the disease, which can last for months or even years (8). The lymphatic system is important in mounting an immune response to foreign antigens in humans and animal models. The liver and spleen were produced a large amount of lymph, this organs overwhelming innate and adaptive immune cells, and it plays important roles in host defense against the invasion of exogenous pathogens. Some studies have indicated that the liver is a lymphoid organ and that the immune response may initiate in the liver (8, 9). These studies hypothesize that the direct or indirect priming of lymphocytes is facilitated in this organs by the potential contact between circulating lymphocytes and antigens displayed by antigen-presenting cells in the sinusoids. The disease is characterized by nonspecific symptoms, including undulant fever, weight loss, depression, hepatomegaly, and splenomegaly. Arthritis, spondylitis, osteomyelitis, epididymitis, and orchitis, as well as other more severe complications as neurobrucellosis, liver abscesses, and endocarditis (10). This paper discusses well-characterized murine models of brucellosis that have been used to study infection and disease caused by *Brucella melitensiss.*

Materials and Methods

- **Animals and History:** The present study was carried out on a total number of 20 female mice (8 weeks of age) obtained from laboratory animals were divided into two groups. Group A: 10 mice injected with *Brucella melitensis* and group B: 10 mice without injected as a control. Mice were kept in conventional animal facilities and received water and food at liberty.
- **Isolation and Identification of *Brucella*:** *Brucella* was isolated from sheep abortion case and identification for Brucella according to the technique recommended by Alton, *et al*. (11). Bacteria was first grown onto Brucella agar under appropriate condition and was used for subsequent experimental infection of mice. Briefly, from Brucella agar, single colony of bacteria was transferred into 10 mL of Brucella broth and incubated at 37C^{o} for 72h. The concentration of bacteria in the broth was adjusted to 0.5 McFarland turbidity standards and from which 1 mL, approximately containing 5×108 cfu was used to infect the mice intraperitonially by the methods described previously by Zerva, *et al* (12). In addition, 10 mice, injected with 1 mL

of Brucella broth, and used as a negative control group. Samples (liver and spleen) were collected over a 6 weeks period of infection and kept in 10% formalin for histopathological study.

- **Sample collection:** After 6 weeks following exposure, 5 mice (group A) and two mice (group B as a control), liver and spleen were prepared for histopathological examination.
- **Histopathological Examination:** specimens included liver and spleen were collected and fixed in 10% formalin solution then washed, dehydrated, embedded in paraffin, sectioned at 4-5 micron thickness and stained with hematoxyline and eosin as a routine work for histopathological studies according to Bancroft & Stevens (13).

Results

- **Histopathological changes:** *Brucella* infection in the mice, the spleen is the most heavily colonized organ, and it showed mild hyperplastic activation of the white pulp with the presence of abundant histocytic and plasma cells around the medullary cords of the red pulp (Fig.1). Active proliferation of reticulum cells was the characteristic picture in most cases. Epithelioid and giant cell microgranuloma was also detected surrounded by the rem of lymphocytes and there are some of fibroblast cells (Fig. 2). The liver is also an important site for colonization and replication of Brucella in the mouse. Usually, mice infected with *Brucella* have mild to moderate hepatitis, which is characterized by neutrophils infiltrate at early stages of infection (Fig. 3), followed by histocytic infiltrate with epithelioid cells and microgranuloma lesions at chronic stages of infection with bacteria localizing intracellular in macrophages within microgranuloma lesions (Fig. 4).

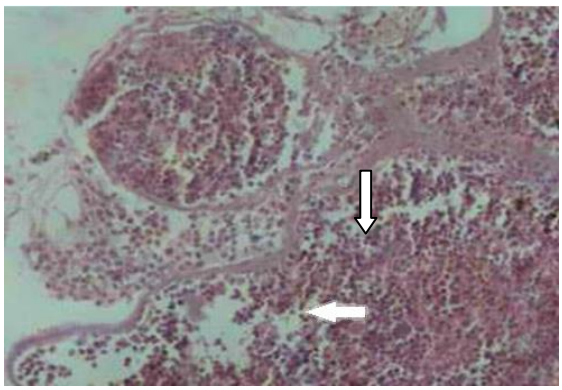

Fig (1) Spleen-histocytic and plasma cell around the medullary cord of the red pulp H & E. stain (15x)

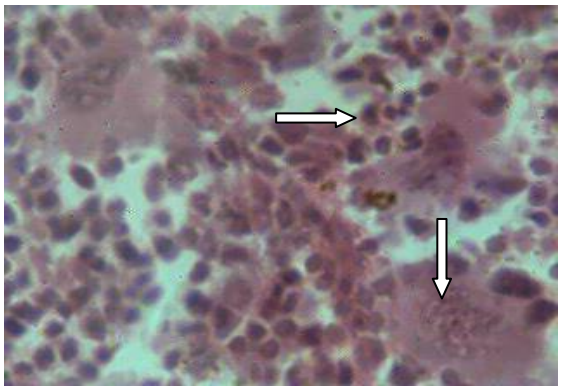

Fig (2) Spleen-Epithelioid and giant cell microgranuloma. H & E. stain (40X)

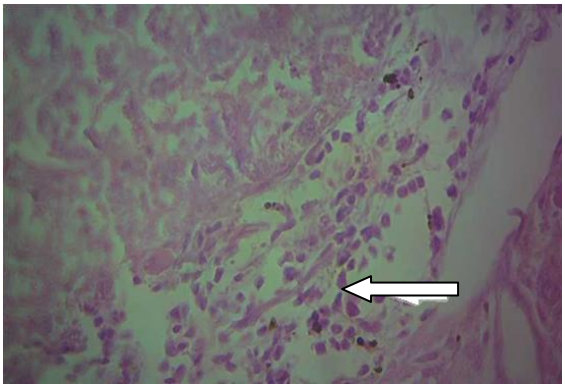

Fig (3) Liver- neutrophilic infiltration at early stages of infection. H & E. stain (40X)

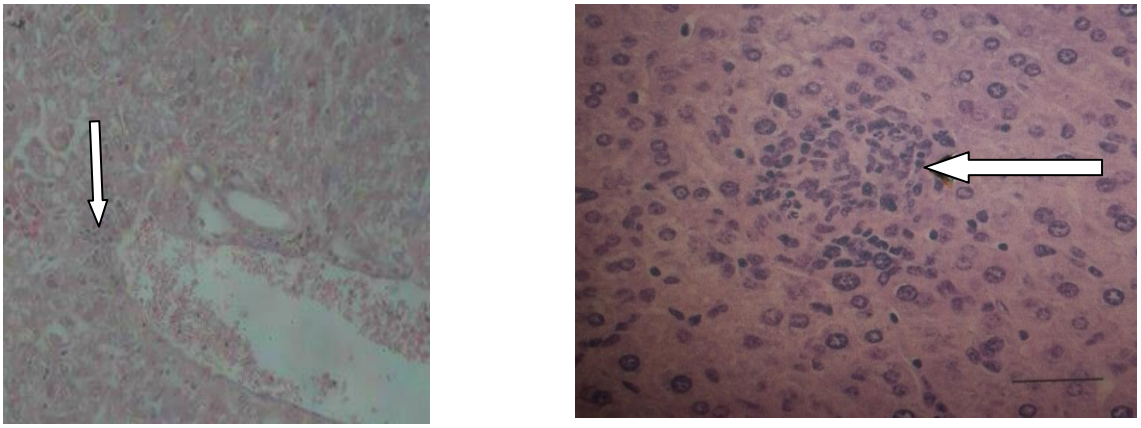

Fig (4) Liver- microgranuloma lesions. H&E. stain (40X)

Discussion

Brucella melitensis is the most invasive species and produces the most serious infection in human and animals (14). Analysis of the results reported here documents that intraperitonial exposure of mice to 105 cfu of *Brucella melitensis* leads to dose-related hepatic and splenic inflammation with persistence of bacteria in tissues of the mononuclear phagocyte system at least 6 weeks. During *Brucella melitensis* infection of the mice, the spleen is the most heavily colonized organ, and it develops hyperplastic activation of the white pulp with the presence of abundant histiocytes and plasma cells around the medullary cords of the red pulp (15, 16, 17) (Fig. 1). Mice intraperitoneally infected with *Brucella melitensis* develop significant proliferation of reticulum cells was the characteristic picture in most cases. Epithelioid and giant cell microgranuloma was also detected (17, 18) (Fig. 2). In another study conducted by our laboratory group, we reported that splenic white pulp to red pulp ratios increased in mice exposed intranasally to *Brucella melitensis*, similar findings in the study reported here require further characterization to ascertain the specific cell populations and subpopulations of the splenic white pulp responsible for the morphologic changes detected (19, 20). Also, investigation of quantitative changes in red and white pulp during the course of infection from onset to resolution may provide insights on the ability of *Brucella* to stimulate the host response (21). Histopathological examination revealed that hepatitis increased in severity with virulent strains of *Brucella melitensis* in mice. The liver is also an important site for colonization and replication of *Brucella melitensis* inside kuffers cells (16, 22, 23). which is characterized by neutrophilic infiltration at early stages of infection (Fig. 3), followed by histiocytic infiltration with epithelioid cells and microgranulomas at chronic stages of infection with bacteria localizing intracellularly in macrophages within microgranulomatous lesions (Fig. 4). The results reported here support this finding in that there was an apparent chronologic variation in lesions evident in the liver, some hepatic lesions were small and consisted of a few intrasinusoidal lymphocytes in contrast to other areas in which larger aggregates of lymphocytes and a mixture of histiocytes and neutrophils expanded and replaced hepatic architecture (15,17). This variation may reflect intermittent hepatic inoculation after periodic bacteremia. We were not surprised to see evidence of hepatitis because the liver is a documented target organ for humans and other animals with brucellosis. Hepatitis clinically manifested as an increase in activity of transaminases is evident in approximately half of the people with brucellosis, although substantial hepatic disease is evident in only a small percentage (24). It is noteworthy that *Brucella* infection in mice results in lesions that mimic those described in chronic infections in humans. Patients with chronic brucellosis may develop splenomegaly and hepatomegaly. Additionally,

multifocal granulomas with epithelioid macrophages are observed in the parenchyma of the liver and spleen in biopsy samples from infected patients (25, 26). However, hepatic and splenic abscess were described as uncommon complication in some patients during the acute phase of *Brucella sp*. infection (27). Brucella sp. chronic infection in humans may also lead to osteoarticular disease, including osteoarthritis, spondliytis, and osteomyelitis (1, 4). A previous study reported that mice may develop bacterial colonization in osteoarticular tissues during chronic stages of *Brucella melitensis* infection (28, 29). It is concluded that the histopathological changes in liver and spleen caused by *Brucella melitensis* in mice are similar to those observed in humans with brucellosis, the animal models, particularly the mice, have can be used and allowed for accumulation of valuable information of pathogenesis of *Brucella spp.* in vivo.

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Detection of *E.coli K99* and *Rota virus* antigens in diarrheic and healthy buffalo of Babil Province, Iraq.

A. M. R. Al-Mafraji
College of Veterinary Medicine/ University of Baghdad

Abstract

This study was conducted to detecte *E.coli K99* and *Rota virus* antigens in both diarrheic and healthy buffalo of Babil province (Iraq) by using ELISA kit.100 Fecal samples were collected from (12 calves aged 3-11 day, 13 calves aged 1-9 months and 75 adults at age 1-5 years) during November 2013-April 2014.Out of the 44 *E.coli* isolates which had growth on EMB agar, 7(58.3%), 5(38.4%) and 18(24%) isolates for calves aged 3-11 day and 1-9 months and adults at age 1-5 years respectively were found to be positive *E.coli K99* antigen. The percentage of infection with *rota virus* was 4(33.3%) in calves aged 3-11 day, 2(15.3%) in calves aged 1-9 months and 1 (1.3%) in 1-5 years. The results revealed a wide spread of *E.coli K99* and *rota virus* between calves and adults buffalo (diarrheic and healthy animals), in which buffalo considered a reservoirs and a potential source of more important zoonotic enteropathogens.

Key words: *E.coli K99, Rota virus,* ELISA, Buffalo, Babil.
Email: Sarmadmazda@yahoo.com

تثبيت مستضدات الاشيريشيا القولونية K99 وفايروس الدوار في الجاموس (المصاب بالإسهال والسليم ظاهريا) في محافظة بابل/ العراق

أماني محمد راضي المفرجي
كلية الطب البيطري/ جامعة بغداد

الخلاصة

أجريت هذه الدراسة لتثبيت مستضدات الاشيريشيا القولونية K99 وفايروس الدوار في براز الجاموس المصاب بالاسهال والسليم ظاهريا في محافظة بابل (العراق) باستخدام فحص الاليزا (المقايسة المناعية المرتبط بالانزيم الممتز). جمعت 100عينة براز من (12 عجل بعمر 11–3 يوم، 13 عجل بعمر 9–1 شهر و75 بالغ بعمر 5–1 سنة) خلال تشرين الثاني 2013 – نيسان 2014. من مجموع 44 عزلة من الاشيريشيا القولونية والتي نمت على أكار الايوزين مثيلين الأزرق، 7 (58.3%)، 5 (38.4%) و18 (24%) عزلة للعجول بعمر 11–3 يوم و9–1 شهر وبالغ بعمر 5–1 سنة على التوالي كانت موجبة للاشيريشيا القولونية k99. كانت نسبة الإصابة بفايروس الدوار 4 (33.3%) في العجول بعمر 11–3 يوم، 2 (15.3%) في العجول بعمر 9–1 شهر و1(1.3%) في 5–1 سنة. النتائج تشير إلى الانتشار الواسع للاشيريشيا القولونية k99 وفايروس الدوار بين الجاموس (العجول والبالغ) المصاب بالاسهال والسليم ظاهريا، لهذا يعتبر الجاموس خازن ومصدر فعال لاهم الممرضات المعوية المشتركة بين الإنسان والحيوان.

الكلمات المفتاحية: الاشيريشيا القولونية K99، فايروس الدوار، الاليزا، جاموس، بابل.

Introduction

Diarrhea is a major problem in live stock production throughout the world (1). Enteritis in newborn calves causes high morbidity and mortality leading to significant economic loses, the principal known etiological agents include bacteria, viruses, protozoa (2,3). Diarrhea due to *Esherichia coli* is one of the most common diseases of

young calves, *E.coli K99* is seen in calves greater than 3-5 days old (4). Verotoxic *E.coli* associated with human diseases can also be isolated from feces of healthy cattle and buffalo(5). *E.coli* population are divided in to serotypes and serogroups according to antigenic composition (Somatic or O antigens, flagellar or H antigens and capsular or K antigens (6). *Rota virus*, the member of family *Reoviridae*, is the most important cause of human and animal diarrhea (2), birds worldwide (7,8). The group A *rota virus* is the most recorded in the cases of diarrhea, the virus has been reported in buffalo of India (9) and in buffalo calves of many countries like Turkey (10) and Iraq (11). The main purpose of the present study was to detect *E.coli K99* and *rota virus* in feces of calves and adult buffalo (diarrheic and non diarrheic animals) of Babil governorate.

Material and Methods

- Animals: The population of this study consisted of 100 buffalo (25 buffalo calves aged between 3-11 days old and 1-9 months) as well as 75 adults buffalo of local breed at Babil governorate, Iraq as in (Table 1).
- Isolation and Identification of *E.coli K99*: Aloopful of fecal sample was inoculated on 5% sheep blood agar, MacConkey agar, Eosin Methylen blue (EMB) agar(Oxiod).After overnight incubation at 37 ºC, colonies of *E.coli* were subculture on TSI, SIM and incubated aerobically at 35-37 ºC for 24 hr. The results of reactions read and compared with charts according to (12).
- Serological test: *Rota virus* was detected in feces of buffalo by commericial Kit (Latex agglutination test, plasmatic Laboratory Products/ United Kingdom).

Sandwich ELISA Kit (Enzyme Linked Immunosorbent Assay) was performed to detect antigens of *rota virus* and *E.coli K99* in fecal samples of buffalo as described by the Kit (*E .coli F5* (*K99*) and *Rota virus* ELISA Kit) Code L 11413.

Table (1) No. of exanimated samples of buffalo of Babil

Age of animals	No. of exanimated samples	Fecal status	
		ND	D
3-11 days	12	4	8
1-9 months	13	9	4
1-5 years	75	55	20

Results

The percentage of isolation of *E.coli* was 83.3% of buffalo calves aged (3-11 days), 77% of buffalo calves aged (1-9 months) and 32% of adults animals as in Table (2). All the isolates of *E.coli* which were growth on EMB agar were analyzed by Sandwich ELISA Kit to confirmed detection of this pathogenic microorganism. *E.coli K99* was found in 58.3% of calves aged (3-11 days), While the percentages was 38.4% at age (1-9 months) and 24% in adults buffalo as in Table (3). Concerning *rota virus* results of present revealed that out of 100 (diarrheic and non diarrheic) fecal samples collected from buffalo, *rota virus* was detected by Latex agglutination test and confirmed by Sandwich ELISA Kit as in Table (4,5) , yielding a prevalence rate of 33.3% and 15.3% in 3-11 day old calves and 1-9 months calves respectively, while the difference was occurred only at aged 1-5 years between the 2 tests, where the rate of infection was 1.33% (1 sample) for ELISA and 2.66% (2 sample) for Latex agglutination test.

Table (2) No. of *E.coli* isolates grown on EMB agar

Age of animals	No. of exanimated samples	*No. of *E.coli* isolates growth on EMB
3-11 days	12	10 (83.3%)
1-9 months	13	10(77%)
1-5 years	75	24(32%)

***Differences were significant (Chi-square value 9.87 P=0.007)**

Table (3) positive results for *E .coli K99* by ELISA Kit

Age of animals	No. of exanimated samples	No. of samples positive for ELISA*	Fecal status	
			ND**	D***
3-11 days	12	7(58.3%)	2(20%)	8(80%)
1-9 months	13	5(38.4%)	4(40%)	6(60%)
1-5 years	75	18(24%)	10(41.6%)	14(58.3%)

***Differences were not significant (Chi-square value 4.42 P=0.11)**
****Differences were not significant (Chi-square value 2.109 P=0.348)**
*****Differences were significant (Chi-square value 10.27P=0.006)**

Table (4) positive results for *Rota virus* by Latex agglutination test

Age of animals	No. of exanimated samples	No. of samples positive for **rota virus*	Fecal status	
			ND **	D***
3-11 days	12	4 (33.3%)	O	4(100%)
1-9 months	13	2 (15.3%)	1 (50%)	1 (50%)
1-5 years	75	2(2.6%)	2(100%)	O

***Differences were significant (Chi-square value 13.17 P=0.001)**
****Differences were not significant (Chi-square value 0.821 P=0.365)**
*****Differences were not significant (Chi-square value 2.05 P=0.152)**

Table (5) positive results for *Rota virus* by ELISA Kit

Age of animals	No. of exanimated samples	No. of samples positive for **rota virus*	Fecal status	
			ND**	D***
3-11 days	12	4 (33.3%)	O	4(100%)
1-9 months	13	2 (15.3%)	1 (50%)	1 (50%)
1-5 years	75	1(1.3%)	1(100%)	O

***Differences were significant (Chi-square value 16.16 P=0.00001)**
****Differences were not significant (Chi-square value 1.97 P=0.16)**
*****Differences were significant (Chi-square value 16.89 P=0.00001)**

Discussion

Out of the 44 *E.coli* isolates which had growth on EMB agar, 7(58.3%), 5(38.4%) and 18(24%) isolates for calves aged 3-11 day and 1-9 months and adults at age 1-5 years respectively were found to be positive *E.coli K99* antigen. The prevalence rate of *E.coli K99* was 8(80%) of diarrheic and 2 (20%) of non diarrheic calves aged (3-11 day), the infection rate was 6(60%) of diarrheic and 4 (40%) of non diarrheic calves aged (1-9 months), these finding was do not agree with (13) in England who record ETEC in 7.51% of diarrheic calves, but not from clinically normal calves. (14) confirm that ETEC and other serogroups had the highest frequency in 1-7 day old calves in winter season and reported prevalence rate 28.41% for ETEC among diarrheic calves in Iran. (15) in Egypt recorded that infection rate of *E.coli K99* was 57.1% in calves aged 0-4 day while the rate was 20.8% in calves aged between (5-14 day, 15-21 day and > 21 day). In a study of (10) done for diarrheic calves in Turkey it is estimated that the prevalence of *E.coli K99* was 9.4%. In another study (16) performed among healthy diary cattle herd in Van in Turkey, out of 235 isolates (28 isolates were found to be positive for *K99*). (17) reported that *E.coli K99* was detected not only as 13.4% in the diarrheic calves but also as 5.6% in the healthy calves by ELISA technique. In a study of (18) recorded 4.95% prevalence rate among healthy water buffalo in India. Results of present study do not agree with (19) who suggesting that *E.coli K99* as the major cause of neonatal diarrhea occurring in the first 4 days of life; however, it rarely lead to diarrhea in older calves or adults animals. In a study of (20) done for diarrheic buffalo calves in Egypt, have recorded the most common *E.coli* serotypes in isolated samples were O26 (23.52%), O103(19.6%) and O119(17.64%). Percentage of infection with

rota virus was 4(33.3%) in calves aged 3-11 day, 2(15.3%) in calves aged 1-9 months and 1 (1.3%) in 1-5 years. The highest rate of infection with *rota virus* in present study was recorded in diarrheic calves at age (3-11 day and 1-9 months), this findings in agreement with (11) suggesting that bovine *rota virus* is the essential cause of neonatal calf diarrhea, on the other hand this findings is different from those reported in diarrheic calves in Turkey (10) and India (21) which recorded low percentage of infection 25% and 4.76% respectively, while the values confirmed in present study in adult buffalo (1-5 years) was lower than (9) who recorded 22.01% in buffalo of India. These differences in incidence rates between two pathogens (*E.coli* and *Rota virus*) among the studies may be attributed to different diagnostic methods used, farm management practices exercised in different regions and related to aging of calf and stress factors. The results obtained in this study indicate the wide spread of *rota virus* and *E.coli K99* between calves and adult buffalo of Babil (diarrheic and non diarrheic), so more studies are required in order to establish precisely the identity and prevalence of these zoonotic pathogens in Iraq, such studies will provide important epidemiological data about this microorganisms.

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Influence of thawing period on some post-thaw semen characteristics of Holstein bulls following catalase addition to Tris extender

T. A. AbdulKareem
Collage of Agriculture/ University of Baghdad

Abstract

This study was undertaken to investigate the influence of thawing period on some post-thaw semen characteristics of Holstein bulls following adding catalase to Tris extender. Five Holstein bulls of 2.5-3 years old were used in this experiment. Semen was collected via artificial vagina at once ejaculate per bull per week for seven weeks experimental period. Pooled semen was equally divided into two groups using Tris extender. Catalase (100 IU/ml) was added to Tris extender as compared with control group (Tris extender). Following one year cryopreservation period, straws were thawed at 37˚C and examined after different thawing time (15, 30, 60 and 120 minutes). Sperm individual motility, live sperm percentage and total sperm abnormality were investigated. Sperm individual motility were superior ($P\leq 0.04$-$P\leq 0.08$) in catalase as compared with the control group during all thawing time. However, 15 and 30 minutes exhibited the highest ($P\leq 0.002$) motility percentage in catalase group. Similarly, higher live sperm percentage was noticed in catalase as compared with control group. The 15 and 30 minutes being the better live percentage for both groups. In contrast, the differences between groups in sperm abnormality percentage lacked significance. The least abnormality percentage was observed following 15 minutes and the highest at 120 minutes thawing time for both groups. In conclusion, the addition of catalase to Tris extender led to improved post-thaw sperm individual motility and live percentage of Holstein bulls following 15 and 30 minutes thawing period. This will in turn enhance fertility rate of artificially-inseminated cows, and owner's economic income consequently.

تأثير وقت الإسالة على بعض خصائص السائل المنوي بعد الإسالة لدى ثيران الهولشتاين بعد إضافة الكاتليز إلى مخفف ترس

طلال أنور عبد الكريم
كلية الزراعة / جامعة بغداد

الخلاصة

أجريت هذه الدراسة لبيان تأثير وقت الإسالة على بعض خصائص السائل المنوي بعد الإسالة لدى ثيران الهولشتاين بعد إضافة إنزيم الكاتليز إلى مخفف ترس. استخدم في هذه التجربة خمسة ثيران هولشتاين بعمر 2.5−3 سنوات. تم جمع السائل المنوي بمعدل قذفة واحدة أسبوعياً لكل ثور ولمدة سبعة أسابيع. تم تجميع السائل المنوي للثيران جميعها وتقسيمه بالتساوي إلى مجموعتين باستخدام مخفف ترس. تم إضافة إنزيم الكاتليز (100 وحدة دولية/ مللتر) إلى مخفف ترس ومقارنتها مع مجموعة السيطرة (مخفف ترس لوحده). وبعد فترة حفظ بالتجميد لمدة سنة، تمت إسالة القصبات بدرجة حرارة 37 درجة مئوية وعلى مدد إسالة مختلفة (15، 30، 60 و120 دقيقة). تم دراسة كل النسبة المئوية للحركة الفردية للنطف والنطف الحية وكذلك النطف المشوهة. تفوقت الحركة الفردية للنطف ($P\leq 0.04$−$P\leq 0.08$) في المجموعة المضاف إليها الكاتليز مقارنة بمجموعة السيطرة ولجميع مدد الإسالة المدروسة، في الوقت الذي أظهرت فيه مدتا الإسالة 15 و30 دقيقة اعلى ($P\leq 0.002$) نسبة للحركة الفردية

للنطف لدى مجموعة الكاتليز. وبشكل مماثل، فقد أظهرت النتائج اعلى نسبة للنطف الحية لدى مجموعة الكاتليز مقارنةً بمجموعة السيطرة، مع تميز المدتان 15 و30 دقيقة بتحقيق افضل نسبة للنطف الحية لكلا المجموعتين. وعلى العكس من الصفتين المذكورتين أعلاه، انعدمت الفروق المعنوية بين المجموعتين في النسبة المئوية للنطف المشوهة. وقد بلغت اقل نسبة للتشوهات عند المدة 15 دقيقة وأعلاها لدى المدة 120 دقيقة ولكلا المجموعتين. يمكن الاستنتاج، بان إضافة إنزيم الكاتليز إلى مخفف ترس أدى إلى تحسن كل من النسبة المئوية للحركة الفردية والنطف الحية لثيران الهولشتاين بعد 15 و30 دقيقة من الإسالة. ان هذا سيؤدي بالتأكيد إلى تحسن نسبة الخصوبة لدى الأبقار الملقحة اصطناعياً بهذا السائل المنوي وبالتالي زيادة العائد الاقتصادي لمربي الأبقار.

Introduction

Bovine semen has been cryopreserved since more than a half century for artificial insemination and nowadays it is being widely used all over the world (1). It is well known that the cryopreservation procedure produced reactive oxygen species (ROS) (2). ROS induced lipid peroxidation (LPO) for sperm membrane, DNA damage and enzyme inactivation which reflected negatively on the decline of sperm motility, viability and fertilizing ability in bull (2, 3, 4). The continuous liberation of ROS from sperm metabolism, abnormal and immature sperms, as well as the output of freezing-thawing processes of semen which is often accompanied by a low concentration of antioxidants in sperm and seminal plasma, causing sperm oxidative stress (5). Moreover, sperm and seminal plasma have low endogenous antioxidants (6). Antioxidants in the semen includes enzymatic and non-enzymatic antioxidants like superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase as well as, vitamin C, E, A and glutathione (5, 6). Catalase is one of the enzymatic antioxidants defense existed in both sperm cytoplasm and seminal plasma, playing an important role in protection of sperm against ROS. Catalase removes or minimizes both intracellular and extracellular H_2O_2 to water and oxygen (7, 8). Adding of catalase to semen extender improved viability and decreased malondialdehyde (MDA) concentrations (9, 10). The post-thaw semen quality is thought to be affected by numerous factors in the cryopreservation procedure such as type of extender, glycerol concentration, packaging method, freezing rate and thawing period (11, 12). A practical thaw for bull spermatozoa, recommended by most AI organizations, is as 35-37˚C water bath for at least 30 seconds (13, 14, 15). It has been shown that an increase in post-thaw viability will result in increased fertility of the semen (16). Very limited trials have been carried out to investigate the influence of thawing period on post-thaw semen characteristics in Holstein (15) and buffalo (12) bulls. However, the interaction between different thawing periods and catalase addition to Tris extender and its effect of post-thaw semen attributes in Holstein bulls was not previously investigated. This prompted us to explore these effects currently.

Material and Methods

- **Animals and semen collection:** Five Holstein bulls of 2.5-3 years old with good quality semen characteristics (>70% forward individual motility and concentrations of at least 1.0×10^9 spermatozoa/ml) were selected to be the semen source. The bulls were clinically proven to be free from any general or genital diseases and were maintained at the Livestock Central Artificial Insemination Department pertaining to the Ministry of Agriculture (Baghdad, Iraq). Ejaculates were collected from the bulls using an artificial vagina at once a week. The ejaculates were pooled to increase the semen volume for replication and to eliminate variability among the evaluated samples.

- **Semen processing and groups:** Following one year cryopreservation period, straws were thawed at 37˚C and examined after different thawing time (15, 30, 60 and 120 minutes). Tris-based extender (24.2 g of Tris, 13.4 g of citric acid, 10 g of fructose, 19.2% v/v egg yolk, 64 ml glycerol and 1000 ml of distilled water at a pH of 6.8) was used experimental extender. The extender with pooled semen were divided into two parts. Catalase (100 IU/ml), Sigma-Aldrich, USA) was added as compared with control group (Tris extender). A drop of semen was placed on a pre-warmed microscope slide and was subjectively assessed at 37˚C for its percentage of individual motility (17). Live sperm percentage was estimated using eosin-nigrosin stain. Following smearing, 200 sperms were counted by 400 × microscope (18). Abnormal spermatozoa were identified following staining with eosin-nigrosine (19) using similar slide for live sperm determination. The percentage head (giant, narrow, pyriform, twin and detached), tail midpiece (Swollen, twin and proximal and distal protoplasmic droplets), tail principal and terminal (bent, coiled, and twin) as well as sperm abnormalities were determined (20).
- **Statistical analyses:** Statistical computations were performed using a general liner model (GLM) procedure in the SAS program (21) to investigate effects the thawing period and addition of catalase to Tris extender on some semen characteristics. The statistical model for analysis of variance was:

$Y_{ijk} = \mu + Ti + P_j + e_{ijk}$

Where:

Y_{ijk} = Dependent variable (individual motility, live sperm percentage and sperm abnormality percentage).

μ = Overall mean.

T_i = Effect of addition (Control and catalase).

P_j = Effect of thawing periods

e_{ijk} = Error term.

Differences among means were computed using the Duncan multiple range test (22)

Results

- **Sperm individual motility:** The catalase group exhibited higher ($P\leq 0.04$-$P\leq 0.002$) sperm individual motility percentage in comparison with the control group during the whole thawing periods (Table 1). Within catalase group, 15 (36.66 ± 4.41 %) and 30 (45.00 ± 5.00 %) minutes post-thawing periods recorded greater ($P \leq 0.002$) individual motility than other periods (Table 1). Concomitantly, higher individual motility was noticed at 15 minutes post-thawing period (18.33 ± 4.41%) as compared with its counterpart periods within control group (Table 1). Furthermore, lesser sperm motility was showed at 120 minutes post-thaw either in catalase (16.66± 1.66 %) or control (5.00 ± 0.00 %) groups (Table 1).

Table (1) Effect of different thawing periods on sperm individual motility percentage of Holstein bulls following adding catalase to Tris extender (Mean ± S.E.)

Thawing period (Minutes) / Groups	15	30	60	120	Level of Significance
Catalase	36.66 ± 4.41a A	45.00 ± 5.00a A	23.33 ± 1.66a B	16.66 ± 1.66a B	P≤ 0.002
Control	18.33 ± 4.41b A	13.33 ± 1.66b AB	11.66 ± 1.66b AB	5.00 ± 0.00b B	P≤ 0.03
Level of Significance	P≤ 0.04	P≤ 0.004	P≤ 0.008	P≤ 0.002	-

Means with capital superscripts within each row indicate comparison among thawing times and small superscripts within each column indicate comparison among groups within each time.

- **Live sperm percentage:** Greater (P<0.02- P<0.0001) live sperm percentage was observed in catalase group in comparison with control one during the whole thawing periods (Table 2). Within catalase group, the 15 and 30 minutes achieved higher (P≤0.05) live sperm percentage (71.63 ± 3.47 and 72.03 ± 6.30 % respectively), as compared with the 120 minutes thawing period (59.06 ± 0.43%) (Table 2). Similarly, the greatest (P≤0.08) live percentage was observed within the control group at 15 (56.33 ± 2.47 %) and 30 (53.33 ± 2.58%) minutes than 60 (47.20 ± 1.13%) and 120 (44.83 ± 0.66%) minutes thawing period (Table 2).

Table (2) Effect of different thawing time on live sperm percentage of Holstein bulls following adding catalase to Tris extender (Mean ± S.E.)

Thawing period (Minutes) / Groups	15	30	60	120	Level of Significance
Catalase	71.63 ± 3.471a A	72.03 ± 6.30a A	62.30 ± 1.13a AB	59.06 ± 0.43a B	P ≤ 0.05
Control	56.33 ± 2.47b A	53.33 ± 2.58b A	47.20 ± 1.13b B	44.83 ± 0.66b B	P ≤ 0.008
Level of Significance	P≤ 0.02	P≤ 0.05	P≤ 0.0007	P≤ 0.0001	

Means with capital superscripts within each row indicate comparison among thawing times and small superscripts within each column indicate comparison among groups within each time.

- **Sperm abnormality percentage:** The differences in sperm abnormality percentage between catalase and control groups during the whole thawing periods lacked significance, however, it tended to be numerically higher in catalase than control group (Table 3). Greater (P≤0.05) sperm abnormality percentage was noticed at 120 minutes thawing period (21.00 ± 2.08%) than those at 15 minutes (12.03 ± 2.27%) within catalase group (Table 3). Concomitantly, similar trend was observed within control group, being higher (P≤0.03) abnormality percentage at 60 (22.76 ± 0.89%) and 120 (24.50 ± 0.55%) minutes as compared with 15 minutes (18.76 ± 1.70%) (Table 3).

Table (3) Effect of different thawing time on sperm abnormality percentage of Holstein bulls following adding catalase to Tris extender (Mean ± S.E.)

Thawing time (Minutes) / Groups	15	30	60	120	Level of Significance
Catalase	12.03 ± 2.27a B	16.53 ± 2,64a AB	18.73 ± 2.08a AB	21.00 ± 2.08a A	P≤ 0.05
Control	18.76 ± 1.70a B	21.46 ± 0.99a AB	22.76 ± 0.89a A	24.50 ± 0.55a A	P≤ 0.03
Level of Significance	NS	NS	NS	NS	

Means with capital superscripts within each row indicate comparison among thawing times and small superscripts within each column indicate comparison among groups within each time. NS= Non-significant.

Discussion

High viability and motility of spermatozoa are important factors for successful artificial insemination, due to the pronounced correlation between post-thawing sperm viability and subsequent conception rate has been documented (23, 24). Motility is one of the most important factors in determining bull sperm because it obtains an indicative information about the sperm cell's energy sources (25). Higher sperm individual

motility of catalase group in comparison with control group is in line with those obtained by (26)(20.8 ± 2.9 vs. 11.6 ± 7.6%) and (10) (44.28 ± 2.76 vs. 21.43 ± 3.03%) who add 200 and 100 IU/ ml catalase to Tris extender in Holstein bulls respectively. These improvements may returns to the antioxidant role of catalase as the first cellular defense against ROS (27). One molecule of catalase has an ability to dissociate two million molecules of H_2O_2 per minute, in addition to its role as NADPH oxidase inhibitor, that may collectively reduce the superoxide O_2^- (28, 29). The catalase activity is dependent on NADPH activity within the sperm plasma membrane, in which the enzyme binds to protect itself from inactivation, consequently increasing its activity (30). On the other hand, these data are disagreed with those reported by Asadpour *et al* (2011) who did not found enhancement of sperm motility after addition of 100 and 200 IU/ml to Tris extender in Holstein bulls. Higher sperm individual motility after 15 and 30 minutes post-thawing period within catalase group explained that slow rate of thawing is beneficial tool to improve Holstein bulls frozen semen characteristics when added catalase to Tris extender. The sperm motility pattern reflects the biochemical environment and physical conditions imposed on bull spermatozoa. These results were contradict with those of (15) who demonstrated that thawing at 37˚C for 30 seconds yielded a higher sperm motility as compared with the other protocols in Holstein bulls. The current data were also disagreed with (12) who recorded recommended 70˚C for 6 seconds thawing rate for buffalo semen to harvest a good sperm motility. Decreasing sperm motility following 60 and 120 minutes may clarifying the negative influence of long thawing rate on sperm DNA integrity and sperm motility consequently (31).

Greater live sperm percentage in catalase as compared with control groups might reflects the role of catalase in the enzymatic metabolism of H_2O_2 and prevent the formation of OH, thus reducing the oxidative stress (32) or improved the plasma membrane integrity and thus increasing survival rate (10). These results were in accordance with those reported by (33), who yielded a good live sperm percentage (86.67 ± 4.41%) of bulls by adding 100 IU/ ml of catalase. The pronounced live sperm percentage following 15 and 30 minutes post-thawing periods currently in catalase group explained that these periods could be safely used to improve survival rate following cryopreservation in Holstein bulls when added catalase enzyme. Declining live sperm percentage after 60 and 120 minutes post-thawing period, explained that leaves straws for a long time may lead to pH fluctuations and consequently protein denaturation and cell death (34).

The lacked significance between catalase and control groups in sperm abnormality percentage confirmed the results of (10) who did not find an obvious effect of adding 100 IU/ml of catalase on total sperm abnormality percentage in Holstein bulls as compared with control group, three months post-cryopreservation (20.60 ± 1.05 vs. 21.65 ± 0.64%). The sperm abnormality percentage was reduced significantly after 15 and 30 minutes post-thawing period as compared with other periods, confirming that these thawing periods are appropriate to prevent sperm DNA damage (35) concomitantly with the protective antioxidant effect of catalase to maintain normal sperm morphology. The higher sperm abnormality percentage at 15 and 30 minutes post-thawing periods in control group (18.76-24.50%) may confirm the protective role of catalase from ROS.

In conclusion, the addition of 100 IU/ml of catalase to Tris extender has improved sperm motility and livability percentages in Holstein bulls following 15 and 30 minutes post-thawing periods.

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Prevalence study of gastrointestinal Nematodes in goats in Baghdad province-Iraq

S. M. K. Al-Dabagh[*],*A. M. A. Al-Amery and D. A. M. Al-Ani[**]
[*]Department of Parasitology- College of Veterinary Medicine\ University of Baghdad -Iraq
[**]Office of Agricultural Research\Ministry of Agriculture- Iraq

Abstract

This study carried out in order to investigation the prevalence of gastrointestinal nematodes in goats (*Capra hircus*) in Baghdad province during the period between October 2014 to March 2015.The results found four genera of GIT nematodes detected from 300 fecal samples of examined goats, which confirmed the highest prevalence rate with *Toxocara vitulorum* was 43.33%, followed by closed rate with *Haemonchus contortus* and *Strongyloides papillosus* which was recorded 22.66%,and the lowest prevalence rate was recorded with *Skrjabinema ovis* 12.66%, the two genera of *Toxocara vitulorum* and *Skrjabinema ovis* were recorded for the first time in Baghdad province. According to the age group, most species of GIT nematodes recorded the highest prevalence rate in age <1-6 months except *S. ovis* detected in age ≥ 3 years. Relation to the sex, the highest percentage for all species of nematodes infection was detected in males more than females without significance differences. According to the months of study, the maximum percentage for *H. contortus* was noticed in March 44% and the lowest in January was 4%, while the highest percentage rate for *S.papillosus* in February was 28% and the lowest in January was 16%, February had highest percentage rate 84% for *T. vituloum* compared to November reached to 24%, *S. ovis* had closed prevalence rate in October, December, January, March which was recorded 16% while the lowest infection in February was recorded 12%.

Key word: *Haemonchus contortus, Strongyloides papillosus, Toxocara vitulorum,* prevalence, goats
E-mail: sahed aldabagh@yahoo.com

دراسة انتشار ديدان المعدة والأمعاء الأسطوانية في الماعز في محافظة بغداد– العراق

سهد محمد كاظم الدباغ[*]،عامر مرحم عبد العامري[*] ودنيا عبد الملك العاني[**]
[*]فرع الطفيليات– كلية الطب البيطري/ جامعة بغداد– العراق
[**]دائرة البحوث الزراعية/ وزارة الزراعة–العراق

الخلاصة

أجريت هذه الدراسة من اجل التحري عن مدى انتشار ديدان المعدة والأمعاء في الماعز في محافظة بغداد ابتداءً من شهر تشرين الأول 2014 ولغاية شهر آذار2015. أظهرت النتائج 4 أجناس من أسطوانيات المعدة والأمعاء المشخصة من 300 عينة من براز الماعز، سجلت اعلى نسبة إصابة بديدان *Toxocara vitulorum* 43.33%، تلتها 22.66% لديدان *Haemonchus contortus, Strongyloides papillosus,* أما اقل نسبه انتشار لاسطوانيات المعدة والأمعاء هي *Skrjabinema ovis* 12.66% وسجلت الدراسة الجنسان *T.vitulorum, S.ovis* لأول مرة في محافظة بغداد. أظهرت الدراسة ان اغلب أجناس ديدان المعدة والأمعاء سجلت بنسبه عالية بعمر اقل من 6–1 اشهر عدا ديدان *S.ovis* سجلت بعمر اكثر من 3 سنة. اعتمادا على الجنس، أظهرت النتائج نسبة الإصابة للذكور اعلى من الإناث لجميع أنواع الاسطوانيات وبدون وجود فروق معنوية. سجلت إصابة *H. contortus* اعلى نسبة لشهر آذار 44% واقل نسبة في كانون الثاني 4%،أما

S.papillosus سجلت اعلى نسبه بشهر شباط 28% واقل نسبه بشهر كانون الثاني 16%.*T. vituloum* سجلت اعلى نسبة إصابة بشهر شباط 84% واقل نسبة خلال شهر تشرين الثاني 24%. أما *S.ovis* سجلت نسب إصابة متقاربة خلال اشهر تشرين الأول وكانون الأول وكانون الثاني وآذار 16% واقل نسبة إصابة خلال شهر شباط 12%.

Introduction

Goats are one of the earliest domesticated animals which have served human longer than sheep and cattle. It is reared for production milk, meat and hair especially in tropical and sub-tropical area (1).The population of goats in the world in 2004 was reached to over 743 million in developing countries, furthermore, goats harbor different species of GIT nematodes infection which effect on production and growth of animals. *H.contortus* known (barber–pole worm), blood suckling parasites present in abomasum of ruminants causes diarrhea, anemia, loss of weight, recumbency, odema, anorexia, death in chronic cases (2). *S.papillosus* is one of the destruction disease in ruminants cause severe disease in goats when slight infection take placed, it is skin penetrating nematodes, kids and lambs can be succumbed lactogenically by larvae invigorated from tissues of the dam during the time of pregnancy (3). It causes dehydration, cachexia, diarrhea, chewing movement with foaming at the mouth, anorexia, bruxism, and nervous signs like ataxia, anemia, wide-based stance, weak growth, loss of appetite, wandering, aimless, pushing the head against solid objects (4) the *T.vitulorum* is principle parasite of Asian *Bos* spp. and *Bubalus bubalis* but it has been recorded in sheep and goats also(5). It cause coughing, fever, paralysis, opisthotonos, conjunctivitis diarrhea (white scours), colic, constipation, dehydration, weight loss, butyrous odour during breath, anorexia, skin eczema(6). While *S.ovis* is small ruminant pin worm belong to the family of *Oxyuridae*, worldwide distribution in Africa, Kenya, Nigeria, Tchad, Reunion. The *Skrjabinema caprae* is recognize for goat but is similar in synonymous with *S. ovis*. The severity of infection with GIT parasites in goats could be due to susceptibility of goats to internal parasites because poor immunity compared to the other species of livestock (7).

Materials and Methods

A total 300 fecal samples from goats were collected randomly from three regions (Abu-Ghraib 96, Al-Tagi 96 and Al- Radwaniyah 108), in different age groups ranged from one to six months, six to twelve months, one to three years and more than three years, also for both sexes (72 males - 228 females) during the period from the beginning of October 2014 to end of March 2015. Fecal samples were collected directly from the rectum, put in a clean plastic container and were tightly closed. All information included case history, age, sex, giving of treatments and date of sampling were reported, and the samples were transported to parasitology laboratory in College of Veterinary Medicine-University of Baghdad.

- Laboratory examination: fecal samples were subjected to macroscopic examination included color, consistency and odor.
- Microscopic examination included direct method, staining methods by using Lugol's Iodine stain and flotation methods by using saturated Sheather's solutions, prepared by dissolving 500 gram sugar in 320 ml distilled water and adding 6.5 gram phenol as preservative and saturated salt solutions, prepared by dissolving 454 gram of salt (NaCl) in 1140 of distilled water to identified eggs of gastro intestinal nematodes(8), also by use fecal culture, taken about 20 gram of feces placed into jar and broken up with spatula, kept sufficient moist and added sphagnum and mixed well, not close the cover completely and allow air enter to the jar, placed the culture in incubator at 25-27c^{o} for seven to ten days to make larvae reached to infective stage (larvae three), the fecal culture was turned every day to inhibit the growth of fungi or by

added sodium carbonate 1%, the culture exposed to light for one hour and filled the mixed culture with warm water 30 c^{o} and inverted into glass petri dish and waiting about 24 hours until the larvae moved towards moat, then by micropipette draw out the larvae into test tube put in centrifugation and taken the sediment, and examined under 10× and 40× by put Lugol's Iodine stain on the border of cover slide to kill the larvae to identification of third stage larvae of goat gastrointestinal nematodes (9). Ocular micrometer was used to measured eggs and larvae of GIT nematodes (8). Statistical analysis, the data were analyzed statistically by use chi square test (SPSS and version) also standard error by use t. test and Anova test (10).

Results

During the period of study, the results showed the highest percentage rate with *T.vitulorum* was 43.33% (Fig. 1) which was diagnosis for first time in Baghdad province, followed by closed rate with *H.contortus* and *S.papillosus* was 22.66% (Fig. 2, 3), the lowest prevalence rate of nematodes infection with *S. ovis* was 12.66% (Fig. 4), which was recorded for first time in Baghdad province. According to the age group, the age of < 1-6 months had highest rate of prevalence 40.38% for *H. contortus* compared with age ≥3 years had lowest prevalence rate of 8.69%. For *S. papillosus*, the highest rate of infection in age < 1-6 months was 36.53% while the lowest in age 6-12 months was 10.34%. The age of < 1-6 months had highest rate by *T. vitulorum* of 46.15% compared with age 6-12 months had lowest rate of 37.93%. For *S. ovis*, had highest percentage of infection in age ≥3 years of 17.39% and the lowest in age 1-3 years of 8.69%. All different species of GIT nematodes recorded significance differences $p \leq 0.05$ between age group (Table 1). Relation to the sex, the highest rate of prevalence for *H. contortus* in males was 30.55% while the lowest in females was 20.17%. For *S. papillosus* the highest percentage rate in males was 27.77% while the lowest in females was 21.05%. Males had highest infection rate of 55.55% by *T.vitulorum* in contrast with females was 39.47%. For *S. ovis*, males had highest infection rate 16. 66% while females had lowest rate 11.40%. All these different species of GIT nematodes recorded no significance differences between sexes (Table 2). According to the months of study, March had highest rate of infection with *H.contortus* of 44% compared to January had lowest prevalence rate of 4%. For *S.papillosus* percentage of infection was highest in February reached to 28% and lowest in January was 16%. For *T.vitulorum* the highest rate in February was 84% compared to November reached to 24%. For *S.ovis*, had closed prevalence rate in October, December, January, March of 16%, and no infection detected in November, while the lowest in February was 12%. All these differences for all spp. of GIT nematodes recorded significance differences between months $p \leq 0.05$ (Table 3).

Table (1) Total rate of prevalence with different gastrointestinal Nematodes according to the age groups

Age groups	No. of samples examined	No. of +ve.with *Haemonchus contortus* (%)	No. of +ve. with *Strongyloides papillosus*(%)	No. of +ve. with *Toxocara vitulorum* (%)	No. of +ve. with *Skrjabinema ovis* (%)
<1 – 6 months	104	42 (40.38)	38 (36.53)	48 (46.15)	14 (13.46)
6 – 12 months	58	12 (20.68)	6 (10.34)	22 (37.93)	8 (13.79)
1 – 3 years	92	10 (10.86)	12 (13.04)	40 (43.47)	8 (8.69)
≥3 years	46	4 (8.69)	12 (26.08)	20 (43.47)	8 (17.39)
Total	300	68 (22.66)	68 (22.66)	130 (43.33)	38 (12.66)
X^2=22.1	**Significance differences p≤ 0.05**				

Table (2) Total rate of prevalence with different gastrointestinal Nematodes according to the sex

Sex	No. of samples examined	No. of +ve. with *Haemonchus contortus* (%)	No. of +ve. with *Strongyloides papillosus*(%)	No. of +ve. with *Toxocara vitulorum* (%)	No. of +ve. with *Skrjabinema ovis* (%)
Males	72	22 (30.55)	20 (27.77)	40 (55.55)	12 (16.66)
Females	228	46 (20.17)	48 (21.05)	90 (39.47)	26 (11.40)
Total	300	68 (22.66)	68 (22.66)	130 (43.33)	38 (12.66)
X^2=3.95	**No Significance differences**				

Table (3) Total rate of prevalence with different gastrointestinal Nematodes according to the month

Months	No. of samples examined	No. of +ve with *Haemonchus contortus* (%)	No. of +ve with *Strongyloides papillosus*(%)	No. of +ve with *Toxocara vitulorum* (%)	No. of +ve with *Skrjabinema ovis* (%)
October	50	14 (28)	12 (24)	16 (32)	8 (16)
November	50	10 (20)	10 (20)	12 (24)	-
December	50	8 (16)	12 (24)	16 (32)	8 (16)
January	50	2 (4)	8 (16)	20 (40)	8 (16)
February	50	12 (24)	14 (28)	42 (84)	6 (12)
March	50	22 (44)	12 (24)	24 (48)	8 (16)
Total	300	68 (22.66)	68 (22.66)	130 (43.33)	38 (12.66)
Chi square=25.4	**significance differences p≤ 0.05**				

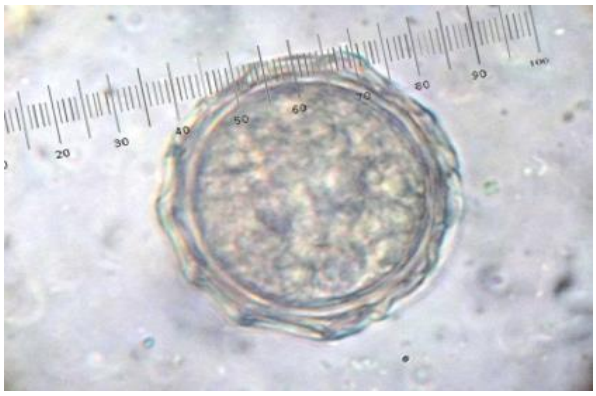
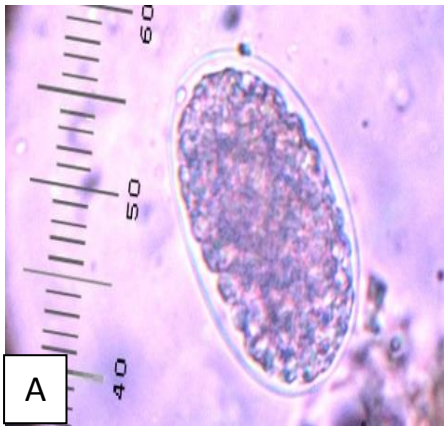
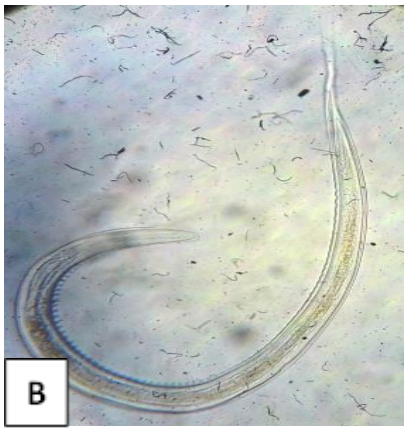


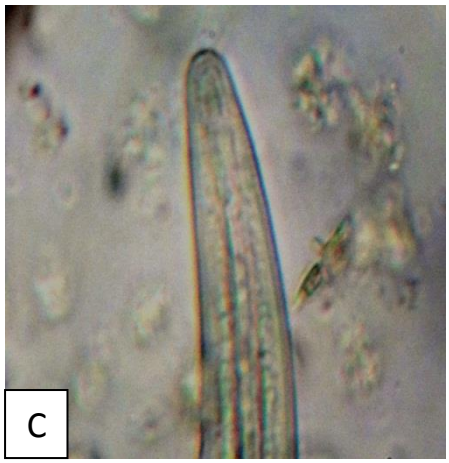
Fig. (1) Egg of *Toxocara vitulorum*, by Sheather's solution flotation method (40 x)



A-Egg of *H. contortus*, by salt solution flotation method (40x)



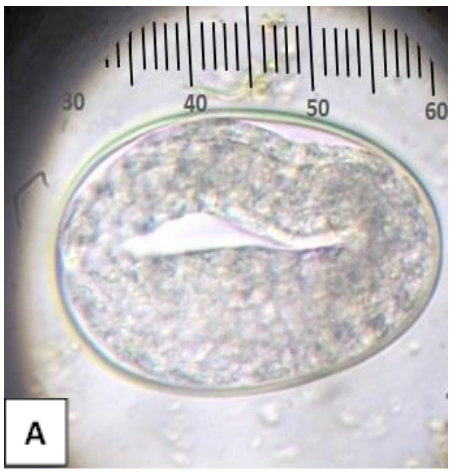
B-Total length of *H.contortus*, by fecal culture



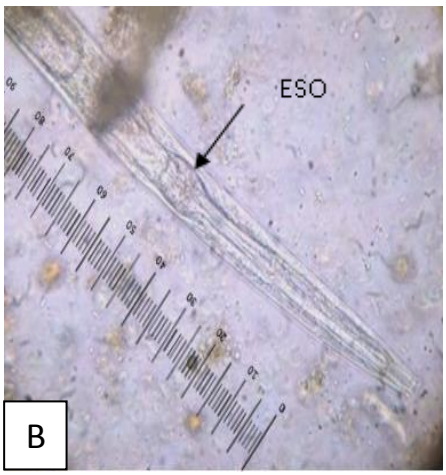
C- Anterior end of L3 of *H.contortus*

D- Posterior end of L3 *H.contortus* (kinked pointed tail)

Fig. (2) *Haemonchus contortus*



A- Egg contain larvae, by direct method



B- Anterior end of free living female, Rhapditiform esophagus (ESO)

C- Egg in the body of free living female **D- Posterior end of free living female**

Fig. (3) *Strongyloides papillosus* (40 x)

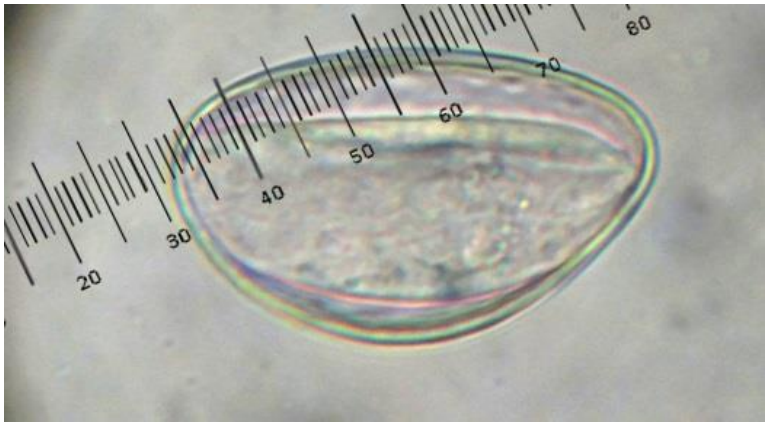


Fig. (4) Egg of *Skrjabinema ovis*, by Lugol's Iodine stain (40x)

Discussion

Current results detected that *T. vitulorum* had highest prevalence rate in infection with GIT nematodes, could be due to thick Albumin's shell of eggs which tend them resistant to various environmental condition and persistence of eggs for at least seven months and more than two years in other studies (6). Young animals take infection by milk ingestion from dams and adult worm present only in young ruminants, so the main transmission by trans mammary routes which play role in epidemiology of *T. vitulorum*, in addition of high biotic potential of females worm excrete 8000-100000 eggs daily per gram of feces (EPG) (11). The percentage rate for *T. vitulorum* was in a compatible with (12) in Egypt who carried out in order to investigation the percentage rate in cattle was 47.9% and disagree with (13, 14) whom detected of 2.4%,14%, respectively. Results were shown the rate of infection by *H. contortus* was 22.66% which in consonance with (13) in Mosul province, was revealed that percentage rate of *H. contortus* was 18.2% in sheep and (15) in Hilla city who recorded of 29.67% in goats, and disagree with (16) who noticed the percentage rate of 3.48% in goats and (17) who recorded the percentage rate was 2.3% in Egypt. Present results were lower than studies showed by (18, 19) whom detected in Baghdad the infection rate of *H.contortus* was 34.94%; 34.66% respectively in goats. For *S. papillosus* the infection rate 22.66% and it was in a harmony with (20) that recorded of 20.45% in goats and in contrary with (21) who found of 66.66% in goats. The rate of infection by *S.ovis* was in compatible with (20,22) whom reported the percentage rate was 11.6%,12% respectively and disagree with (17)

who reported the percentage rate was 1.1% in goats. These differences in the percentage rate for different species of GIT nematodes may be due to physiological factor of animals (lactation and pregnancy), immune system of host, taken of fecal matter and litter during wet seasons, age, breed, sex of host, mal nutrition effect on development of acquired immunity against GIT parasites, number of samples collection, overcrowding, illiteracy of keepers of host, short generation interval of most nematodes infections, and practice of measurement. Regarding to the age group of months, present results noticed the highest rate of *H. contortus* in age <1 – 6 months and lowest in age ≥3 years, many studies have been carried out the prevalence rate represented as our results found by (23) who reported the highest rate in age < 1 years of 80.48% and the lowest in age > 4 years of 23.80%. And disagree with (18, 19) whom recorded the highest percentage in adult 41.12% while kids had percentage of 23.14%, and 92.35% in age 1-2 years and 31.70% in age >2 years respectively. Lowest rate of prevalence in age ≥3 years result of when animals cross one years of age, the infection reduce due to phenomenon self-cure and resist to infection although permanent exposure to some level of infection is acquired to sustain their resistant status (24). Age <1-6 months had highest percentage for *S. papillosus* compared to age 6-12 months had lowest percentage rate, this was in line with (25), that recorded age 1-6 months of 71.5%,67.9% in age 7-18 months in Nigeria, and did not agree with (17) who confirm that adult goats had highest rate of 24.9% while in kids was 11.8%. There is known that *S. papillosus* transmission by trans mammary routes during period of parturition and lactation, therefore in age 6-12 months lowest prevalence was noticed may attributed due to separating of young animals in this age from their dams and acquired immunity. The *T. vitulorum*, the highest rate of infection occurred in age< 1-6 months in contrast with the lowest occurred in age 6-12 months, and this was in compatible with (17) in Egypt, that recorded the infection rate of 0.65% in kids,0.5 % in adults, and with (26) in Florida, who detected age< 3 months had highest rate was 17.6% compared to age 3-4 months and age 5-6 months was 0.4%, 0.9% respectively and not noticed eggs of *T.vitulorum* in age 7-9 months in calves. These finding disagree with (14), who revealed age 10-30 day had prevalence rate of 7.14% in goats while age 31-90 days was 85.71%, but at age 90-180 day was 7.14%. The highest rate in age <1-6 months may could due to larvae 3 of *T.vitulorum* transmission by colostrum milk to new born calves in 2-5 day after calving and evolution to new mature worm in intestine of calves after 10 days of age of host, then eggs passed in three weeks in feces of calves (27). At age≥3 years had highest rate of infection by *S. ovis* in contrast with age 1-3 years had lowest percentage and this in compatible with (17) that recorded highest rate of 1.5% in adults and 0.65% in kids and (20) in Sudan that explained that adult goats particularly bear high worm burden, and in contrary with (22) who carried out the highest rate in age 1-1.5 years and the lowest in age > 3 years in goats. The highest rate in age ≥3 years may be due to highly humidity and moderate temperature play role to subsequences development of life cycle of nematodes infection lead to pasture contamination around the years and presence of carrier animals in herd also open grazing lead to recycle infection and resistant animals to treatment (28). The lowest percentage in age 1-3 years in adult goats due to evolution of immune system and elimination of worm burden when they reach 12 months of age and resistant to reinfection (29). According to the sex, The highest rate of *H. contortus* in males was 30.55% compare to females was 20.17%, this was in agreement with (21), who carried out the highest rate in males was 75% and the lowest in females was 64.10% in goats without significance differences, and in contrary with (18, 19) whom detected the highest rate in females more than males.

The highest rate in males due to males more susceptible to infection than females and different in resistant level were significant after puberty only due to stimulation of estrogen effect on immune response against GIT nematodes while androgen suppress immune response (30). Present results showed highest rate for *S. papillosus* in males

was 27.77% in contrast with females was 21.05% and this was in line with (21), who explained the highest rate for *S.papillosus* in males was 66.66% in goats and 61.54% in females without significance differences, and also with (20) that recorded the highest worm burden in males more than females. For *T. vitulorum* infections, highest rate in males was established of 55.55% and the lowest in females 39.47%, and this in agree with (31) who confirm the highest rate of nematodes prevalence in males was 82.8% more than females was 75.3% without significance differences, and disagree with (32), who recorded highest rate in females was 80.43% while in males of 47.45% in buffalos. (33) was explained that females exposure to stress factor during pregnancy and parturition and lowered resistant due to in balance of hormones and insufficient diet against higher needs, suppression of immunity and Spring in apparent increase of number of worm by resumption of development of previously inhibited larvae and occurred of newly acquired larvae and failure of elimination of existing mature worm which lead to fecundity of eggs laying by adult females nematodes. For *S. ovis* infection, males had highest rate of 16.66% while in females was 11.40% and this was in accordance with (34), who detected the maximum rate in males was 57.69% while the lowest in females was 32.3%. All these variation in the percentage rate for different spp. of GIT nematodes according to the sex due to differences in geographical distribution, number of samples collection, age, breed, seasons of samples collection, health status of animals, grazing habits, nutritional status of host.

Regarding to the months of study, current results were shown the highest rate for *H. contortus* in March and lowest in January, which in compatible with (16, 18) whom confirm the highest percentage rate was 22.75%, 80.95% respectively in March. Low prevalence rate in January due to cold weather lead to inhibition L4 (hypobiosis), according to study of (35), who reported the late Autumn and beginning of Winter seasons when temperature low, L4 hypobiosis in abomasum of sheep, and least inhibition take place in warm rainy seasons and Spring. Current results disagree with (19), who detected the highest rate in December 57.33%, 17.10% in October, and with (34) who recorded the highest rate 33.30% in Autumn in goats. For *S. papillosus* recorded in present results, the highest percentage in February was recorded 28% and the lowest in January was16%, this was in accordance with (21), who recorded highest rate in February was 100% and lowest in June was 61.11% in goats with significance differences. The highest rate of *S. papillosus* in February may be attributed to period of parturition and lactation in this month. There is known that larvae of *S. papillosus* transmission by trans mammary route by colostrum milk and can noticed eggs in feces of young animals after 6-7 days, in addition to favorable climatic condition in February month for evolution of free living stage (3).

The lowest prevalence rate in January may due to adverse climatic condition in winter subsequences to arrested evolution of larvae in host and environment, short photo period in winter and reduce period of grazing support in reduce chance of contact between host and parasite, also phenotype or strain have difference response to temperature changes and high temperature shortened their evolution while low temperature prolong developed of free living stage (36). (17) was confirmed that *Strongyloides* spp. infections increase gradually in Autumn and reach peak in Winter and Spring and declined in Summer in goats, and didn't agree with (13) who noticed all nematodes infection reach peak in Spring seasons and also with (29) that detected the highest rate in Spring and Summer seasons was 95.23% and lowest in Winter was 52.77%. For *T.vitulorum*, results were shown maximum prevalence rate in February was 84% compared to November was 24%. The highest rate in February may be due to period of parturition and lactation. The main source of transmission by trans mammary routes and suckling ruminants ingestion milk contain L3 from dams and developed to adult worm in the small intestine of young animals and can noticed eggs in feces in 29 day after birth (37). The highest rate in consonance with (38), who recorded all

nematodes eggs count increase in parturition period due to stress factor and poor nutrition and per parturient relaxation which synchronized with climatic condition lead to develop of free living stage. These finding disagree with (17) in Egypt, who recorded highest rate of *T.vitulorum* in goats was 1.2% in Spring and Autumn and with (26), Who detected that no significance differences between months with infection of *T.vitulorum* in ruminants. For *S.ovis*, October, December, January and March had closed prevalence rate compared to February had lowest rate and no infection detected in November with *S.ovis*. The highest rate of *S.ovis* was in harmony with (34), who carried out the highest rate of *S.ovis* in Autumn seasons was 72.20%.

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Some Protocol Treatments for Retained Placenta in local Iraqi Buffalo (*Bubalus bubalis)*

A. H. N. Aal-Said
Animal Production and Fish Center Resources- Agriculture Directorate Research\ Ministry of Science and Technology

Abstract

The study aimed to evaluate different protocol treatments efficiency on fetal membranes retention in local Iraqi buffaloes breed (*Bubalus bubalis)* and some reproductive efficiency criteria for the treated animals under field condition. A study was conducted on 80 local buffalo cows breed *(Bubalus bubalis)* in the south of Baghdad suffered from retained placenta after parturition or abortion, they treated with different protocols (PGF2α+Ut.Pes+Placenta removed manually, PGF2α+ GnRH +ut.Pes+Placenta removed manually+systemic antibiotics + mineral inj, PGF2α+ systemic antibiotics and PGF2α), according to healthy status (if there was any complications or hypocalcaemia or magnecimia injuries and others). Results revealed that the mean duration days treatment were (3.25, 2.5, 3.5, 4.2), mean duration days open were (99.5, 73.3, 106.5, 109.0) and the pregnant percentages were (80.0, 90.0, 70.0, 60.0)%, respectively for the four groups (G1,G2,G3 and G4). We concluded that treatment with PGF2α+ GnRH+Ut.Pes+Placenta manually removed + systemic antibiotics+meniral inj, was the highly effective for buffalo retention fetal membranes at ($P \leq 0.05$) and PGF2α+ GnRH hormones preferred to be given after delivery.

Key words: Treatment, retained placenta, Iraqi buffaloes.
E-mail:vetabbashn@yahoo.com

بعض البرامج العلاجية المستخدمة لمعالجة احتباس الأغشية الجنينية في الجاموس العراقي المحلي*(Bubalus bubalis)*

عباس حسين نايف آل سعيد
مركز الثروة الحيوانية والسمكية– دائرة البحوث الزراعية/ وزارة العلوم والتكنولوجيا

الخلاصة

هدفت الدراسة إلى تقييم كفاءة بعض البرامج العلاجية المستخدمة لمعالجة مشكلة احتباس الأغشية الجنينية في الجاموس العراقي المحلي جنوبي بغداد من خلال تأثيرها بكفاءتها التناسلية. أجريت الدراسة الحالية على 80 أنثى جاموس عراقية محلية في مناطق جنوب بغداد كانت تعاني من احتباس الأغشية الجنينية بعد الولادة أو بعد الإجهاض. استخدمت لها بعض البرامج العلاجية المختلفة اعتمادا على حالتها الصحية هي (PGF2α+Ut.Pes+Placenta removed manually, PGF2α+ GnRH +ut.Pes+Placenta removed manually +systemic antibiotics + mineral inj, PGF2α+ systemic antibioticsand PGF2α). أظهرت نتائج الدراسة التي أجريت على مجاميع حيوانات التجربة الأربعة (م1، م2 وم3 و م4) أن استخدام المعالجة الهرمونية (بهرموني البروستاكلاندين ومحرض القند) مع الإزالة اليدوية للأغشية الجنينية واستخدام المضادات الحياتية الجسمانية العامة والموضعية (تحاميل رحميه تحوي هرمون الاسترادايول) أعطت نتائج معنوية وعلى مستوى (أ ≤ 0.05)، وكان معدل الأيام المفتوحة (99.5، 73.3، 106.5 و109.0) ومعدل أيام المعالجة (3.25، 2.5، 3.5 و4.25) ونسبة الحمل (80، 90، 70 و60)% على التوالي. نستنتج أن هرموني

البروستاكلاندين ومحرض القند والإزالة اليدوية للأغشية الجنينية ذات كفاءة علاجية عالية وبمستوى (أ ≤0.05) ولذا ننصح باستخدام المعالجة الهرمونية خصوصا هرموني البروستاكلاندين ومحرض القند مابعد الولادة.

الكلمات مفتاحية: معالجة، احتباس الأغشية الجنينية، الجاموس العراقي.

Introduction

Buffalo is one of the most important animal to many farmers in Iraq for its productivity of high fat content milk, meat, and other products. In the south of Baghdad large number of small commercial herds 3-150 animals of buffaloes are maintained, most of these animals belong to (*Bubalus bubalis*) breed which named popularly (Furaty or Babylon Buffaloes), milk of these animals was supplied directly or collected by middlemen and supplied to towns and cities in Baghdad (A personal note). Buffalo is 1). (considered a low reproductive efficiency animal as it achieves long calving intervals Retention of fetal membranes (RFM) is one of the most common conditions occurring in animals after parturition, It is observed mainly in cattle and buffaloes (2). RFM is one of the major pathological problems faced by the farmers and field veterinarians in practice, and to maintain a calving interval of 13-14 months in buffaloes, successful breeding must take place within 85-115 days after calving (3). Disturbances during this period due to delay of uterine involution or resumption of estrous activity are likely to prolong the calving interval and reduce the lifetime reproductive and productive efficiency (4). RFM is one of the common diseases after delivery in buffaloes in this from all reproductive disorders which reached %region of Baghdad which reached 4.66 59.0% (5). The main causes of RFM are nutritional, physiological, mechanical, pathological, (6, 7), and immunologically that is one theory suggests the fetal placenta must be recognized as "foreign" tissue and rejected by the immune system after parturition to cause expulsion of the placenta (8). The prognosis indicated that mortality rate not exceed 1-2% in uncomplicated cases (9). Different methods of treatments to RFM were be applied to include manual removal and hormonal programs accompanied with antibiotics (10, 11). The aims of study were to evaluate different treatment protocols upon retention of fetal membranes and the reproductive efficiency for the treated animals, number of animals responded, days open and duration days for treatment till the animal recovered.

Material and Methods

The study was conducted on 80 local buffalo cows (*Bubalus bubalis*) aged 3-8 years in south of Baghdad, where farming system is semi-intensive and the animals are fed with concentrate, wheat husk, cotton seeds and seasonal green fodders (maize, alfalfa and other green grass), these animals suffered from RFM after 24hrs from delivery (naturally or aborted), during the period from 2011-2012. Animals divided into four groups according to healthy status, 1st group (G1) included 20 buffalo cows treated by PGF2α (Estrumate, Schering Plough Animal Health-Germany) 750µg (I\M)). plus (Ut.Pes) Uterine Pesory (contain Oxytetracycline plus estradiol hormone) plus Placenta removed manually (Pl .Rem.). 2nd group (G2) included 40 buffalo cows treated with Estrumate 2 ml.plus GnRH (Receptal) 5ml plus Ut.Pes. plus Pl.rem plus antibiotics (20% Oxytetracycline 1ml\10kg.B.W.) plus mineral inj. (calcium plus phosphorus), 3rd group (G3) (10) treated with Estrumate 2ml plus systemic antibiotics and the 4th group (G4) included (10) cows buffaloes treated with PGF2α alone. Responses of animals and duration from treatment till the animals be recovered was recorded. As well as we recorded the first postpartum estrus (days open). The manual removal as reviewed by (12). Cows showed estrus were naturally inseminated from an experienced fertile bulls and examined for pregnancy detection after 2 months rectally. Statistical analysis included mean, st.de. and st.er and the significances tests were done according to (13) by using (14). Results were considered significant at ($P<0.05$) or less.

Results and Discussion

Table (1) Show types of treatment groups programs and MDT, MDO and pregnant cows percentages

Group	1	2	3	4
Protocol treatment	PGF2α+Ut.Pes+Placenta manual remove	PGF2α+ GnRH +ut.Pes+Placenta manual remove +systemic antibiotics+meniral inj	PGF2α+ systemic antibiotics	PGF2α
No. cows treated	20	40	10	10
Mean duration days treat.	3.25 b	2.5a	3.5b	4.2c
Mean duration days open	99.5b	73.3a	106.5c	109.0 c
St.De.	3.88	3.1	4.18	4.28
St.Er.	1.97	1.8	2.1	2.04
% of cows pregnant	80.0% b (16/20)	90.0% a (36/40)	70% c (7/10)	60% c (6/10)

Values with different superscripts in the same row differ significantly (P<0.05).

Table (1) shows a significant results at (P<0.05) when hormones and antibiotics used and this may be due to deficiency in secretions of PGF2 and serum Ca concentration, which maintain adequate contraction of the uterus, that cause RFM, when increase the risk of dystocia and delay the involution of the uterus (15, 16), or may be due to an imbalance in the synthesis of PGF2α and PGI2 during the first 60 minutes postpartum conducting to a lack of PGF2α and that the relative increase in PGI2 is associated with placental retention in bovine(17). Researchers (18, 19) obtained that treatment with antibiotics were so useful due to uterine bacterial infections during the postpartum period which associated with lower conception rates, increased intervals from calving to first service or conception and more animals culled for failure to conceive. Also infection of uterus invariably causes damage to the endometrial epithelium; thus, the uterus becomes unable to secrete luteolytic pattern of PGF2α, and hence the corpus luteum is retained and self perpetuating infection results (20). While 21 showed that treatment with GnRH or $PGF_2\alpha$ helps the normalization of electrolytes and vitamins, as well early treatment of abnormal cows with GnRH improves the reproductive efficiency through normalization of serum progesterone. The reproductive performance of treated buffalos with GnRH improved as was shown by the significant (P<0.01) decrease in days to first service, days open and calving interval, so GnRH treatment had relatively ameliorated the metabolic function in treated buffaloes via increasing concentrations of blood total protein, glucose, creatine, creatinine, calcium and inorganic phosphorus (22). Intrauterine infusion of Rifampicin and systemic administration of Oxytetracycline positively affected the clinical cure and uterine involution of buffaloes with toxic puerperal metritis in local Iraqi buffalo (23). Also (24) showed that serum progesterone concentration was significantly higher (P<0.05) in buffaloes with RFM compared to buffaloes without RFM, and mean serum estradiol-17-β level in buffaloes with RFM was significantly lower (P<0.05), so that treatment of RFM with estradiol is preferred. Combined GnRH and PGF2alpha application in cows with endometritis puerperalis treated with antibiotics, clinical recovery was 96.6% in the experimental groups and 82.5% in the control group (p<0.05), first service pregnancy rate was significantly better in hormone treated than control cows 51.7 versus 36.4% (p<0.05), total pregnancy rate and insemination index values were not significantly improved following GnRH and PGF2alpha treatment. The average service

period was 89.8 +/- 21.2 days in cows after hormone treatment, and 112.6 +/- 24.5 days in control cows, the difference were significant ($p<0.05$), these results indicate that the sequential GnRH and PGF2alpha application in cows with puerperal endometritis positively affected ovarian function and uterine involution in Kundhi buffaloes (25). It has been proved that treatment with PGF2 alpha or its analogue in early postpartum cows and buffaloes in reproduction order to hasten early resumption of cyclic ovarian Information activity and thereby to increase the reproduction (25, 26). The study was agreement with (27), treatment with GnRH plus PGF2α increased significantly ($P<0.05$) and accelerate the process of uterine involution postpartum, reduce the time period of first postpartum estrus and induce early expulsion of fetal membrane in Kundhi buffalo. Also agree with (28) that the hormonal treatment is a good method and gives appositive reproductive result reaches to 80% response in Iraqi buffaloes. Our conclusions that Iraqi local buffaloes suffered from bad management especially ration imbalance which lead to hormonal disturbance. We recommended hormonal treatment especially PGF2α+ GnRH hormones accompanied with manual removal to retained placenta and control incorrect managements especially feed imbalance.

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Synthesis, Characterization, and Antibacterial Evaluation of New N-Phenylnaphthalimides linked to Benzothiazole moiety

A. S. Hamed
College of Veterinary Medicine/ University of Fallujah

Abstract

In this work several new naphthalimides linkd to substituted benzothiazole moiety were prepared. Preparation of the new naphthalimides was performed via multistep synthesis which include reacted of naphthalic anhydride with 4-aminobenzoic acid, then the product subsequently introduced in reaction with thionyl chloride producing 4-(N-naphthalimidyl) benzoyl chloride which in turn introduced in reaction with substituted benzothiazoles affording the target new naphthalimides. The prepared compound were screened for their antimicrobial activity against gram positive (*Staphylococcus aureous*, *Streptococcus pyogenes*), gram negative (*Escherichia coli* and *Pseudomonas aureginosa*) and yeast (*Candida albicans*). The results indicated that they exhibit good antimicrobial activity against the tested organism.

تحضير وتشخيص وتقدير الفعالية البايولوجية لـ N- فنيل نفثال ايمايدات جيدة مرتبطة بمكونة البنزوثايازول

احمد سليمان حمد

كلية الطب البيطري / جامعة الفلوجة

الخلاصة

تم في هذا البحث تحضير عدد من النفثال ايمايدات الجديدة المرتبطة بمكونة البنزوثايازول المعوضة. تم تحضير النفثال ايمايدات الجديدة بطريقة التحضير متعدد الخطوات، تم في الخطوة الأولى مفاعلة انهيدريد النفثالك مع 4- امينو حامض البنزويك ومن ثم تم مفاعلة الناتج مع كلوريد الثايونيل لتكوين 4- (N- نفثال ايميديل) كلوريد البنزويل والذي يدخل في تفاعل مع بنزوثايازولات معوضة لتكوين النفثال ايمايدات الجديدة. قيست الفعالية البايولوجية للنفثال ايمايدات الجديدة ضد بكتريا موجبة لصبغة غرام (ستافيلوكوكس اريوس وستريبتوكوكس بايوجينز) وبكتريا سالبة لصبغة غرام (اشريشيا كولاي وبسيدوموناس اوريجينوزا) والخميرة كانديدا البيكانس، حيث أثبتت النتائج امتلاكها فعالية بايولوجية ضد الأحياء المجهرية قيد الدراسة.

Introduction

Cyclic imides represent a very important compounds in drug discovery due to their wide spectrum of biological and pharmacologicl properties (1, 2, 3, 4). Naphthalimides first discovered by Brana and co-workers (5, 6), are DNA-targeted chemotherapeutic agents acting primarily by attacking DNA at some level (synthesis, replication, or processing) (7). Therefore plenty of naphthalimide based anticancer drugs have been synthesized (8, 9, 10), and promising results have been obtained (11). The benzothiazole scaffold is prevalent in a variety of pharmacologically active synthetic and natural compounds exhibiting antimicrobial (12, 13, 14, 15, 16, 17), anticancer (18, 19, 20), anthelmintic (21), and anti-diabetic activity (22). They are widely found in bioorganic and medicinal chemistry with application in drug discovery (23, 24). Taking into consideration the above described beneficial effects of cyclic imides and benzothiazole, we realized that it would be of interest to synthesize novel structural hybrids containing both heterocyclic ring systems.

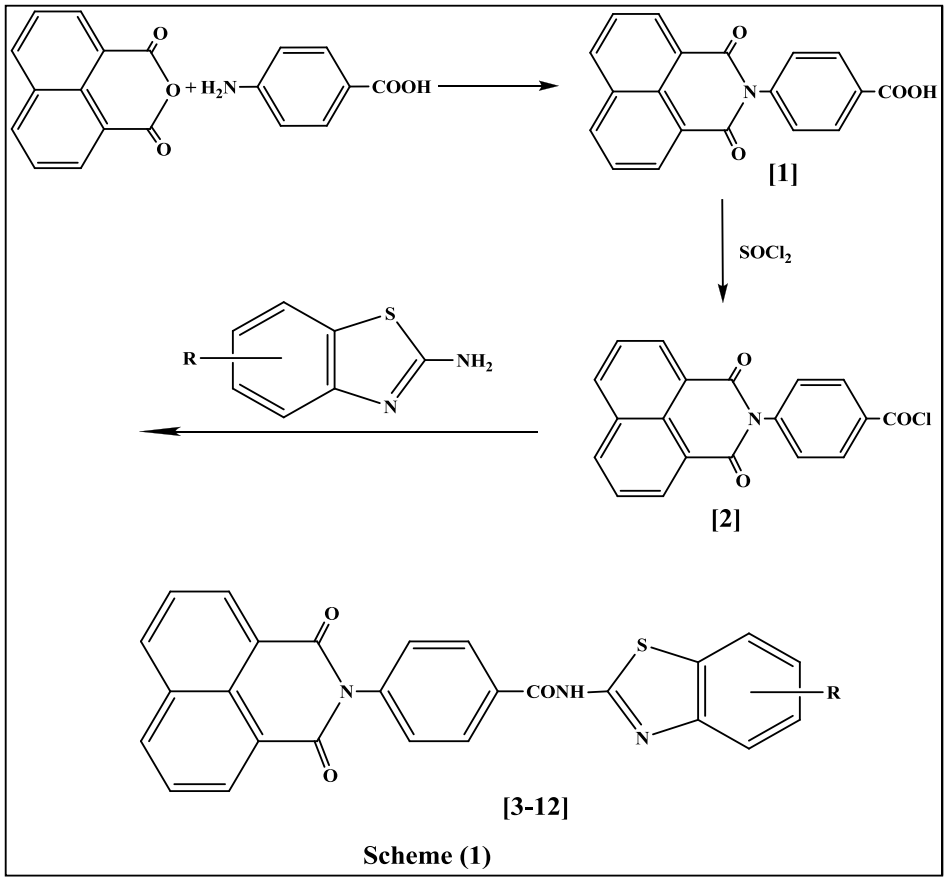
Materials and Methods

Chemicals used in this work are supplied from BDH and Fluka companies and are used without further purification. Melting points were determined on Gallenkamp capillary melting point apparatus and were uncorrected. FTIR spectra were recorded on SHIMADZU FTIR-8400 Fourier Transform Infrared spectrophotometer using KBr discs. ^{1}HNMR and ^{13}CNMR spectra were recorded on Bruker 300MHz instrument using deuterated dimethyl sulfoxide (DMSO-d_{6}) as a solvent and trimethylsilane (TMS) as internal reference.

- **Synthesis of 4-(N-naphthalimidyl) benzoic acid [1]**: The titled compounds were prepared according to literature procedures (25). (0.01mol,1.98g) of naphthalic anhydride and (0.01mol, 1.37g) of 4-aminobenzoic acid were placed in a wide dry Pyrex tube which was immersed in an oil bath and provided with a thermometer. The oil bath was heated until fusion of the amic acid then temperature was maintained at ten degrees above the melting point of the used amic acid for (30-45) minutes. After cooling to room temperature the obtained solid was boiled with 10% sodium bicarbonate for 20 mints and purified by recrystallization from ethanol. Yield 85%, melting point > 300 ºC.
- **Synthesis of 4-(N-naphthalimidyl)benzoyl chloride [2]:** A mixture of 10g of 4-(N-naphthalimidyl) benzoic acid and (1.25g, 7.7mL) of thionyl chloride in 100mL round bottomed flask fitted with a double surface reflux condenser carrying a calcium chloride guard tube was heated on water bath with occasional shaking for 1 hour. The excess of thionyl chloride was distilled at 77ºC (26) .
- **Synthesis of 4-(N-naphthalimidyl)-N-(substitutedbenzothiazol-2-yl) benz- amide [3-12]:** To the solution of (0.01 mol) of 4-(N-naphthalimidyl)benzoyl chloride in 100mL. of ethanol was added (0.01mol) of substituted benzothiaol. The mixture was refluxed on a water bath for 6 hours. After cooling, the precipitate was collected, washed with distilled water, and recrystallized from suitable solvent(27). Physical properties of prepared compounds are listed in Table (1).
- **Biological Study:** The cup plate method (28) using nutrient agar medium was employed in studying the antimicrobial activity of the prepared imides against four types of bacteria including *Staphylococcus aureous*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aureginosa* respectively and yeast *Candida albicans* fungi. Dimethyl form amide (DMF) was used as sample solution which fixed at (0.1 mL) for all compounds using a sterilized cork borer cups were scooped out of agar medium contained in a petri dish which was previously inoculated with the microorganisms. The test compound solution (0.1 mL) was added in the cups and the petri dishes were subsequently incubated at 37°C for 48 hrs. Zones of inhibition produced by each compound was measured in (mm) and the results are listed in Table (3).

Results and Discussion

- **Chemistry:** Since both naphthalimides and benzothiazoles are biologically active components having wide spectrum of medicinal and pharmacological applications, the present work is directed toward synthesis of new compounds containing these two active moieties with expected biological activity. The target of present work is synthesis of new naphthalimides linked to benzothiazole moiety. Performing of this target involved many steps, the first one involved synthesis of 4-(N-naphthalimidyl) benzoic acid [1] via direct reaction between 1,8-naphthalic anhydride and 4-aminobenzoic acid under fusion condition. Compound [1] was introduced in reaction with thionyl chloride in the second step producing 4-(N-naphthalimidyl) benzoyl chloride [2]. Reaction of compound [2] with different substituted benzothiazole in the third step gave the corresponding 4-(N-naphthalimidyl)-N-(substitutedbenzothiazol-2-yl) benzamide [3-12]. The synthetic route of the new compounds is outlined in Scheme (1).



Scheme (1)

Structures of the prepared compounds were confirmed by FTIR, ^{1}HNMR and ^{13}CNMR spectra data. FTIR spectrum of compound [1] showed appearance of ν(C=O) of carboxylic acid at (11687) cm^{-1} and ν (C=O) imide at (1700) cm^{-1} absorption bans indicating success of imidation reaction. Besides the spectrum showed characteristic absorption bans at (3325-3461) cm^{-1} due to ν (OH) carboxylic acid and ν (NH) amide and at (1666) cm^{-1} due to ν(C=O) amide. FTIR spectra of imides [3-12] showed clear absorption bands at (3270-3365) cm^{-1}due to ν(NH) amide, bands at (1643-1682) cm^{-1} due to ν(C=O) amide and bands at (1680-1718) cm^{-1} due to ν(C=O) imide. Other bands appeared at (1588-1665) cm^{-1},(1542-1595) cm^{-1} and at (625-710)cm^{-1} due to ν(C=N) thiazol, ν(C=C) aromatic and ν(C-S) thiazol respectively. All details of FTIR spectral data of compounds [3-12] are listed in Table (2)(29). ^{1}HNMR spectrum of compound [1] showed clear signals at (δ = 7.7-8.5) ppm and at δ = 12.3 ppm belong to aromatic protons and (OH) proton while ^{13}CNMR spectrum of the same compound [1] showed signals at (δ = 124.2-136.3) ppm and 162.8ppm belong to aromatic carbons and (C=O) carbons respectively. ^{1}HNMR spectrum of compound [4] showed clear signals at δ = 2.32 ppm, (δ = 7.2-8.6) ppm and δ = 11.8 ppm due to CH_3 aromatic protons and (NH) proton, while ^{13}CNMR spectrum of the same compound [4] showed signals at δ = 22 ppm, (δ = 118.5-157) ppm, 162.8 ppm and 164.1ppm belong to CH_3, aromatic carbons,(C=N) and (C=O) carbons respectively. ^{1}HNMR spectrum of compound [7] showed signals at δ = 3.85 ppm, (δ = 7.31-8.72) ppm and δ = 10.9 ppm due to (OCH_3), aromatic protons and (NH) proton respectively, while ^{13}CNMR spectrum of the same compound [7] showed signals at δ = 55.8 ppm, (δ = 106.5-153.9) ppm, 157.8 ppm and 165.2 ppm belong to (OCH_3), aromatic carbons, (C=N) and (C=O) carbons respectively. ^{1}HNMR spectrum of compound [11] showed clear signals at δ = 2.2 ppm, (δ = 7.43-8.56) ppm and δ = 11.2 ppm belong to two methyl groups, aromatic protons and (NH) proton respectively, while ^{13}CNMR spectrum of the same compound [11] showed signals at δ = 15.4 and 18.3 ppm belong to two methyl groups and signals at (δ = 113.5-140.1) ppm, 141.28ppm and 163.82ppm belong to aromatic carbons, (C=N) and (C=O) carbons respectively.

- **Biological Study:** Antimicrobial activity of the synthesized imides were tested against four types of bacteria and *Candida albicans* yeast using cup plate method.

Most of the tested bacterial organisms showed sensitivity to synthesized imides compared to standard antibiotics. Inhibition zones caused by each compound was measured in (mm) and the results are listed in Table (3). The results indicated that compounds (8, 9) are highly active against all types of tested bacteria. Compound [10] also highly active against *S. aureus* and *S. pyogenes* and moderate active against *E. coli*. Compound (10) is highly active against *Candida albicans* yeast while compound (8) showed moderate activity against *Candida albicans* fungi. The rest of imides were found to be moderately active against the tested organisms.

Table (1) Physical properties for prepared imides [3-12]

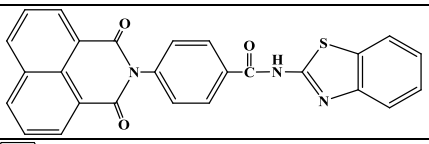
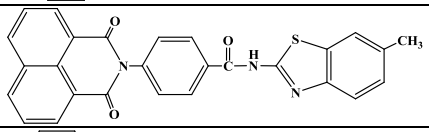
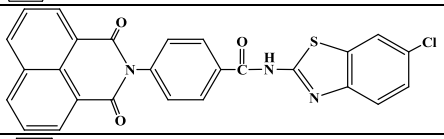
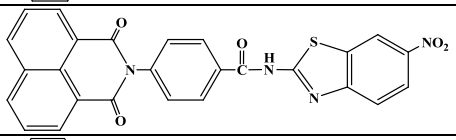
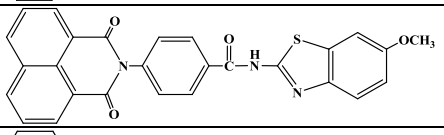
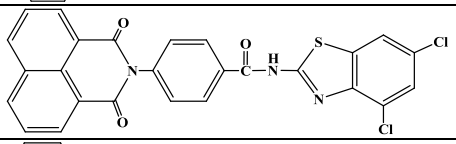
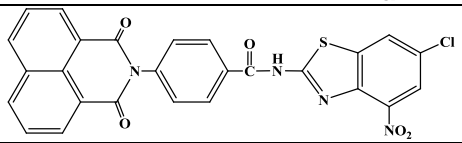
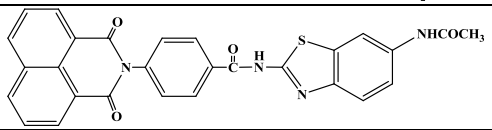
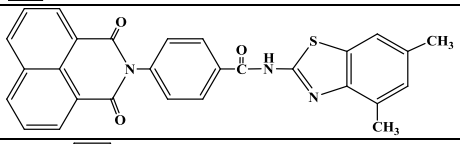
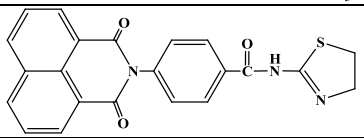
Comp. No.	Compound structure	Color	Melting point °C	Yield %	Solvent of Recr.
3		White	230-232	75	Ethanol
4		Light brown	255-257	83	Ethanol
5		Off white	295-297	70	Ethanol
6		Yellow	>300	65	Ethanol
7		Violet	>300	85	Ethanol
8		Gray	220-222	75	Ethanol
9		Deep yellow	>300	68	Ethanol
10		Greenish yellow	>300	78	Ethanol
11		Brown	>300		Ethanol
12		Brown	245-247	87	Ethanol

Table (2) FTIR spectral data of naphthalimides [3-12]

Comp. No.	FTIR spectral data cm^{-1}						
	ν(N-H) amide	**ν(C=O) amide**	**ν(C=O) imide**	**ν(C=N) thiazole**	**ν(C=C) aromatic**	**ν(C-S) thiazole**	**others**
3	3310	1678	1680	1610	1585	670	-
4	3382	1682	1715	1638	1560	675	-
5	3270	1675	1718	1600	1589	677	**ν(C-Cl)aromatic** 1095
6	3285	1666	1685	1625	1593	688	**ν(C-NO_2)** 1500, 1410
7	3360	1665	1718	1665	1542	710	**ν(C-O-C)** 1220,1175
8	3295	1650	1695	1635	1589	708	**ν(C-Cl)aromatic** 1068
9	3310	1670	1689	1615	1593	678	**ν(C-NO_2)** 1440, 1327 **ν(C-Cl)** 1055
10	3325	1678 1643	1698	1595	1581	668	-
11	3365	1678	1710	1645	1578	673	-
12	3305	1680	1705	1588	1595	625	-

Table (3) Inhibition zones for some of the prepared compounds measured in (mm)

Comp. No.	Gram-positive bacteria		Gram-negative		Yeast
	S. aureus	*S. pyogenes*	*E. coli*	*P. aeuroginosa*	*Candida albicans*
3	12.5	10	9.5	8.6	8.8
5	13	11.2	10.8	11.5	9.5
6	10.2	8.5	9.5	8.5	7.2
8	14.6	12.5	14	12.4	12
9	14.8	12.5	15.2	13.4	8.5
10	16	11.5	10.5	8.2	15.7
ampicillin	17	12.5	12	14	-
DMSO	-	-	-	-	-

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Effect of Boron on broiler productive traties and immune response of Newcastle disease vaccine

B. H. Ali*, M. F. Hmud and E. J. Ali***

***Dep. of Pathology and Poultry disease\ College of Veterinary Medicine\ University of Baghdad**

****Dep. of Veterinary Public health / College of Veterinary Medicine\ University of Baghdad**

Abstract

This study was performed to investigate changes in growth performance, blood pictures, weights of internal organs and immune response to Newcastle disease vaccine (ND vaccine) of broilers fed different levels of Boron (75,150 and 250mg/kg) in feed from 5 weeks. 120 broiler chicks (one day old) were randomly divided into four equal groups (each group contain 30), as following: first group (G1) was received 75 mg/kg of Boron, second group (G2) was received 150 mg/ kg of Boron, third group (G3) was received 250 mg/ kg of Boron and fourth group (G4) was not received Boron in feed and consider as control, All groups were vaccinated with ND vaccine at 10 and 20 days old, Growth performance of the chicks (Body weight, weight gain and mortality rate) were weekly determined, at the end of experiment weights of internal organs (liver, kidney and bursa of fabricius) were determined and Blood samples were collected to measured Ab titer against ND vaccine by ELISA test and to determined blood pictures (RBCs, PCV, HB), The results of this study showed that body weight, weight gain, blood pictures and Ab titers were highly significant increase ($p<0.01$) in G2 as compared to control group followed by G3 and G1 respectively in all weeks. The mortality rate was significantly decrease ($p<0.01$) in G1, G2 and G3. Whereas the weights of internal organs (liver, kidney and bursa of fabricius) were significantly increase in G3, G2 and G1 respectively. In conclusion, the results indicated that all levels of Boron supplemented diet of broiler chicken caused improvement of growth performance, blood parameters, and without inhibition of the immunity response of chicken against Newcastle disease vaccine.

Keywords: Boron, broiler, growth performance, blood cells, immune response

E-mail: helenjassim@yahoo.com

دراسة تأثير عنصر البورون على الصفات الإنتاجية للدجاج اللاحم والاستجابة المناعية للقاح مرض نيوكاسل

بلقيس حسن علي*، مهند فلحي حمود وابتسام جواد علي***

***فرع الأمراض وأمراض الدواجن/ كلية الطب البيطري/ جامعة بغداد**

****فرع الصحة العامة البيطرية/ كلية الطب البيطري/ جامعة بغداد**

الخلاصة

أجريت الدراسة لبحث التغيرات في أداء النمو، الصورة الدموية، أوزان الأعضاء الداخلية والاستجابة المناعية للقاح نيوكاسل في أفراخ دجاج لاحم أعطيت تراكيز مختلفة من عنصر البورون (75، 150 وملغم لكل كغم) عن طريق العلف ومن عمر يوم واحد ولغاية انتهاء التجربة، تم استخدام 120 فرخة دجاج وبعمر يوم واحد وقسمت عشوائيا إلى أربعة مجاميع متساوية وكالاتي المجموعة الأولى أعطيت 75 ملغم لكل كغم، المجموعة الثانية أعطيت 150 ملغم لكل كغم، المجموعة الثالثة أعطيت 250 ملغم لكل كغم والمجموعة الرابعة لم تعطى بورون

بالعلف واعتبرت مجموعة سيطرة تم قياس الصفات الإنتاجية للأفراخ (وزن الجسم، الزيادة الوزنية) ونسبة الهلاكات وكذلك تم إجراء الفحوصات لبعض الصفات الدمية (حساب العدد الكلي لخلايا الدم البيض WBC، خلايا الدم الحمر RBC، الهيموغلوبين Hb وحجم خلايا الدم المرصوص PCV) وأيضاً تم قياس أوزان بعض الأعضاء الداخلية (الكبد والكلية وجراب فابريشيا) وكذلك قياس معيار الأضداد المناعية للقاح مرض نيوكاسل بواسطة اختبار الاليزا، أظهرت نتائج الصفات الإنتاجية والصفات الدمية والاستجابة المناعية معيار الأضداد المناعية تفوق مجموعة G2 معنويا عن باقي المجاميع تليها مجموعة G3 ومجموعة G1 مقارنة بمجموعة السيطرة G4 وفي جميع الأسابيع أما نسبة الهلاكات الطبيعية فقد شهدت انخفاضا معنويا في مجموعة G2 ومجموعة G1 ومجموعة G3 في الأسبوع الثالث والرابع أما أوزان الأعضاء الداخلية (الكبد والكلية وجراب فابريشيا) فقد شهدت زيادة معنوية في كل من المجموعة G3 والمجموعة G2 والمجموعة G1 على التوالي، نستنتج من ذلك ان كل التراكيز المستخدمة والمضافة لعلف الأفراخ في هذه الدراسة كان ذا تأثير إيجابي على الأداء الإنتاجي، الاستجابة المناعية والصفات الدمية.

Introduction

Boron is the fifth element in the periodic table, it's the only non metal in the group IIA elements, but contain characters between those of metal and non metal(1). Boron is ubiquitous in nature and its commonly found in soils, rocks and surface of ocean water in the form of boric acid and inorganic borates(2). In nature B is found either bound to oxygen or in the borate form, organic compound containing Boron bound to oxygen or nitrogen groups are referred to as organoboron compounds these complex found in plant, animal and human tissue (3). Since boron has not been consistently accepted as an essential element for higher animals and plants, its importance in poultry nutrition has been recognized only since the late 1980s were some functions and relation ships of boron were suggested by Hunt and Nielsen (4) including improvement of the growth rate, nutritional efficiency, calcium and phosphorus retention in broilers besides reduction of vit. D deficiency symptoms (5). Boron play an important role in the metabolism of cro-minerals in broilers, mainly calcium, improving the balance of the mineral and bone fractures mainly in processing plants (6) Although boron is not considered essential for poultry or most animals the inclusion of 2 ppm in poultry feed was recommended by NRC (7) such suggestions are not present in the requirements for poultry in 1994(8). Boron may be toxic in high doses, addition of 400 mg/L boron in drinking water has been shown to inhibit the development of intestinal villi in Gushi chickens(9). Also high doses of boron can cause damage of reproductive and digestive organs, and high doses of boron (>200mg/l) could significantly inhibit the growth of immune organs and even exhibit toxic effect but appropriate supplements of boron (<100mg/l) could improve the growth of immune organs in broiler after 4 weeks (10). thus, the purpose of this study was to investigate the effect of different levels of Boron on growth performance, blood pictures, weight of internal organs and immune response of broiler to Newcastle disease vaccine.

Materials and Methods

- **Broilers Chicks:** The experiment was done at the Poultry house at the department of Veterinary Public Health, College of Veterinary Medicine-Baghdad University, after cleaning and disinfecting, Feeders and water utensils were cleaned and disinfected, newly hatched chicks of the Ross breed, were brought in good condition from Al-Gamieea Hatchery-Baghdad-Abu-Ghraib. Chicks were vaccinated with NDV vaccine Lasota strain (Intervet-Germany) by manual oral drench at 10 days old, and 20 days. The basal diet was formulated for chicks according to National Research Council. Table (1) shows the ingredients and calculated chemical analysis of this diet, Boron was weighted individually and supplemented to diet in homogenous form.

Table (1) Ingredients of feed used in this study

Constituents	Percentages (%) of ingredients in D1
Wheat	34.2
Yellow corn	35
Soybean meal(45%protein)	20
Animal Protein (40% protein) (Meat and bone meal)	10
Calcium (CaCo3)	0.7
Salt (NaCl)	0.1
Total	100%

- **Design of experiment:** Boron powder was at the level (75,150, 250 mg/kg. feed) was tested at first day to the end experimental period (35 days). A total of 120 chicks at age of one day old were divided randomly into four groups each group have 30 chicks as follow:

- Group 1: broiler fed boron (75 mg /kg. feed)
- Group 2: broiler fed boron (150 mg/kg. feed)
- Group 3: broiler fed boron (250 mg/kg. feed)
- Group 4: Control chicks fed the basal diet no boron

- **Parameters included in this study:**

- **Live body weight:** The weights of each group of chicks separately were taken at weekly intervals by weighting chicks individually at the end of every week and the total weights of all chicks were divided by the number to find the average weight of the chicks in gram (11).
- **Weekly body weight gain:** The weight gains were calculated depending on the difference in body weight between the beginning of the week and the end of it, depending on the following equation:

Weekly body weight gain = Body weight at the end of the week - body weight at the beginning of the week (11)**.**

- **Estimation of relative weights of liver, kidney, bursa weight to the total body weight (relative weight):** At the end of each experiment 15 birds were chosen from each group randomly and there live weight was taken then slaughtered, and samples from the liver, kidney, bursa were collected after slaughtered the chicks. Carcass chicks were cleaned and weighted. The relative weight of each organ was measured according to the following equation:

$$\text{Relative weight of the organ } (\%) = \frac{\text{Weight of organ}}{\text{Weight of live bird}} \times 100 (12)$$

- **Blood sampling for Hematological tests:** Blood samples were randomly collected at the end of the experiment from (20) chicks from each group. The procedure of blood collection was carried out by slaughter of chicks followed and blood samples were taken to determine antibody titers against ND in blood serum by using ELISA test (Synobiotics-USA), Total red blood cells counting (cell/ mm^3), Total White Blood cells counting (cell/mm^3), Packed cell volume PCV% were measured by hemocytometer method described by (13), also measured of Hemoglobin concentration (g/dl)(14).
- **Statistical Analysis:** The statistical Analysis System-SAS(15) program was used to detect differences best different groups. least significant difference-LSD test was used to significant compare between means in this study.

Results

The effects of boron supplemented in feed on the Body Weight (BW) and Weight Gain (WG) of broilers are shown in Table (1) and in Table (2). The addition of boron (75, 150, 250 mg/kg) to the diets caused a significant increased ($p<0.01$) in BW compared to the control group for all weeks. The BW G2 was higher than that of

chicken in G1 and G3 in all weeks except 4th week showed significant differences, Also there was a significant differences ($p<0.01$) between G3 and G1 in 3rd and 5th week. The results of GW are presented in table (2) which revealed a significant increased ($p<0.01$) in G2 compared to control group for all weeks, also G1 exhibited a significant increased ($p<0.01$) in GW in comparison to control group for all weeks, except 3rd week, while G3 showed significant increased ($p<0.01$) relative to control group except 2nd and 4th week. Mortality rate of chicken are shown in Table (3), the results revealed significant differences between all groups in 1st and 2nd weeks, while in 3rd and 4th weeks there was a significant decreased ($p<0.05$) of mortality rate in groups supplemented with boric acid compared with control group, also in 5th week there was in significant decreased in G2 and there was no mortality rate in G1and G3 compared with the control group. The results of blood parameters are summarized in Table (4), G2 showed a significant increased ($p<0.01$) in PCV%, RBCs and WBCs count and HB concentration followed by G1 and G3 respectively, compared to control group Also, G1 revealed a significant increase in PCV%, WBCs and RBCs number and HB concentration compared with control group. While G3 showed a significant increase ($p<0.01$) in PCV%, RBCs, WBCs count and HB concentration in comparison to control group and other groups. The effect of boron supplementation in diet on the weight of internal organs are shown in table (5) the weight of liver, kidney and bursa of fabricious are significantly increased ($p<0.01$) in G3 and G2 compared with control and G1. while G1 showed a significant increased ($p<0.01$) in weight of kidney and bursa of fabricious and in significant increased in weight of liver compared with control group. The effect of B supplementation on immune response of chicken against Newcastle disease vaccine (ND) vaccine are presented in table (6). The results revealed a significant increased ($p<0.01$) in Ab titers of G1, G2 and G3 as compared with control group, also there was a significant differences ($p<0.01$) in G1 compared with G2 and G3, the best immune response was seen in G1 followed by G2 and G3 respectively.

Table (1) Effect of boron supplemented in feed on body weight (g) of broilers (Mean±SE)

Group	Mean ± SE (gm)				
	1st week	2nd week	3rd week	4th week	5th week
G1	154.80±5.52 b	386.40±12.55 b	728.60±9.47 c	1197.10±74.25 a	1651.60±13.3 c
G2	147,80±2.63 a	426.00±5,53 a	812.50±3.86 a	1204.60±13.54 a	2083.40±13.38 a
G3	157.50±3.64 b	334.40±8.01 c	771.50±5.61 b	1157.50±24.12 a	1957.20±32.90 b
G4	142.00±4.38 c	312.80±2.95 d	639.10±1.97 b	982.40±3.41 b	1392.90±2.71 d

Small litters means significant differences ($p<0.01$) between groups

Table (2) Effect of boron supplemented in feed on gain weight (g) of broilers (Mean±SE).

Group	Mean ± SE (gm)				
	1st week	2nd week	3rd week	4th week	5th week
1	109.80±5.52 b	231.60±13.35 a	342.20±75.36 c	468.50±75.36 a	454.50±78.26 b
2	130.80±2.63 a	251.20±4.29 a	386.50±6.70 b	392.10±6.70 a	878.80±31.70 a
3	115.50±3.64 b	176.90±8.47 b	437.10±12.77 c	386.00±23.72 ab	799.70±39.93 a
4	101.00±4.38 c	170.80±6.11 c	326.30±4.57 c	343.30±2.31 b	410.50±4.81 bc

Large litters means significant differences ($p<0.01$) between groups and small litters means significant differences ($p<0.05$) between groups.

Table (3) Effect of boron supplemented in feed on Mortality percentage% of broilers

Group	Mortality percentage%				
	1st week	2nd week	3rd week	4th week	5th week
G1	0.00	0.00	3.33	3.33	0.00
G2	3.33	0.00	0.00	3.33	3.33
G3	0.00	0.00	6.66	0.00	0.00
G4	0.00	0.00	9.99	6.66	3.33
Level of significant	NS	NS	*	*	NS

***(P<0.05).**

Table (4) Effect of boron supplemented in feed on blood parameters of broilers

Group	Mean ± Se			
	(%) PCV	RBCs×10^6	WBCs×10^3	Hb(g/dl)
G1	29.88±0.38 b	2.40±0.01 c	19.74±0.08 C	10.84±0.03 c
G2	36.23±0.17 a	3.59± 0.02 a	23.14±0.15 A	13.50±0.01 a
G3	28.94±0.26 c	2.61±0.03 b	20.87±0.02 B	11.74±0.04 b
G4	28.99±0.14 c	2.36±0.01 c	18.18±0.12 D	9.15±0.02 d

Small litters means significant differences (p<0.01) between groups

Table (5) Effect of boron supplemented in feed on relative weight (gm) of organs

Group	Mean ± Se (gm)		
	Relative weight of liver	Relative weight of Kidney	Relative weight of bursa of fabricius
G1	3.22±0.04 c	0.855±0.01 c	1.13±0.01 c
G2	3.65±0.02 b	0.956±0.01 b	1.25±0.001 b
G3	3.73±0.02 a	1.08±0.01 a	1.52±0.02 a
G4	3.05±0.05 c	0.760±0.013 d	0.877±0.02 d

Small litters means significant differences (p<0.01) between groups

Table (6) Effect of boron supplemented in feed on antibody titers against ND vaccine in broilers (M±SE).

Groups	Mean ± SE
	ND titer
G1	3553.40±13.80 b
G2	4153.80±8.81 a
G3	3393.20±3.46 c
G4	3111.50±2.86 d

Small litters means significant differences (p<0.01) between groups

Discussion

In the present study, the results showed that three levels of boron (i.e. 75,150 and 250 mg/kg) induced improvement of the growth by significantly increase of BW and WG especially the addition of 150 mg/kg, followed by 75 and 250mg/kg. These results are in agreement with other reports that found that boron supplementation had positive effect on growth performance (16) who pointed out that 5 and 25 mg/kg B added to

diets containing an adequate vitamin D3 caused a significant increase in body weight on days 14 and 28 compared to the inadequate D3 diet which is not supplemented with boron, in the same way, the same researcher have not observed any effect on growth in laying hens supplemented with boron (50 to 200mg/kg) for 120 days (17) Eliot and Edwards (18) stated that the body weight gains were not affected in broilers fed with boron diets containing 0, 20, 40, and 80 mg/kg. By contrast, (19) reported that the body weight significantly increased in broilers fed diet supplemented with 37.4 mg/kg for the first 21 days of experiment as well as, in birds supplemented with 57 mg/kg boron during the whole experimental period. While (10) showed that body weight and weight gain significantly reduced by addition of 200 and 400 mg/kg. The discrepancies observed among the different studies could be related to the use of various boron sources or may be due to environmental experimental conditions. The positive effect of boron on body weight and weight gain may be attributed to the role of boron on certain metabolic processes of enzymes, minerals of chickens (20), boron supplementation tended to increased bone ash and that there was a significant interaction between B and vitamin D bodyweight gain(21). The result of effect of boron supplementation on blood pictures showed that there was a positive effect on all parameters, it seems that also could affected blood cell counts and composition because blood cell formation and maturation are influenced by changes in cell membrane or kidney function or in calcium metabolism (20). However, there were only limited data on blood cell variables affected by boron supplementation in chicks or other animal spp, boron increase the response of cell membrane of blood cell to erythropoietin and this hormone synthesized in the kidney which plays a role in the maturation of red blood cell precursors in bone marrow (22) for the mortality rate the addition of boron caused significant decreased of mortality percentage of chicken, this result are in line with previous study (23, 24) this can be attributed to the doses used in this study, as 75,150 and 250 mg/kg were tolerated by the broilers because of boron is absorbed rapidly and virtually completely from gastrointestinal tract and rapidly excreted (23), the weight of internal organs (liver and kidney) influenced by boron supplementation and there was significant effect in the weight of them and this result are in agreement with (25) who found that boron supplementation at 10 and 20 ppm determined a significant effect on the liver weight, also the weight of bursa and fabricius have positive effect by boron supplementation and this result are in agreement with (10), The improvement of growth performance that caused by addition of B in diet of chicken reflexed on the health status of the chicken which enhance immune response of Newcastle vaccine, the significant increased of Ab titers in the present study perhaps attributed that the addition of boron could obviously improve the cellular and humeral immune response and immune function by enhance Fc receptor, expression and interleukin-6 production of cytokines by increasing the y production of tumor necrosis factor and interferon-y after a stress or disease challenge (26), This result are in agreement with (10) who found that the supplemented with 100 mg/kg of boron by drinking could obviously promote boron thymus development of chicken also found that higher boron (exceed 200 mg/litter) has inhibition and toxicity effects on the growth and on the thymus development. In conclusion, this study have shown that supplementation of 75,150 and 250 mg/kg of B in the feed of chicken cause improvement of growth performance, blood parameters, and do not inhibition the immune response of chicken against Newcastle disease vaccine.

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