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Contents

Epidemiological and Molecular Studies of Hydatid Cyst in Slaughtered Cattle and Sheep in Toukh, Egypt

Reham, S. EI- Madawy[1], Nashwa, O. Khalifa[2] and Jehan, S. A. Afify [3]

Faculty of Veterinary Medicine, Departments of Parasitology[1], Zoonoses[2] and Food Hygiene[3], Benha University-Kalubia Egypt.

Abstract

Hydatidosis is one of the most important parasitic zoonoses all around the world. The hydatid cysts were collected from slaughtered cattle and sheep in Toukh abattoir, Kaliobia governorate, Egypt. Cyst fluid was obtained from hepatic and pulmonary cysts for demonstration of protoscolices and hooklets. The prevalence of infection of hydatid cyst was 12.71 % and 7.87% among examined cattle and sheep respectively, 42.66% and 38.46% had hydatid cysts in liver respectively, while the infection rate was 36% and 46.15% in the lung respectively. The rate of fertile cysts was found to be 32 (61.53%) in liver and 33(64.70%) in lung of slaughtered cattle and sheep. PCR amplification was used for identification of internal transcribed spacer gene 1 (lTS1) of fertile hydatid cysts obtained from cattle and sheep by using specific primer. The amplified DNA fragment was further analyzed by PCR mediated restriction fragment length polymorphism (PCR-RFLP) using two restriction enzymes (MSPl and RSAl).The PCR yielded similar amplified DNA band of the same molecular size marker at 1115 bp in different isolates of Hydatid. No band variation of ITS1 gene could be detected by PCR- RFLP by using two restriction enzymes. Amplification product of I1'SI after digestion with MSP1 showed at 661 bp and 406 bp, while those restricted with RSA1 enzyme appeared at 745 bp and 360 bp.

Introduction

Echinococcosis caused by Echinococcus granulosus is one of the most zoonotic parasites in the Middle East and Arabic North Africa from Morocco to Egypt (23). The adult worm lives in the small intestine of carnivore (definitive hosts) and the larval form (hydatid cyst) found in the internal organs of a wide range of mammals including human who acquires infection through accidental ingestion of tape worm eggs (26) . The incidence of human infestation is about 1-2 / 1000 person in rural areas of infested regions (1). The disease has a considerable impact in both human and animal health, with important economic consequences arising out of the cost of medical treatment

and morbidity for human cases and losses in animal productivities (27). These losses can take the form of a reduction in liver weight gain, reduced yield of milk, reduction in the fertility rate and reduction in the value of wool or other products. Also totally or partially discard of infected organs cause annual largest costs' could be as high as 10% or more (28). Estimation of cyst fertility rate is highly desirable because it provides valuable information on the epidemiology of the disease (25).The higher percentage of fertile cysts were in sheep and goat, indicating that sheep and goat are the most important intermediate hosts for Echinococcus granulosus (15). Regarding its molecular characterization E.granulosus poses a high degree of genetic diversity based on genome pattern, morphology and host specificity have allowed the differentiation of at least 10 strain genotypes (Gl-G10) (20). There are two main groups of genotypes sheep strain (G 1) and camel strain (G6) were found in North Africa (3), Eastern Africa (7) and in Tunisia (19).

The present epidemiological study was conducted in slaughtered cattle and sheep and the prevalence of hydatid infection was determined, aiming to identify ITS 1 gene concerning hydatid cyst isolated from cattle and sheep in Egypt by using PCR followed by further identification By PCR- RFLP by using two digestive enzymes MSP 1 and RSA1.

Material and Methods

Sampling:

The hydatid cysts were collected from slaughtered 590 cattle and 660 sheep in Toukh abattoirs, Kaliobia go vernorates, Egypt. The Slaughter houses were visited twice a week from first January 2009 to the end of December of the same year. Carcasses were thoroughly examined for detection of hydatid cysts according to the technique recommended by (10), including observation, palpation and examination of the liver and lung.

Microscopic Identification of Hydatid Cysts:

The suspected infected organs were collected from slaughtered animals for routine microscopic examination according to (14) . Cyst fluid was obtained from pulmonary and hepatic cysts for demonstration of protoscolices and hooklets. Protoscolices were isolated from the fertile cysts and then washed three times by phosphate buffer saline (PBS), pH7.2 and preserved in 70% alcohol (v/v) for isolation of DNA (30).

PCRAssay:

Amplification of ITS 1 gene was done by using of primers described by (S), The primer was designed as forward 5' GTC GTA ACAAGG TTT CCG TA'3 and reverse 5' TCT AGA TGC GTT CGA A(G/A) TGT CGA

TG'3 .The specific primer was supplied from Jena, Bioscience, Germany. A100-bp DNA was used as molecular size marker.

Isolation of DNA:

Total genomic DNA from cystic E. granulosus was isolated according to (2). In brief the protoscolices were suspended in 500ul of CTAB buffer and transferred to a microfuge tube, incubated for 15 min. at 55°C in water bath, then the mixture was centrifuged at 12000 r.p.m for 5 min. and the supematant transferred to a clean microfuge tubes. To each tube 250ul of chloroform: IsoAmyl alcohol (24:1) was added and the solution then mixed by inversion. The mixture span at 13000 r.p.m for 1 min. Here the upper aqueous phase contained the DNA which is transferred to a clean microfuge tube. To each tube 50 ul of 7.5 M Ammonium. Acetate was added followed by 500 ul of ice cold absolute ethanol to precipitate the DNA. The precipitate was transferred into a microfuge tube containing 500 ul of ice cold 70% ethanol and then centrifuged at 13000 rpm for 1 minute supernatant discarded and the remaining DNA pellet washed by adding 70% ethanol, then centrifuged at l3000 rpm for 1 min. and again the supematant removed and the DNA re suspended in DNAse and incubated at 65°C for 20 min and stored at 4 °C.

PCR Procedure for Amplification of DNA:

The amplification reaction was carried in 25 ul volume containing 500mM Kcl, 10 mM Tris- Hcl (PH9.0), 1% Triton x- 100,4 mM Mgcl, 100uM dNTPs each, 15-20 ng of ITS 1 primer, 25 ng of DNA and 1.5 units of Tag DNA polymerase.For data analysis PCR assay was performed in thermal cycler (Teche TC - 512UK) .The DNA was denaturated for 6 min. at 95°C. The mixture was then' subjected to 30 cycles of denaturation at 94 °C for 45 sec., annealing of primers at 55°C for 60 sec.and primer extensions at 72°C for 90 second. The final extension was held at 72°C forI min. PCR products were analyzed after electrophoresis in 1.5% (W/v) agarose gel and visualized in ethidium bromide. The data analyzed by using GelPro analyzer V4.

PCR - Mediated RFLP:

PCR product derived from hydatid cyst were digested with MSP1 and RSA1 (10u) using buffer recommended by the manufacture (Jena Bioscience, Germany). Restriction fragments were separated by gel electrophoresis through 2% TBE agarose gel.

Results

Table (1) declared the prevalence of infection of hydatid cyst in slaughtered cattle and sheep, a total of 75(12.71 %) of 590 cattle and 52 (7.875%) of 660 sheep were infected with hydatid cysts. Postmortem examination revealed that hydatid cyst was found in 32(42.66%) examined

liver and in 27(36%) examined lung of slaughtered cattle, while it was 20(38.46%) and 24(46.15%) of liver and lung of sheep respectively. The rate of fertile cysts was 32(61.53%) in livers and 33(64.70%) in lungs of cattle and sheep. The result of PCR amplification of ITS 1 gene of hydatid cysts showed similar pattern of PCR product of all isolates with amplified DNA band of the same molecular size at 1115bp on agarose gel (Fig. 1) Molecular analysis by PCR-RFLP patterns of ITS 1 gene of cattle and sheep isolates, all showed no variation and produce identical pattern in all examined isolates with Msp I and Rsa1 (Fig. 2and 3). Amplication product of ITSI after digestion with MSP 1 showed at 661 bp and 406 bp, while those restricted with RSA1 enzyme appeared at 745 bp and 360 bp.

Discussion

Echinococcus granulosus is medically and economically one of the most important zoonoses. Hydatid cyst develops in the internal organs of human and herbivore intermediate hosts, mainly in the liver and lung (21). In Egypt the prevalence of hydatidosis has been highlighted by surveys among animals in Assiut and Aswan governorates showed that hydatid cyst in camel was 107(7.67%) out of 1395, but no infection in cattle and buffaloes (8), whereas (22) found the incidence rate was 6.4% and 5.27% in sheep and goat respectively. (13) reported it as 0.3 % in sheep and 6.4% in cows in Mansoura official abattoirs. In the present study conducted in Toukh abattoir, Kaliobia governorate, the prevalence of infection was found to be 12.71 % in cattle and 7.87% in sheep .The higher rate of infection in examined animals is attributed to cattle and sheep are widely raised outdoor and high number of stray dogs involving as definitive host and cattle and sheep as intermediate hosts.

A study previously performed in the same locality of this work, reported that hydatid cyst was localized in liver (5.7 %) and lung (6.4 %) of sheep (22). It was (0.21 %) and (0.34 %) in liver of slaughtered cattle and sheep in EI- Bassatin abattoir, Cairo (29). The current study indicated that the rate of infection in cattle was higher in liver 32 (42.66 %) than in lung 27 (36 %). An observation in accordance with that noticed in Turkish cattle by (18). Hydatid cyst was 20 (38.46 %) in liver and 24 (46.15 %) in lung of infected sheep .It appears that hydatid cyst in Egyptian sheep has seen most frequently in lung followed by liver .A finding previously observed by (12) and (22) and attributed to the easy passage of oncosphere through the relatively wide liver capillaries to settle in narrow sized lung capillaries developed to hydatid cyst (9).

Based on the epidemiology and molecular studies, the fertility of cyst is one of the most important factors in the epidemiology of Echinococcus granulosus. The fertility of cyst varies depending on the hosts and geographical

situations (17). The sheep strain (G1) is the' predominanting Echinococcus species in the Mediterranean countries (24). Another factor which determines the fertility rate of hydatid cyst is the type of strain (17). It is reported that sheep strain (G 1) of E. granulosus produces fertile cyst in cattle (6).(24) has found the fertility rate was 63% and 82% in liver and 72% and 79% in lung of sheep and cattle respectively. In the present work the fertility rate of hydatid cyst in slaughtered cattle and sheep has been found to be 32(61.53%) in liver and 33(64.70 %) in lung of examined animals. The high rate of fertile cyst may indicate that the cause of infection in investigated animals might be due to sheep strain (Gl). As such genotype is commonly recognized as a predominating species of E. granulosus in Mediterranean countries (16).

Molecular genetics study has been carried out to identify the genetic characters of hydatid cysts in cattle and sheep where DNA fingerprints were indistinguishable from one another and PCR yielded similar amplified DNA band of the same molecular size marker at 1115bp in different isolates of hydatid. This may be due to the samples were collected from the same locality. Our finding coincided with that of (7) in Eastern Africa, (3) in North Africa and (19) in Tunisia.

In the present work the use of RFLP technique of ITS1 gene indicated that all isolates of examined cattle and sheep produced identical patterns with the individual enzymes used MSPl and RSA1. DNA amplification product of ITS I gene showed at 661 bp and 406 bp after restriction with MPS 1, while those restricted with RSA1 enzyme appeared at 745 bp and 360 bp. This result is indicative of absence of ITS 1 variant which could not be discriminated by using these two restriction enzymes. Our results agree with that gained by (4). (11) found that the molecular characterization of human and animal isolates by using PCR- RFLP of ITSI and morphological criteria, the sheep strain was the most common genotype in Iran.

We concluded that the obtained epidemiological results as well as molecular approach indicated high fertility rate and absence of variation in amplified of ITS I and indistinguishable genetic character in PCR -RFLP, revealed that cattle and sheep are infected with E. granulosus assumed to be sheep strain. In the future more researches are required to determine genotypes of E.granulosus in human and animal.

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Table (1) the prevalence of infection of hydatid cyst in slaughtered animals.

Slaughtered animals	Total examined number	Positive hydatid cysts		Liver Hydatid cyst				Lung Hydatid cyst			
				positive		fertile		positive		fertile	
		No.	%	No.	%	No	%	No.	%	No	%
Cattle	590	75	12.71	32	42.66	19	59.3.7	27	36	14	51.85
Sheep	660	52	7.87	20	38.46	13	65	24	46.15	19	79.16
Total	1250	127	10.16	52	40.94	32	61.53	51	40.15	33	64.70

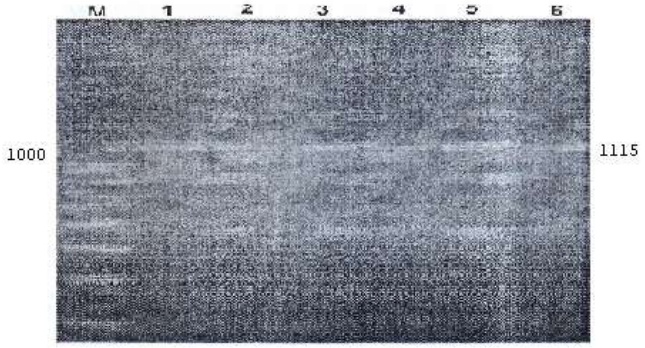

Fig.l Ethidium bromide stained agarose (2%) gel showing amplification product of ITSI of E.granulosus by PCR .lane M: a 100 bp molecular size marker; lane +1115 bp; lane 1,2,3 cattle host DNA; lane 4,5,6 sheep host DNA.

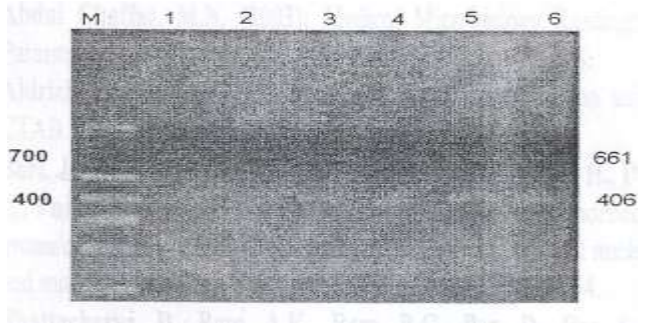

Fig.2: Ethidium bromide stained agarose (2%) gel showing amplification product of ITSI of E.granulosus by PCR after digestion with MSP1 .1ane M: a 100 bp molecular size marker; top to bottom arrows:661 bp ,406 bp; lane 1,2,,3 cattle host DNA; lane 4,5,6 sheep host DNA.

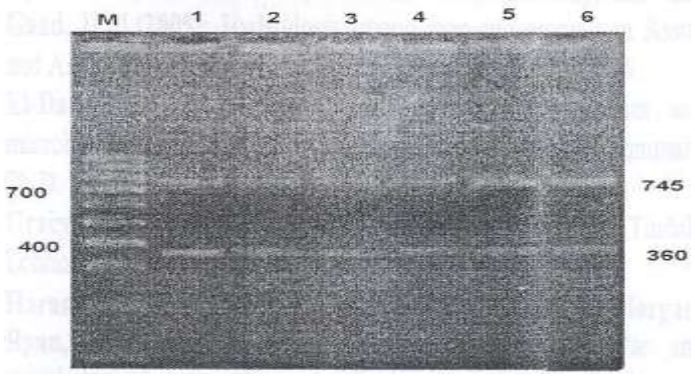

Fig.3 Ethidium bromide stained agarose (2%) gel showing amplification product of ITSI of E.granulosus by PCR after digestion with RSA1 .lane M: a 100 bp molecular size marker; top to bottom arrows:745 bp ,360 bp ; lane 1,2 ,3 cattle host DNA; lane 4,5,6 sheep host DNA.

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الملخص العربى

دراسات وبائية وجزيئية عن داء الاكياس المائية فى الماشية والاغنام المذبوحة بطوخ – مصر

و جيهان سيد أحمد عفيفى[23] ونشوى عثمان خليفة [1]ريهام سمير المعداوى

قسم تنمية الطفيليات، قسم الامراض المشتركة[3] و قسم المراقبة الصحية على الاغذية [3] [1]
كلية الطب البيطرى، جامعة بنها

داء الاكياس المائية من الامراض المشتركة ذات الاهمية الصحية العامة على مستوى العالم. لذلك اجريت هذه الدراسة على الماشية والاغنام المذبوحة عام 2009 بمجزر طوخ– قليوبية – مصر. تم الحصول على الاعضاء الداخلية الاكباد والرئات بعد اجراء الكشف البيطرى عليها وتم نقلها الى المعمل لاجراء التجارب المعملية. حيث تم الحصول على السائل الداخلى لهذه الاكياس للكشف الميكروسكوبى والتمييز بين الاكياس الخصبة والعقيمة. وكانت نسبة الاصابة 12.71 % فى الماشية و 7.87 % فى الاغنام ووجد ان نسبة الاصابة بالحويصلات اكبر فى كبد الماشية عن الرئة بينما وجد العكس فى الاغنام. و اسفر الكشف الميكروسكوبى عن وجود الحويصلات الخصبة بنسبة 61.53 % فى كبد و 64.70 % فى رئة الماشية والاغنام. تم الاحتفاظ بالمحتوى الداخلى للحويصلات الخصبة لاجراء اختبار البلمرة المتسلسل لتشخيص البصمة الجينية الخاصة بالاكياس المائية. وقد اسفرت نتائج اختبار البلمرة المتسلسل عن عدم وجود اختلاف فى البصمة الجينية للحامض النووى الديوكسى فى عينات الاكياس المائية فى كلا من الماشية والاغنام المصابة . وقد تم مناقشة الاهمية الصحية العامة للمرض واقتراح اجراء اختبارات جزيئية مستقبلية للتوصل الى النوع الجينى لداء الاكياس المائية فى الانسان والحيوانات المصابة فى مصر.

Combined intravenous anaesthesia with midazolam and propofol for performing abdominal surgery in sheep.

Adel M. Badawy[1], Mohamed M. Ghanem[2]

[1] Surgery Dept., Faculty of Veterinary Medicine, Benha University
[2]Animal Medicine Dept., Faculty of Veterinary Medicine, Benha University

Abstract

The aim of this study was to evaluate the combination of midazolam and propofol anesthesia as a safe anesthetic protocol for performing abdominal surgery in sheep. To achieve this purpose, five mature non-pregnant ewes were administered intravenously with a dose of 3mg/kg propofol, 10 min after administration of 0.6mg/kg midazolam as a premedication. During the period of anaesthesia, these animals were subjected to the standard rumenotomy technique. The evaluation criteria included changes in heart and respiratory rates, rectal temperature, different body reflexes, and changes in hepatic enzymes activities. Scoring for sedation, induction and recovery were also recorded. The result showed that midazolam induced good sedation in 4/5 of the injected sheep. In addition, propofol induced profound smooth anaesthesia in 4/5 of the injected sheep. The average full recovery time was 48.6 minutes and the average period of surgical rumenotomy was 28.2 minutes. The heart rate was significantly increased after propofol anesthesia while the respiratory rate was significantly reduced. Rectal temperature was not significantly changed. These parameters returned to normal values after recovery. Serum hepatic enzymatic activities of ALT and AST were non significantly increased during the period of anaesthesia and then return to decline at the recovery time (start with the walking and swallowing). It was concluded that, the intravenous anesthesia with midazolam and propofol combination in sheep, is a feasible protocol to perform short term surgical procedures under field conditions with smooth, rapid recovery and without causing adverse effects on clinical status and the hepatic enzymatic activities of the anaesthetized sheep.

Introduction

Sedatives used for calming small ruminants include U2 adrenoceptor agonists, such as xylazine, phenothiazines (like acepromazine), enzodiazepines (like diazepam) and midazolam and opoids (like butorphanol) (8) and (32). Sedatives are used pre-operatively to induce sedation, improve the quality of induction of anaesthesia and more importantly, minimize drug-related adverse

effects by reducing the amount of injectable or inhalation anaethetics required to induce and maintain general anaesthesia (16), (18) and (34).

Midazolam is water soluble benzodiazepines that are considered to be fast acting with a short elimination half-life (3) and (20). Unlike diazepam, it can be administered by intramuscular route as well as the intravenous route (8) and (18). Midazolam has mild cardiovascular and respiratory effects and is commonly used as a mild tranquilizer, muscle relaxant and anticonvulsant (18) and (24). Benzodiazepines have agonistic effects on specific benzodiazepines receptors located in the postsynaptic nerve endings within the central nervous system (3) and (23). The resultant increase in availability of the inhibitory neurotransmitter glycine leads to the anxiolytic and muscle relaxant effects. The sedative and hypnotic effects of midazolam are dose-dependent as well as dependant on route of administration. Midazolam can produce maximal sedative effects in 20 minutes after intramuscular administration of 0.6 mg/kg and 5 minutes after intravenous administration of the same dose (13) and (37). Midazolam has more hypnotic, anticonvulsant muscle relaxing and amnesic effects than other benzodiazepines; it is 1.5-2 times as potent as diazepam (24) and (39). Furthermore, midazolam in comparison with diazepam, it is 4 times more potent in goats (36). The administration of midazolam premedication in small ruminants has greatly led to overcoming and preventing occurrence of many adverse effects that encountered when an anaesthetic administered without such premedication (25). Salivary levels appeared to be related to depth of sedation. This has been particularly noted with benzodiazepines sedation, in which sedation is significantly correlated with saliva level (5). Benzodiazepines are known to infrequently, cause dry mouth (13) and (26).

Propofol (2, 6-diisopropyl-phenol) is one of the induction agents commonly used in goats because it has a rapid, smooth onset of action and is cleared rapidly from the tissues (10), (17) and (32). Propofol is slightly soluble in water and is marketed as an aqueous emulsion containing 10 mg of propofol, 100 mg of Soya bean oil, 22.5 mg of glycerol and 12 mg of egg lecithin per ml. Propofol emulsion is capable of supporting microbial growth and endotoxin production (2) and (18). Propofol has been used extensively in human beings and animals. It has a high volume distribution, rapid metabolism and rapid clearance when given by repeated doses or continuous intravenous (IV) infusion (2), (12) and (32). The administration route has little effects on the magnitude of propofol uptake into titration to effect, rather than producing more anaesthesia for a given dose (20), (21) and (38). The rapid onset and short duration of action, with rapid recoveries make the drug potentially useful in ruminants, in which these features are particularly desirable (4), (27) and (33). Propofol causes a dose- related

decrease in blood pressure due to peripheral vasodilatation and myocardial depression, bradycardia, epileptic form, seizures and true convulsions (2) and (35). The indicated dose of 3mg/kg of propofol was sufficient for induction of anaesthesia. Propofol when administered at a dose 4-7 mg/kg intravenously in unpremedicated goats and sheep induces sufficient anaesthesia for endotracheal intubation (10), (29) and (32), while a dose of 3mg/kg was proved sufficient in premedicated goats (I). In a very few studies in sheep, propofol was studied from some certain aspects and experimented either alone or with other anaesthetic or premedication. The anaesthetic propofol infusion was used either alone or combined with ketamine in sheep (25).

The objective of this study was to evaluate the effect of the combination of midazolam and propofol anesthesia as a safe anesthetic protocol in sheep for performing abdominal surgery with minimal risk and without adverse effect on the clinical and biochemical status of the anaesthetized animals.

Material and Methods

Animals:

Five mature non-pregnant ewes were used in this study, their body weight ranged from 38-47 kg. The animals were deprived of food for at least 12hours but had access to water for up to 2hours before the anaesthesia. The skin overlying the left jugular vein was clipped and a catheter was introduced into the jugular vein and fixed in place by adhesive bandage.

Anaesthetic protocol:

Ten minutes after intravenous administration of the midazolam (Dormicum)* by a dose of 0.6 mg/kg as a premedication, propofol (Diprivan)** was injected intravenously by dose rate of 3 mg/kg to induce a level of adequate anaesthesia for performing rumenotomy in these animals (8).

Surgical procedures :

The animals were prepared aseptically for rumenotomy (7) for assessment of the injected drugs through evaluation criteria, blood analysis and measurement of the duration of the operation, anesthesia and recovery time.

Dormicum*; F. Hoffmann-La Roche Ltd Basel, Switzerland by CENEXI SAS Fontenay-sous - Bois, France)

Diprivan **; 1 %; AstraZeneca SpA, Caponago, Milan, Italy for AstrZeneca UK Limited, Macclesfield, Cheshire, SKlO 2NA, United Kingdom).

Evaluation criteria :

Heart and respiratory rates and rectal temperature were measured immediately before administration of the drugs (-1), immediately after injection of the drugs (at 0 time) and at la-minute intervals during the period of drugs effect until full recovery was obtained (6) and .(14)

The different body reflexes were evaluated at 1 a-minute intervals after administration of the midazolam and after induction of anaesthesia by propofol. These reflexes included pedal and palpebral reflexes, swallowing reflex (31), cough reflex (30) and tongue reflex (15).

The quality of sedation, general anaesthesia and recovery times were assessed by sedation, induction and recovery scoring .(19)The degree of sedation was scored 10 minutes after administration of midazolarn as it is most probable that the sedative effects were optimum at this time.

Sedation scoring:

- Good sedation =the animal assumes sternal recumbency, and may raise the head without holding it up.
- Fair sedation = cough reflex was positive.
- Poor sedation = most of response to the body reflex were positive.

Induction scoring:

- Good induction =smooth, rapidly without signs of excitement.
- Fair induction =slightly prolonged, mild excitement.
- Poor induction =obvious excitement, attempts to stand after recumbancy.

Recovery scoring:

- Good recovery =smooth, easily resume sternal position, stand in a reasonable amount of time and able to walk with minimal degree of ataxia.
- Fair recovery = transient restlessness, excitement some struggling and paddling with moderate degree of ataxia.
- Poor recovery = Prolonged paddling and struggling, unsuccessful attempts to stand.

Blood analysis :

The activities of hepatic enzymes were determined at basal time (-1), 10 minutes after induction of anaesthesia and after recovery to evaluate the effect of propofol anesthesia on these enzymes. Therefore, peripheral blood samples were collected into 4-ml Vacuette tubes without anticoagulant (Greiner Bio-one GmbHy, Kremsmunster, Austria) and the serum was harvested after centrifugation at 5000 rpm for 5 minutes. The serum samples were then stored at -20°C until assayed. Commercial kits were used for spectrophotometric determination of the activity of alanine aminotransferase

(ALT) and aspartate aminotransferase (AST) (Diamond company, Egypt) on a selective chemistry analyzer (Abbott Alcyon 3001, USA) (11).

Statistical analysis :

The results were presented as means ± standard error (M ± SE). The changes of heart rate, respiratory rate, rectal temperature as well as, serum hepatic enzymes were compared with their baseline values (before administration of midazolam and propofol) using student t-test. The statistical analyses were all performed using SPSS (version 13 for windows; SPSS Inc., Chicago, IL. USA). The results were considered statistically significant when $P < 0.05$.

Results

Midazolam intravenous injection in a dose of 0.6 mglkg resulted in sedation of the animals. The sedation was good in 4/5 of injected animals, where ataxia occurred after 10.2 ± 0.3 seconds after injection of midazolam. These animals fall to the ground within 16.4 ± 0.4 seconds, while 1/5 of animals revealed poor sedation as it suddenly falls to the ground with obvious excitement, struggling and attempts to stand.

In respect to the tested reflexes, 2/5 of the sedated animals lost the pedal reflex, 4/5 of the animals lost the cough reflex ten minutes after midazoalm administration, while all animals lost the swallowing and tongue reflex. The results of responses of the animals to the tested reflexes are showed in table 1.

Table (1): The effect of midazolam on the tested reflexes ten minutes after its intravenous injection (N= 5).

Reflex	Pedal reflex	Palpebral reflex	Cough reflex	Swallowing reflex	Tongue reflex
A	2/5	0/5	4/5	5/5	5/5
Percent	40%	0%	80%	100%	100%

A= number of animals lost the reflex

Profuse salivation was obvious in 115 of animals five minutes after midazolam administration. No evidence of regurgitation of the ruminal contents detected in the pharynx or exit through the mouth or nostril.

The induction of anaesthesia by intravenous administration of propofol in a dose of 3 mglkg was good in 4/5 of anaesthetized animals, while only one animal showed fair prolonged induction as it appeared with mild

excitement which disappeared 2 minute later to injection of propofol, Regurgitation was not observed after propofol administration. Profuse salivation was observed in 1/5 of injected animals and disappeared after 15 minutes.

The results of responses of the animals to the tested reflexes after intravenous administration of propofol and the time of their disappearance were showed in table 2.

Table (2): The effect of propofol induction on the tested reflexes five minutes after its intravenous injection and duration of its disappearance (Mean± SE) (N= 5).

Reflex	Pedal reflex	Palpebral reflex	Cough reflex	Swallowing reflex	Tongue reflex
A	5/5	4/5	4/5	5/5	5/5
Percent	100%	80%	80%	100%	100%
D	28.5±1.2	23±1.3	36.3±1.5	65±1.8	15.5±0.8

A= number of animals lost the reflex
D= duration (minutes) of disappearance of the tested reflexes after propofol induction
of anaesthesia.

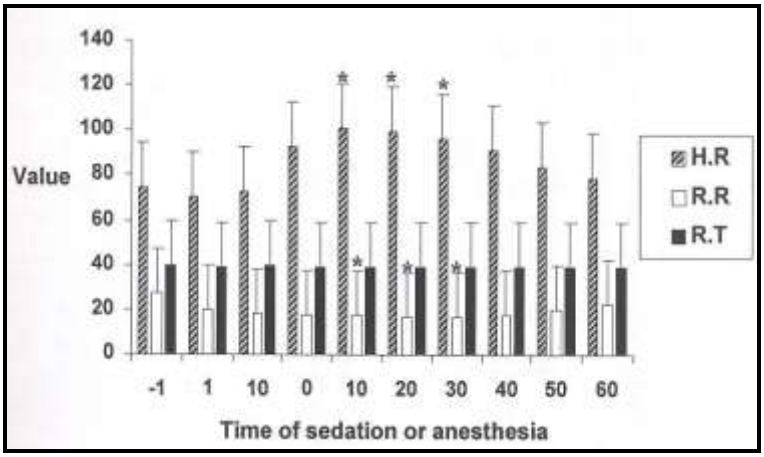

Fig (1): The mean of heart rate (H.R) (beat/minute), respiratory rate/minute (R.R) and rectal temperature (R.T) C" and their relation to time before and during the period of sedation by midazolam and anesthesia by propofol (*= significant at P<O.05) (Mean± SE) (N= 5)

Table (3): The heart rate (H.R.) (beat/minute), respiratory rate (R.R.) (movement/minute) and rectal temperature (R. T) CO before and during the period of sedation and anesthesia (Mean ± SE) (N= 5).

T / P	-1	0_1	10	0_2	10	20	30	40	50	60
H.R	74.2 ±1.4	70.6 ±1.3	*72.8 ±1.3*	92.6 ±1.9	101 ±1.5*	99.6 ± 1.3 *	95.5 ±1.3*	90.8 ± 1.8	83.6 ±1.4	78.4 ±1.7
R.R	27.4 ±0.2	20 ±0.4	18.4 ±0.6	17.6 ±0.4	17 ±0.3*	16.4 ±0.4*	16.6 ±0.2*	17.6 ±0.2	20 ±0.7	22 ±0.5
R.T	39.2 ±0.1	39 ±0.1	39.4 ±0.1	39 ±0.05	38.8 ±0.l	38.8 ±0.1	38.8 ±0.l	38.8 ±0.1	38.8 ±0.l	38.8 ±0.1

P= parametaer T= Time

-1 = before administration of midazolam,

0_1andO_2=immediately after administration of midazolam and propofol, respectively.

***= significant at P<0.05**

Table (4): Showed the duration of stages of anaesthesia and full recovery (minutes) (from the start of induction of anaesthesia) (Mean± SE) (N= 5).

Item	Surgical anaesthesia	Sternal recumbency	Full recovery
Duration	28.2±1.3	33.3±1.6	48.6±1.5

Table (5): Effect of midazolaml propofol anaesthesia on liver enzymatic activities (Mean± SE) (N= 5).

Time	-1	10 min	After recovery
ALT(U/L)	17.89±2.4	19.87±3.9	19.41±3.2
AST(U/L)	66.98± 4.8	70.52± 5.7	70.27± 3.2

-1 =before administration of midazolam or propofol (Basal time). 10 min =10 minutes after propofol administration.

The results of heart and respiratory rates and rectal temperature before and after intravenous administration of midazolam and also propofol were showed in table 3 and figure 3.

Heart rate showed a non-significant decrease after midazolam sedation, but later on it was significantly increased at 10, 20 and 30 minutes after propofol administration. The respiratory rate was significantly reduced at 10,20 and 30 minutes after propofol anaesthesia. There were non significant reduction in the rectal temperature after sedation and anaesthesia; its values remained within normal limits.

The period of surgical anaesthesia and total recovery time were showed in table 4. The duration of rumenotomy was 28.2 ± 1.3 minutes from the total recovery time 48.6 ± 1.5 minutes. The animal assumed sternal recumbancy 33.3± 1.6 after the induction of anaesthesia.

Serum hepatic enzyme activities including AL T and AST were non significantly increased after injection of midazolam and propofol, (Table 5).

Discussion

The objectives of this study is to provide a reversible unconsciousness, analgesia, muscle relaxation with rapid and smooth onset of action and adequate duration of anesthesia with minimal risk to the animal. Salivation of the anesthetized animals in this study was not profuse and recorded only in 20% of animals. This might be attributed to failure of swallowing; this observation supported by other studies (5) and (26). Sedation produced after intravenous administration of 0.6mg/kg of midazolam was smooth, feasible without excitement. This result is in agreement with different authors (12), (28) and (40) who reported that; midazolam has hypnotic, anticonvulsant, muscle relaxing and amnesic effects. Poor grade of sedation with obvious excitement and struggling and paddling and attempts to stand was found in 20% of animals after midazolam administration. This finding might be due to the rapid onset of the drug action and their sudden falling to the ground, this results has a great similarity with (9). Propofol is one of the induction agents commonly used in goats, as it has a rapid onset and short duration of action and cleared rapidly from tissues (10) and (32). The induction dose of propofol used in this study was 3mg/kg IN as it administered after midazolam sedation. This event is supported by (8), (27) and (29), who reported that the dose of propofol for induction of general anesthesia in unpremedicated goats 5.1-5.5 mg/kg, however, a significant decrease (39.7%) in propofol induction dose requirements were observed in goats received midazolam. In the present study, it was observed that, the full analgesic effect of the propofol attained after 5 minutes; this result is in the same line with (8), who reported that the full anaesthetic effect of propofol takes 5 minutes to be happened. As rapid recovery is desirable in ruminants because extended recumbency enhances the risk of tympany and hypoxaemia .(10)Rapid recovery of swallowing reflex minimizes the risk of aspiration of regurgitated rumen content. In this study, the full recovery time was 48.6± 1.5 minutes and it is shorter than that reported by (9), 54.6 minutes after ketamine/diazepam anaesthesia in goats but resembling that recorded by (29), who reported that recovery time is significantly shorter after propofol compared with thiopental or ketamine. Furthermore, propofol anaesthesia was maintained and persisted for 45 minutes (25).

Regarding the recovery grade in the present study, it was good,smooth and eventful without regurgitation or excitement, this finding is similar to that recorded by (29) and disagrees with (9), who reported paddling during recovery of goats after diazepam I ketamine anaesthesia as ketamine characterized by poor muscle relaxation and muscle tone often increased. Also in contrast to that recorded by (25) who stated that, recovery was obtained with observation of many adverse effects like struggling, convulsions.

The period of rumenotomy was 28.2 ± 1.3 minutes from a total recovery time (start with the walking and swallowing) of 48.6 ± 1.5 minutes. This indicated that more than 50% of the total recovery time was used as a period of surgical anaesthesia. These results proved that, this anesthetic protocol has a good, feasible anesthetic and analgesic effect. A similar finding was previously reported (4) and (8). The primary disadvantages of propofol are cost, lack of FDA approval (Food and Drug Administration), limited shelf life once the ampoule is opened and risk of iatrogenic sepsis (18).

Many selected body reflex were examined during the period of sedation or the period of anesthesia in attempt to determine accuracy of these reflexes in observation of these anesthesia (15), (30) and (31).

Concerning the clinical and biochemical changes, heart rate showed a non-significant decrease after midazolam sedation, but later on it was significantly increased at 10, 20 and 30 minutes after propofol administration. These findings are in agreement with (29). The decrease in heart rate after midazolam sedation might be attributed to decrease in the muscle tone and vasodilatation (22). On the other hand, the cause of increased heart rate during propofol anaesthesia is not clear. It is possible that compensatory tachycardia could occur as a result of reduced respiration to avoid tissue anoxia. This could be true especially the respiratory rate was significantly reduced 10 minutes after propofol anaesthesia. There were no significant changes in the rectal temperature after sedation or anaesthesia; its values remained within normal limits. Serum hepatic enzyme activities including ALT and AST were not significantly changed after injection of the combination of midazolam and propofol, which support the use of this combination for anaesthesia in sheep due to their minimal adverse effects This result supported by (2)(12) (32) Reid Bettschart 1 and Prassinos 3).

Conclusions

It was concluded that, the intravenous anesthesia with midazolamlpropofol combination in sheep, is a feasible protocol to perform short term surgical procedures under field conditions with smooth, rapid recovery and without causing adverse effects on clinical status and the hepatic enzymatic activities of the anaesthetized sheep.

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الملخص العربى

التخدير الوريدى المجتمع لعقارى الميدازولام و البروبوفول لاجراء جراحة بالبطن فى الاغنام

عادل محمود بدوى [1] ومحمد محمدى غانم [2]

قسم الجراحة، كلية الطب البيطرى، جامعة بنها[1]
قسم الامراض الباطنة، كلية الطب البيطرى، جامعة بنها[2]

الهدف من هذه الدراسة هو تقييم التخدير المجتمع لعقارى الميدازولام و البروبوفول كطريقة أمنة للتخدير لاجراء جراحة بالبطن فى الاغنام. لاجراء هذه الدراسة تم استخدام خمسة اناث من الاغنام ليست عشار، حقنت عن طريق الوريد بعقار البروبوفول بجرعة 3 مجم /كجم بعد 10 دقائق من حقن عقار الميدازولام بجرعة 0.6 مجم/كجم . تم اجراء عملية فتح الكرش المعتادة للحيوانات خلال فترة التخدير. تم تقييم هذه الطريقة عن طريق قياس معدل ضربات القلب والتنفس ودرجة حرارة المستقيم و ردود الفعل المنعكسة للجسم والتغيرات فى نشاطات انزيمات الكبد. تم تسجيل درجات التهدئة و تقييم التخدير و كذلك الافاقة. أظهرت النتائج ان عقار الميدازولام احدث تهدئة جيدة فى عدد 5/4 من الحيوانات المحقونة ، بالاضافة الى ان حقن عقار البروبوفول احدث تخدير ناعم وعميق فى 5/4 من الحيوانات المحقونة. كان متوسط وقت الافاقة الكلى 48.6 دقيقة ومتوسط الوقت الجراحى 28.2 دقيقة. كان هناك زيادة جوهرية فى معدل ضربات القلب بعد حقن البروبوفول بينما حدث انخفاض جوهرى فى معدل فى معدل التنفس. درجة حرارة المستقيم لم تتغير تغيرا جوهريا. هذه المعدلات عادت واقتربت من القيم الطبيعية بعد الافاقة. هناك ازدياد غير جوهرى فى نشاطات انزيمات الكبد اثناء فترة التخدير والذى تناقص عند بدء وقت الافاقة. فقد استنتج ان التخدير الوريدى بعقارى الميدازولام و البروبوفول فى الاغنام هو بروتوكول ملائم لاجراء العمليات الجراحية على المدى القصير تحت الظروف الميدانية مع افاقة ناعمة و سريعة و بدون تأثيرات سلبية على الحالة الاكلينيكية ونشاطات انزيمات الكبد فى الاغنام المخدرة.

Effect of Rosemary on Shelf-Life of Fish Fillets During Cold Storage

[1]Fatma , H. M.Ali and [2]Jehan , S.A. Afifi

[1]Food Hygiene department, Faculty of Veterinary Medicine , Beni-Suef university, Beni-Suef, Egypt.
[2]Food control department, Faculty of Veterinary Medicine ,Benha university, Benha, Egypt, P.O. 13736, Moshtohour, Tokh, Kaliobia, Egypt.

Abstract

This work was done to study the effect of rosemary on quality parameters of fish fillets. Experimentally, processed fresh fish fillets of lates niloticus species were prepared and dipped in rosemary extract 2%for 60 minutes. The control and treated samples were divided into two parts .The first part was chilled till spoilage had occurred and the second part was frozen for 12 months. The chilled and frozen samples were sensory, chemical and bacteriological examined. The sensory evaluation of treated fillets showed that chilled samples were kept desirable for appearance, odor and texture till 12 days and rejected at 15th day in corresponding to the control rejected at the 6th day. The frozen samples were desirable to the 9th months and rejected at 12th months in corresponding to control rejected at 6th months. The Peroxide value (PV),Thiobarbituric acid value (TBA, mg malondialdehyde /kg) and Total Volatile Basic Nitrogen (TVB-Nmg/100g) in treated fillets were significantly lower than these of the control(P<0.05). The aerobic plate count at 35oC, psychrotrophic count, Coliform (MPN), mould and yeast counts were 2.87+ 0.14,2.46 + 0.08, 4.19 + 0.32 in fresh fillets, respectively. These counts were significantly lower at p< 0.05 than frozen fillets. Thus, the dipping of fish fillets in rosemary extract 2% is recommended as it is efficient anti-oxidant and keeps the quality parameters of fish fillets stable during chilling and frozen storage.

Introduction

Freezing and frozen storage have largely been employed to retain fish sensory and nutritional properties ***(8).*** Cold storage and freezing are the normally employed methods for fish preservation, but they don't completely inhibit the quality deterioration of fish ***(14).*** Nowadays, it is essential to test new preservation and decontamination procedures using natural occurring

chemicals against important pathogenic bacteria in meat ***(19).*** Beside microbial reduction capacity, additional criteria, such as consumer acceptance, human health aspects, development of antimicrobial resistance and environmental safety have to be considered in implementation of chemical decontamination ***(20).*** Decontamination treatments also must be considered as part of an integral food safety system ***(16).*** Plant extract treatments have largely shown a positive effect on inhibiting the quality loss during the frozen storage of minced and filleted fish products ***(15).*** In this respect,it is important that consumers prefer naturally occurring agents, since green consumerism and use of plant extracts is becoming more and more popular ***(6)*** .Therefor,there is a great demand to screen for suitable natural decontaminants and alternative treatment methods ***(7).*** For the assurance of food safety, nowadays, consumers are demanding food products with natural alternatives to chemical additives and but with increased safety, quality and shelf-life. Much attention has been focused on extracts from herbs and spices which have been traditionally used to improve the sensory, odor or pigment characteristics and extend the shelf-life of foods ***(12).*** Recent research has been focused on the positive role of antioxidant molecules present in the plant ***(18).*** Successful applications of plant extract treatments have been carried on fish fillets ***(21).*** Rosmol-p is a permitted ingredient composed from polyphenol compounds and rosmarinic acid that has been reported to retard lipid oxidation in fish fillets ***(3)*** when employed as a pretreatment of a further frozen storage. Therefore, this work was done to study the effect of rosemary extract as anti-oxidant and antibacterial by dipping fish fillets in rosemary extract with 2% before storage and examined for quality parameters during chilling and frozen storage.

Materials and Methods

Preparation of fish fillets:

Whole fishes ***(Lates niloticus,***a fresh water fish) were freshly harvested, gutted and filleted at fish market, Giza governorate. The fillets were rapidly transferred to the Laboratory with minimum of delay.

Preparation of rosemary extract 2% :

It was prepared at faculty of Agriculture, Department of Chemistry with concentration 2% according to the technique recommended by ***(15).***

Experimental technique :

The prepared fillets were divided into two parts. First part was kept as control, the second part was dipped in rosemary extract 2% for 60 minutes. Each control and treated part was subdivided into two parts. The first part was chilled at refrigerator storage at 4oC and examined every 3 days till spoilage, the other part was frozen in deep freeze at –18 ^{o}C for 12 months and examined every 3 months. Both control, chilled and frozen samples were examined for the following:

1-Sensory evaluation:

It was done according to the technique recommended by ***(10)*** as follow:

Sensory attributes	Desirable	Undesirable
Appearance	Translucent, glossy, natural color	opaque, dull, blood stained, discolored
Texture	Firm, elastic	soft, plastic
Odour	Marine, fresh, neutral	sour, stale, spoiled, putrid

2. Chemical examination:

Estimation of peroxide value (PV meq/kg) by iodine titration method ***(5),*** thiobarbituric acid (TBA) value using the distillation method ***(9)*** and determination of total volatile basic nitrogen (TVB-N) using the distillation method ***(1)*** were applied.The TBA values were expressed as mg malondialdehyde/ kg, while TVB-N were expressed as mg/100 g.

3-Bacteriological examination:

Samples homogenate was prepared by homogenization of 10 g of the examined sample with 90 ml sterile peptone water 0.1%. From fish fillets homogenate, serial decimal dilutions up to 10^{6} were performed. The microbiological procedures recommended by the American Public Health Association ***(2***) were applied as follows Aerobic plate count at 35 C (mesophiles) on standard plate count agar (Oxoid; CM 325) with incubation at 35 ^{o}C for 48 hrs, Psychrotrophic count on standard plate count agar (Oxoid;

CM 325) with incubation at 25 °C for 48 hrs, Coliforms (MPN) on Lauryle sulphate treptose broth and mould and yeast counts on Sabroud dextrose agar media.

Results

Table (1): Sensory evaluation of the examined control and treated fillets during refrigeration storage at 4°C.

Parameters (Samples)	**Desirable** (day)	**Undesirable** (day)
Control	0-6	9
Treated	0-12	15

*=significantly differ at $p<0.05$

Table (2): Mean values of PV mEQ/kg, TBA value mg mal/ kg and TVB-N mg/100 g in control and treated fish fillets samples during refrigeration storage at 4 °C.

Samples	Day 0	Day 3		Day 6		Day 9		Day 12		Day 15
		C	T	C	T	C	T	C	T	T
PV	3.50+ 0.011	8.34 ± 0.02	4.85± 0.2	9.5± 0.42	6.5±0 .2*	17.7 ±0.5 7	7.60 ±0.5	--	8.5± 0.45	11.2± 0.05
TBA value	0.34 ± 0.02	0.96 ± 0.014	0.35 ± 0.013	1.98 ± 0.02 1	0.41 ± 0.06*	2.6± 0.34	0.65 ± 0.02 1	--	0.78 ± 0.01 3	0.89 ± 0.07
TVB-N	13.6 ± 0.23	15.3 ± 0.27	14.5 ± 0.70	19.9 ± 0.60	16.8 ± 0.31*	28.9 ±0.2 5	17.6 ± 0.04	--	19.5 ± 0.22	21.8 ± 0.21

*Significant differences between the control and treated samples at $p<0.05$
C: control fish fillets sample.
T: treated fish fillets sample.

Table (3): Mean values of aerobic plate count at 35oC, psychrotrophic count, coliforms (MPN) and mould and yeast counts log10CFU/g of control and treated fish fillets samples during cold storage at 4oC.

Samples	Day 0	Day 3		Day 6		Day 9		Day 12		Day 15
		C	T	C	T	C	T	C	T	T
Aerobic plate count at 35 °C	3.6± 0.40	3.2± 0.31	2.9± 0.22	5.5± 0.30	4.7± 0.51 *	6.8± 0.23	5.4± 042*	-----	6.5± 0.30 *	6.9± 0.71
Psychrotrophic count	1.57 ± 0.20	2.31 ± 0.012	2.00 ± 0.50	4.76 ± 0.41	3.35 ± 0.31 *	6.73 ± 0.13	4.63 ± 0.33 *	------	4.72 ± 0.81 *	5.86 ± 0.16
Coliforms (MPN)	0.9 ± 0.20	1.20 ± 0.11	1.00 ± 0.50	2.7 ± 0.21	2.1 ± 0.40 *	3.6± 0.20	2.9 ± 0.32 *	------	3.3 ± 0.30 *	3.5 ± 0.40
Mould and yeast count	2.0± 0.55	2.2± 0.40	2.1± 0.52	3.6± 0.17	3.40 ±0.4	3.8± 0.19	3.5± 0.61	3.9± 0.13	3.9± 0.14	4.2± 0.53

*= significantly lower at $p<0.05$

Table (4): Sensory evaluation of control and treated fish fillets samples during frozen storage.

Parameters (samples)	**Desirable** (month)	**Undesirable** (month)
Control	0-3	6
Tested	0-9	12

Table (5): Mean values of PVmEq/kg, thiobarbituric acid value mg mal/ kg and TVB-N mg/100 g in control and treated fish fillets samples during frozen storage.

Samples	Day 0	Month-3		Month 6		Month 9		Month 12	
		C	T	C	T	C	T	C	T
PV	2.50+ 0.011	8.78 ± 0.30	5.45 ± 0.45*	13.01 ± 0.5	6.5 ± 0.3*	21.0± 0.67	7.4 ± 0.5*	-----	8.8 ± 0.25
TBA value	0.34 ± 0.02	0.795 ± 0.002	0.545 ± 0.032 *	0.990 ± 0.030	0.561 ± 0.12*	2.68 ±0.55	0.695 ± 0.010 *	------	0.918 ± 0.022
TVB-N	13.6 ± 0.23	19.8 ± 0.51	18.2 ± 0.82*	23.9 ± 0.75	18.9 ± 0.77*	30.25 ± 0.83	19.3 ± 0.65*	-----	22.7 ± 0.27

* Significant differences between the control and treated samples at $p<0.05$

C : control fish fillets sample.

T : treated fish fillets sample.

Table (6): Mean values of aerobic plate count at 35°C, psychrotrophic count, coliforms (MPN) and mould and yeast counts $\log_{10}$CFU/g of control and treated fish fillets samples during frozen storage.

Micro-organisms	Day 0	Month 3		Month 6		Month 9		Month 12	
		C	T	C	T	C	T	C	T
Aerobic plate count at 35°C	3.6± 0.40	2.77 ± 0.26	2.30 ± 0.28	4.40 ± 0.54	2.75 ± 0.27*	-----	2.87 ± 0.22*	-----	3.95 ± 0.63
Psychrotrophic count	1.57 ± 0.20	2.50 ± 0.32	2.22 ± 0.10	3.25 ± 0.11	2.45 ± 0.32*	-----	2.80 ± 0.31*	-----	3.97 ± 0.12
Coliforms (MPN)	0.9 ± 0.20	4.23 ± 0.18	3.00 ± 0.14	5.50 ± 0.60	3.00± 0.06*	------	3.45 ± 0.46*	-----	4.92 ± 0.50
Mould and yeast counts	2.0+ 0.55	2.85 ± 21	2.55 ± 0.50	3.72 ± 0.92	3.52 ± 0.41	4.35± 0.13	4.16 ± 0.62	4.62 + 0.52	4.00 ± 0.22

* Significant differences between the control and treated samples at $p<0.05$

C: control fish fillets sample.

T: treated fish fillets sample.

Discussion

The data obtained in table (1) revealed that the sensory evaluation of treated fresh fish fillets with rosemary extract 2% during chilling at 4^{o}C were significantly lower than that of the control at $p< 0.05$, the treated samples was desirable for 12 days in corresponding to 6 days for the control. PV ,TBA (malondialdehyde mg /kg and TVB-N (mg/100g) in table (2) declared that the PV were 3.50± 0.011, 8.34± 0.02, 9.5± 0.42 at zero day, 3days and 6th day , while at the 9th day it was 17.7 ± 0.57 which exceed the limit recommended by (10-20 rejected) for the control samples. In corresponding the treated samples peroxide value were 4.85 ± 0.2, 6.5± 0.2,7.60 ± 0.5,8.5 ± 0.4 and 11.2± 0.05 meq/g ,rejected at 15th day. The TBA-value in control samples were 0.34± 0.02, 0.96 ± 0.014, 1.98 ± 0.021 and 2.6± 0.34 at 0time, 3 ,6 and 9th days respectively while in treated fish fillets with rosemary extract 2% were 0.35± 0.013, 0.41 ± 0.06, 0.65 ± 0.021, 0.78 ± 0.013 and 8.9 ± 0.07 mg malondialdehyde /g fish respectively at 3, 6, 9, 12 and 15th day. The TVB-N in control samples were 13.6 ± 0.23, 15.3 ± 0.27, 19.9 ± 0.60 and 28.9 ±0.25 mg/100g fish at zero time,3 ,6 and 9th days respectively in corresponding to 14.5± 0.27, 16.8 ± 0.31, 17.6 ±0.04, 19.5 ±0.22 and 21.8 ± 0.21 mg/100g fish fillets at 3, 6, 9, 12 and 15th days respectively in the treated fillets . It was found that the PV, TBA and TVB-N in control samples were significantly higher $p<0.05$ at 6th and 9th day than treated fillets which spoiled at 15th day. The data obtained in table (3) showed that the aerobic plate count at 35^{o}C, psychtrotrophic count, coliforms MPN and mould/yeast counts in log $_{10}$ were significantly lower $p<0.05$ in treated fish fillets with rosemary 2% at 6th day and 9th day when the control was spoiled . These counts were significantly lower in fresh fillets at $p< 0.05$ than counts of frozen one.As the aerobic plat count at 35^{o}C of control samples during cold storage at 4 ^{o}C were 3.6±0.04,3.2±0.31,5.5±0.30 and 6.8±0.23 at zero day, 3, 6 and 9 days, respecthvely, while spoilage occur at 12 and 15 days corresponding to 2.9±0.22, 4.7±0.15, 5.4±0.42, 6.5±0.30 and 6.9±0.71 for treated samples at 3, 6, 9, 12 and 15 days. Psychrotrophic count were 1.57±0.20, 2.31±0.012, 4.76±0.41, 6.73±0.13 for control fish fillets at zero day 3, 6, and 9 days and spoilage occur at 12 and 15 days, while for treated samples the count varies from 2.00±0.050, 3.35±0.31, 4.63±0.33, 4.72±09.81 and 5.86±0.16 at 3, 6, 9, 12 and 15 days of cold storage at 4 ^{o}C. The coliforms count (MPN) for control samples were.9±0.20, 1.20±0.11, 2.7±0.21, 3.6±0.20 at zero day ,3 ,6 and 9 days before spoilage occur at 12 and 15 days, while the treated samples showed range from 1.00±0.50, 2.1±0.40, 2.9±0.32, 3.3±3.30 and 3.5±0.40 respectively at 3, 6, 9, 12 and 15 days . Mould and yeast count log10 cfu/g of control samples during cold storage at 4 ^{o}C were 2.0±0.55, 2.2±0.40, 3.6±0.17,

3.8±0.19, 3.9±0.13 ,respectively, at zero day ,3 ,6 ,9 and 12 days, corresponding to 2.1±0.52, 3.40±0.4, 3.5±0.61, 3.9±0.14, and 4.2± 0.53 for treated samples at 3, 6, 9, 12 and 15 days of storage at 4 ^{o}C.

It was cleared that the quality parameters of fresh fish fillets were significantly differ than the frozen fillets. This agrees with that reported by ***(23)*** who stated that there are two main problems associated with frozen storage of fish: hydrolysis and oxidation of lipids and protein denaturation. These problems cause an off taste and a dry and tough texture. Various factors, such as the freezing temperature, the rate of freezing and vacuum packaging or packaging materials can affect frozen fish quality .Frozen fish are often stored in the form of fillets , however ,filleting operations can affect frozen fish quality. In this respect***, (22)*** reported that the surface flesh of live healthy fish is considered bacteriologically sterile. The largest concentrations of microorganisms are found in the intestine, gills and surface slime. The numbers and types of microorganisms found on freshly caught fish are influenced by the geographical location of the catch and the season and method of harvest.

All the examined samples were within the permissible limits recommended by ***(11) and (13***) criteria which is 2.0 x10^{5} /g for psychrotrophic count. These organisms are capable of causing spoilage because of two important characteristics. First, they are psychrotrophic and thus multiply at refrigeration temperatures. Secondly they attack various substances in the fish tissue to produce compounds associated with off flavor and off odor ***(17).***

The data obtained in table (4) showed that the sensory evaluation of fish fillets dipped in rosemary extract 2% were significantly differ from the control samples . The treated samples stay for 9 months desirable and the control samples for 3 months. Lower figures recorded by ***(15)*** as the treated fish fillets with rosemary extract stay for 7months very good and the control for 5 months. This result agrees also with the chemical analysis as the pH, TBA (mg malondialdehyde / kg) and TVB-N (mg/100g) of the treated samples were significantly differ at $p<0.05$ than control samples (table 5) where table (5)showed that PVwere 2.50±0.011, 8.78± 0.30, 13.01±0.5, 21.0± 0.67 at zero day, 3, 6, and 9 months, respectively for control samples during frozen storage at -18 ^{o}C, while it was rejected at 12 month of storage. Incorresponding to the PV of the treated samples were 5.45±0.45, 6.5± 0.3, 7.4± 0.5 and 8.8±0.25 at 3, 6, 9 and 12 months, respectively. The TBA-value in control samples under freezing storage were 0.34±0.02, 0.722± 0.002, 0.990± 0.030, 2.68±0.55 at zero day, 3, 6 and 9 months, resp.,while the treated samples with rosemary extract 2% were 0.545± 0.032, 0.561±0.12, 0.695±0.010 and 0.918±0.022 mg malondialdehyed/g fish respectively at 3, 6, 9 and 12 months. The TVB-N in control samples were 13.6± 0.23, 19.8± 0.51, 23.9±0.75 and 30.25±0.83mg/100g at zero time, 3, 6 and 9 months, respectively, in

corresponding to 18.2±0.82, 18.9± 0.77, 19.3±0.65 and 22.7±0.27mg/100g fish fillets at 3, 6, 9 and 12 month for treated samples. The results obtained in table (6) showed that the mean value of aerobic plat count during freezing storage of control samples at -18^{o}C varies from 3.6±0.04, 2.77±0.26 and 4.40±0.54 during zero day, 3 and 6 months, respectively, while there were spoilage of the samples at 9 and 12 months. The psychrotrophic count varies from .57±0.20, 2.50±0.32 and 3.25±0.11 at zero day, 3 and 6 months, resp., and there were also spoilage at 9 and 12 months. Coliforms count at zero day, 3 and 6 months respectively were 0.9±0.20, 4.23± 0.18 and 5.50±0.60 and spoilage occur at 9 and 12 months. Mould and yeast count log10cfu/g of control samples during freezing storage at zero time, 3, 6, 9 and 12 months were 2.0±0.55, 2.85±0.21, 3.72± 0.92, 4.16±0.62 and 4.62±0.52, respectively. It was found that there was significant differences between the control and the treated samples at<0.05,as the values of the aerobic plat count at 35^{o}C of the treated samples by rosemary extract 2% during 3 , 6 , 9 and 12 months were 2.30±0.28, 2.75±0.27, 2.87±0.22 and 3.95±0.63, respectively. Psychotrophic count varies from 2.22±0.10, 2.45±0.32, 2.80±0.31 and 3.97±0.12 respectively at zero day, 3, 6, 9 and 12 months. For coliforms count 3.00±0.14, 3.00±0.06, 3.45±0.46 and 4.92±0.50, corresponding to 2.55±0.50, 3.52±0.14, 4.16±0.62 and 4.00±0.22 for mould and yeast count log10cfu/g of treated samples at 3, 6, 9 and 12 months, respectively**. (4)** stated that the plant substances affect microbial cells by various antimicrobial mechanisms, including attacking the phospholipids bilayer of the cell membrane ,disrupting enzyme systems, compromising the genetic material of bacteria ,and forming fatty acid hydroperoxidase caused by oxygenation of unsaturated fatty acids. Also, ***(24)*** reported that rosemary (Rosmarinus officinalis) was effective against gram-positive and gram-negative bacteria including listeria monocytogenes, aeromonas hydrophila and escherichia coli O157:H7. From the present study, it could be concluded that the dipping of fish fillets in rosemary extract 2% is recommended as it improves the quality parameters (sensory, chemical and microbial spoilage) during frozen storage.

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الملخص العربى

تاثير حبة اكليل الجبل على مدة الصلاحية فى الاسماك الفيلية المبردة

فاطمة حسن محمد على[1] و جيهان سيد احمد عفيفى[2]

قسم مراقبة الاغذية كلية الطب البيطرى جامعة بنى سويف1

قسم مراقبة الاغذية كلية الطب البيطرى جامعة بنها2

تعتبر الأسماك من أهم مصادر البروتين الحيواني للإنسان كما تتميز بمحتواها العالي من الكالسيوم والفسفور والفيتامينات و العناصر الغذائية الأخرى. لذلك أجريت هذه الدراسة لتسحين الحالة الصحية لفيلية سمك قشر البياض وذلك باستخدام وقد تم جمع هذه العينات المواد الطبيعة مثل محلول مائى من حبه اكليل الجبل 2%. من عدد من السوبرماركت بمحافظة الجيزة وكذلك تم تحضير محلول اكليل الجبل 2% بكلية الزراعة جامعة القاهرة(قسم الكيمياء) وذلك لدراسة الحالة الحسية والميكروبيولوجية والكيميائية أثناء التبريد عند 4 درجة مئوية لمدة15يوم وأيضاً أثناء التجميد عند -18 درجة مئوية لمدة 12 شهر.

وقد دلت نتائج الدراسة ان العينات المعالجة بحبة اكليل الجبل كانت صالحة للاستهلاك حتى 12 يوم مقارناً بـ 6 أيام في العينات غير المعالجة وأصبحت العينات المعالجة غير صالحة للاستهلاك بعد 15 يوم مقارناً بـ 9 أيام للعينات غير المعالجة.

وبالنسبة للاختبارات الكيميائية (البيروكسيد،حمض الثيوباربيتيوريك والنيتروجين الكلى المتطاير) فقد اوضحت النتائج ان جميع العينات الغير معالجةاصبحت فاسدة بعد12 يوم من التبريد عند54م او6 شهورمن التجميد عند -518م .بينما استمرت الصلاحية فى العينات المعالجة حتى 15 يوم من التبريدعند54م او 9 شهور من التجميدعند-518م. كما اثبتت نتائج الفحص البكتيريولوجى ان العدد الكلى للميكروبات كان يزداد باستمرار بزيادة مدة التخزين سواء بالتبريد او التجميد ،وكان معدل الزيادة اكبر كثيرا فى العينات الغير معالجة مقارنة بالعينات المعالجة بزيت حبة اكليل الجبل. وأخيراً كان العدد اللوغاريتمى للفطريات والخمائر في العينات الغير معالجة والمحفوظة عند درجة حرارة 4°هو 2.0± 0.55 ، 2.2 ± 0.40 ، 3.6±0.17 ، 3.8± 0.19 ، 3.9 ±0.13 خلال الأيام 1 ، 3 ، 6 ، 9 ، 12 على التوالى مقارناً بتركيز 2.1± 0.52 ،40و3± 0.4 ، 3.5± 0.61 ، 3.9± 1.4 ، 4.2± 0.53 ، فى العينات المعالجة خلال اليوم 3 ، 6 ، 9 ، 12 من الفحص.

بينما تراوح متوسط العدد الكلى للفطريات والخمائر في العينات الغير معالجة - والمحفوظة عند درجة حرارة -18° من 2.0± 0.55 ، 2.85 ± 21 ، 3.72± 0.92 ، 4.35± 0.13 ، 4.62± 0.52 خلال اليوم الأول والشهر 3 ، 6 ، 12 علي التوالي، بينما كان العدد في العينات المعالجة يتراوح من 2.55±0.50 ، 3.52± 0.41 ، 4.16± 0.62 ، 4.00± 0.22 في الشهر 3 ، 6 ، 9 ،12. وبذلك وجد أن غمس فيليه الأسماك في محلول حبه اكليل الجبل بتركيز 2% لمدة 60 دقيقة له تأثير فعال في تحسين حالتها الصحية والكيميائية والميكروبيولوجية سواء اثناء التبريد او التجميد.

Comparative Evaluation of Inactivated IBR Vaccines Formulated with Montanide ISA 25, 50 and 206.

G.F. El-Bagoury[1], A.S. El-Habbaa[1], E.M. El-Nahas[1], E.A. El-Ebiary[2] and D.M. Darwish[2]

[1]Faculty of Veterinary Medicine, Departments of virology, Benha University-Kalubia Egypt.
[2] Central Laboratory for Evaluation of Veterinary Biologics, Abbassia , Cairo, Egypt.

Abstract

Inactivated IBR vaccines were prepared using montanide ISA 25, 50 and 206. Purity, safety and sterility of the prepared vaccines were ensured then their potency was evaluated in vaccinated calves using SNT. Humoral immune responses to the prepared vaccines were evaluated in calves after two doses with two weeks intervals. Protective serum neutralizing antibody titer started at 3 weeks post vaccination for both montanide ISA 25 and ISA 50 adjuvant IBR vaccines but started at 2nd week post vaccination for montanide ISA 206 adjuvant IBR vaccine. These protective serum neutralizing antibody titers persisted to 24, 32 and 36 weeks post vaccination for montanide ISA 25, 50 and 206 adjuvant IBR vaccines, respectively. It was concluded that IBR vaccine adjuvant with Montanide ISA 206 gave the earliest and longer duration protective neutralizing antibody titer.

Introduction

Infectious bovine rhinotracheitis (IBR) /infectious pustular vulvovaginitis (IPV), caused by bovine herpesvirus 1 (BHV-1), is a disease of domestic and wild cattle (OIE 2008). IBR is a worldwide distributed disease. It causes significant economic losses to the livestock industry either alone or in association with other respiratory cattle pathogens [1].

In Egypt, IBR virus was isolated for the first time from a respiratory syndrome [2].The role of IBR virus infection in cattle and buffalo calves was investigated through survey in cattle and ruminants indicated that virus infection is wide spread all over Egypt [3], [4], [5], [6] and [7]. Many investigations have attempted the use of inactivated vaccines either singly or in combination with other viral agents [8] and [9]. They concluded that the use of inactivated vaccines for prevention of IBR produced good results. An ideal vaccine would be one that could provide more than 90% efficacy within a few weeks of single administration of vaccine; protection would be of long duration and of low cost [10].

The present work aimed to prepare and evaluate the comparative potency of an inactivated monovalent IBR vaccine adjuvanted with montanide ISA 25, 50 and 206 in susceptible calves.

Materials and Methods

1- **Virus:**

A local Egyptian IBR virus (Abou-Hammad strain) which was previously isolated and identified by [2] and [11]. It was kindly supplied from the Rinderpest Like Diseases Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. IBR virus was propagated on MDBK cell line culture, and the virus suspension was checked for sterility then virus titration according to [12]. The virus titer used for vaccine production was 108 TCID50/ml. It was used in the preparation of a reference inactivated IBR virus vaccine.

2- Vaccines:

The virus suspension was inactivated by 0.01M of Binary ethyleneimine (BEI) for 6 hours at 37°C then neutralized by sodium thiosulphate (20%) with a final concentration of 10 percent was added to stop the action of binary ethyleneimine. Vaccine formulation was done according to [13] as follows: the oil phase consisted of mantanide ISA 50 and 206 mixed as equal parts of an aqueous and oil phase weight/ weight and mixed thoroughly. While mantanide ISA 25 mixed as 25/75 of an aqueous and oil phase weight/ weight and mixed thoroughly.

3- Calves and experimental design:

Twenty susceptible Friesian calves (6 months old), clinically normal, healthy and free from antibodies for IBR were used (12 calves for potency, 6 calves for safety and 2 calves were kept as a control for the experiment). Calves used for evaluation of vaccine potency were inoculated with 2ml intramuscularly (I/M) from each vaccinal batch and the inoculation was repeated after 2 weeks (booster dose), were kept under close observation during the whole time of experiment and subjected for serum samples collection.

4- Serum samples:

Serum samples were collected from vaccinated calves weekly for 48 weeks. The sera were collected and stored at -20°C and inactivated at 56°C for 30 minutes before being used in the test.

5- Serum neutralization test (SNT):

SNT was carried out after [14].

Results

Table (1): The antibody titre of vaccinated calves with oily prepared ISA 25 adjuvanted inactivated IBR vaccine as measured by SNT.

Weeks post vaccination	Vaccinated animal					Non-Vaccinated animal		
	1	2	3	4	mean	5	6	mean
0	0	0	0.3	0.3	0.15	0.3	0.3	0.3
1st vaccination								
1	0.3	0.45	0.3	0.3	0.3375	0.3	0.3	0.3
2	0.6	0.6	0.45	0.9	0.6375	0.3	0.3	0.3
2nd vaccination(Booster dose)								
3	0.9	0.9	0.9	0.9	0.9	0.3	0.3	0.3
4	1.2	1.2	0.9	1.2	1.125	0.3	0.3	0.3
6	1.2	1.5	1.2	1.2	1.275	0.3	0.3	0.3
8	1.5	1.65	1.2	1.2	1.3875	0.3	0.3	0.3
12	1.75	1.65	1.2	1.5	1.525	0.3	0.3	0.3
14	1.75	1.5	1.5	1.5	1.5625	0.3	0.3	0.3
16	1.65	1.5	1.5	1.2	1.4625	0.3	0.3	0.3
18	1.5	1.2	1.15	1.2	1.2625	0.3	0.3	0.3
20	1.2	1.15	1.15	1.15	1.1625	0.3	0.3	0.3
24	0.9	1.15	0.9	0.9	0.9625	0.3	0.3	0.3
28	0.6	0.9	0.9	0.6	0. 75	0.3	0.3	0.3
32	0.45	0.6	0.45	0.45	0. 4875	0.3	0.3	0.3
36	0.45	0. 45	0.45	0.45	0.45	0.3	0.3	0.3
40	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
44	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
48	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

* Log10 serum neutralizing antibody titer.

Table (2): The antibody titer of vaccinated calves with oily prepared ISA 50 adjuvanted inactivated IBR vaccine as measured by SNT.

Weeks post vaccination	Vaccinated animal					Non-Vaccinated animal		
	7	8	9	10	mean	5	6	mean
0	0.3	0	0.3	0.3	0.225	0.3	0.3	0.3
1st vaccination								
1	0.45	0.3	0.75	0.45	0.4875	0.3	0.3	0.3
2	0.75	0.45	0.75	0.75	0.675	0.3	0.3	0.3
2nd vaccination(Booster dose)								
3	1.2	0.75	1.2	1.5	1.1625	0.3	0.3	0.3
4	1.75	1.2	1.5	1.75	1.55	0.3	0.3	0.3
6	1.8	1.5	1.75	1.8	1.7125	0.3	0.3	0.3
8	1.8	1.8	1.8	1.95	1.8375	0.3	0.3	0.3
12	1.8	1.85	1.8	2.1	1.9125	0.3	0.3	0.3
14	1.8	1.8	1.8	1.95	1.8375	0.3	0.3	0.3
16	1.65	1.65	1.65	1.8	1.6875	0.3	0.3	0.3
18	1.5	1.5	1.65	1.2	1.4625	0.3	0.3	0.3
20	1.5	1.5	1.5	1.2	1.425	0.3	0.3	0.3
24	1.2	1.2	1.15	1.15	1.175	0.3	0.3	0.3
28	1.15	1.15	0.9	1.15	1.0875	0.3	0.3	0.3
32	1.15	1.15	0.9	0.9	1.025	0.3	0.3	0.3
36	0.9	0.75	0.75	0.75	0.7875	0.3	0.3	0.3
40	0.6	0.6	0.6	0.3	0.525	0.3	0.3	0.3
44	0.3	0.3	0.6	0.3	0.375	0.3	0.3	0.3
48	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

* Log10 serum neutralizing antibody titer.

Table (3): The antibody titer of vaccinated calves with oily prepared ISA 206 adjuvanted inactivated IBR vaccine as measured by SNT.

Weeks post vaccination	Vaccinated animal					Non-Vaccinated animal		
	7	8	9	10	mean	5	6	mean
0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
1st vaccination								
1	0.6	0.3	0.45	0.6	0.4875	0.3	0.3	0.3
2	0.9	0.6	0.9	1.2	0.9	0.3	0.3	0.3
2nd vaccination(Booster dose)								
3	1.5	1.2	1.5	1.2	1.35	0.3	0.3	0.3
4	2.1	1.75	1.75	1.2	1.925	0.3	0.3	0.3
6	2.4	2.1	2.4	2.1	2.25	0.3	0.3	0.3
8	2.4	2.4	2.4	2.4	2.4	0.3	0.3	0.3
12	2.25	2.4	2.4	2.25	2.325	0.3	0.3	0.3
14	2.1	2.25	2.25	2.25	2.2125	0.3	0.3	0.3
16	2.1	2.1	2.1	2.25	2.1375	0.3	0.3	0.3
18	1.8	1.95	1.8	1.8	1.8375	0.3	0.3	0.3
20	1.5	1.8	1.5	1.8	1.65	0.3	0.3	0.3
24	1.35	1.5	1.35	1.35	1.3875	0.3	0.3	0.3
28	1.2	1.2	1.2	1.2	1.2	0.3	0.3	0.3
32	1.2	1.2	0.9	1.2	1.125	0.3	0.3	0.3
36	0.9	0.9	0.9	1.2	0.975	0.3	0.3	0.3
40	0.6	0.6	0.45	0.6	0.5625	0.3	0.3	0.3
44	0.45	0.45	0.3	0.45	0.4125	0.3	0.3	0.3
48	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

* Log10 serum neutralizing antibody titer.

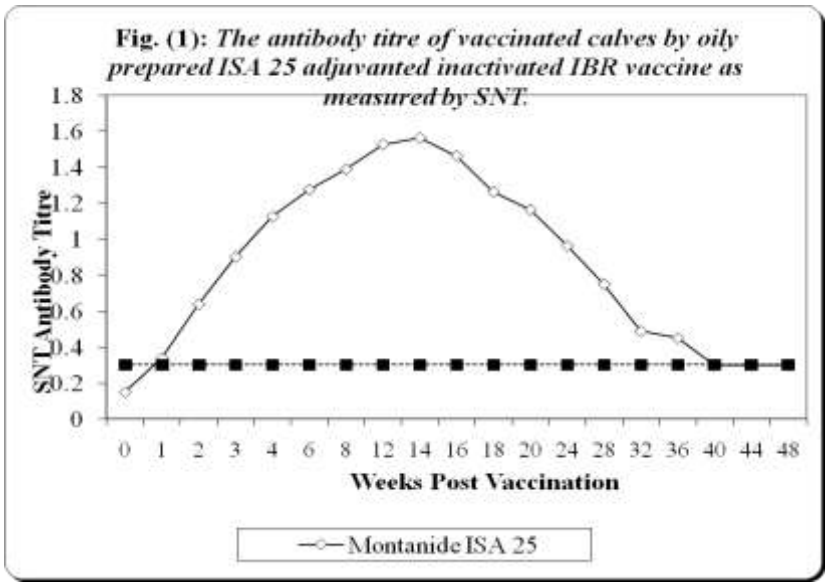


Fig. (1): ***The antibody titre of vaccinated calves by oily prepared ISA 25 adjuvanted inactivated IBR vaccine as measured by SNT.***

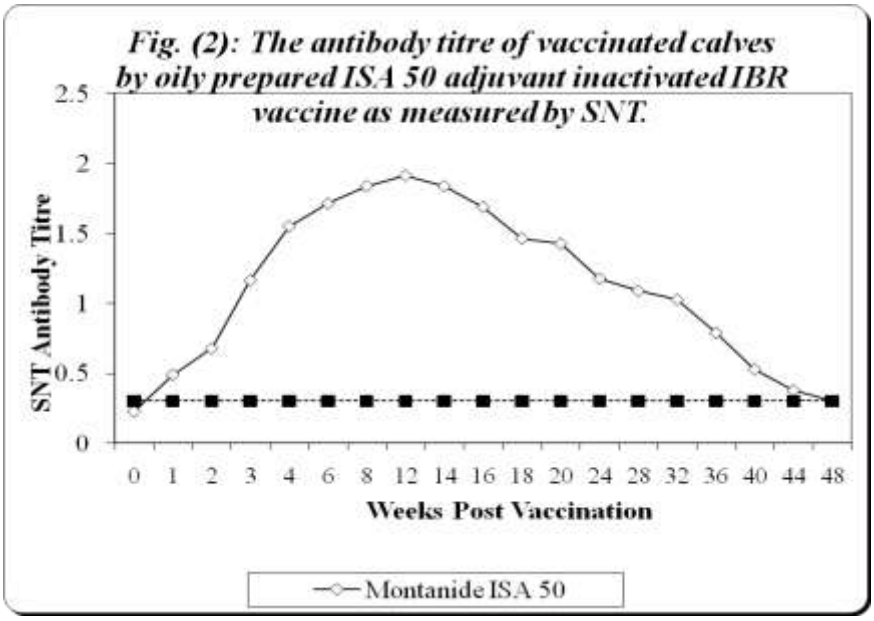
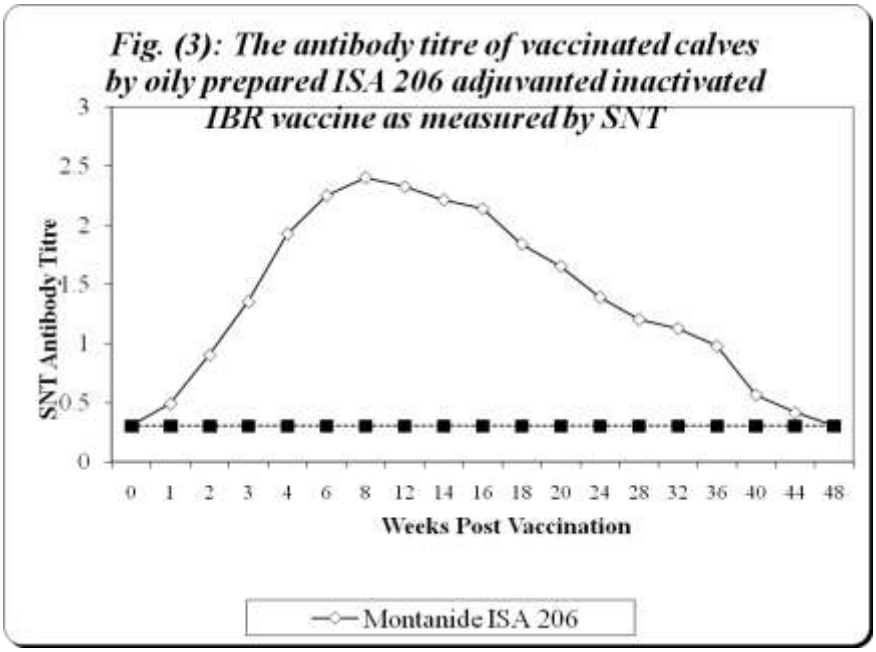


Fig. (2): The antibody titre of vaccinated calves by oily prepared ISA 50 adjuvant inactivated IBR vaccine as measured by SNT.



Discussion

Adjuvants are considered one of the important factors in vaccine formulation so, the progress in vaccine production is directed towards selection of the proper adjuvant that can elaborate high and long lasting immunity [15].

In the present study we have attempted to make comparative evaluation of three ready to formulate oil adjuvants of Montanide series. Montanide ISA 25, 50 and 206 for development of IBR vaccine production in Egypt.

The humoral immune response to IBR vaccine adjuvant on Montanide ISA 25 in vaccinated calves using SNT showed that protective neutralizing serum antibody titer (0.9) started from 3rd week post vaccination and persisted in protective level until 24 weeks (6 months) as shown in tables (1) and figure (1). These results were similar to those obtained using SNT came along with [16], [17] and [18] who reported that the protective level of neutralizing titre 0.9 against IBR.

Sera from calves vaccinated with ISA 50 using SNT showed that protective neutralizing serum antibody titer started from 3rd week post vaccination and persisted in protective level until 32 weeks (8 months) as shown in tables (2) and figure (2). These results were similar to those obtained

using SNT came along with [19] who reported that the protective level of neutralizing titer 1.02 against IBR, and adequate titers of vaccine induced anti BHV-1 antibodies could be demonstrated both by SNT up to 180 days post vaccination.

The humoral immune response to IBR vaccine adjuvant on Montanide ISA 206 in vaccinated calves using SNT showed that protective neutralizing serum antibody titer started from 2nd week post vaccination and persisted in protective level until 36 weeks (9 months) as shown in table (3) and figure (3).These results obtained using SNT came along with [16], [17] and [18] who reported that 0.9 is the protective level of neutralizing titer against IBR virus.

From the above studies for SNT titers for sera of vaccinated calves with different adjuvant we can conclude that the ISA 206 adjuvant vaccine gives high, long peak of titer and also get early and long term protective immune responses.

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الملخص العربى

تقييم مقارن للقاحات مثبطة لفيروس إلتهاب الانف والقصبة البقرى المعدى و206 . ISA 25 , 50 باستخدام ممتزجات زيت المونتانيد

جبر فكرى الباجورى[1] و أيمن سعيد الهباء[1] و إيهاب مصطفى النحاس[1] و إلهام عطا الابيارى[2] ودرويش محمود[2]

1 قسم الفيرولوجيا كلية الطب البيطرى بمشتهر جامعة بنها –القليوبية -مصر
2 المعمل المركزى للرقابة على اللقاحات البيطرية بالعباسية -القاهرة –مصر

تم تحضير لقاحات مثبطة لفيروس إلتهاب الانف والقصبة البقرى المعدى و206 . تم تقييم الاستجابة ISA 25 , 50 باستخدام ممتزجات زيت المونتانيد المناعية للقاحات المحضرة فى العجول بعد اعطائها جرعتين من اللقاح بفاصل زمنى 2 أسبوع. تم التأكد من نقاوة وأمان وتعقيم اللقاحات المحضرة ثم تم تقييم فاعلية اللقاح فى العجول المحصنة بإستخدام اختبار التعادل المصلى. بدأ مستوى الحماية لعيارية الاجسام المضادة المعادلة للفيروس فى السيرم عند الاسبوع الثالث بعد الحقن لكلا من ولكن بدأ بعد ISA 25 , 50 اللقاحان المثبطان باستخدام ممتزجات زيت المونتانيد 206 للقاحات ISAالاسبوع الثانى للحقن للقاح المثبط باستخدام ممتزج زيت الونتانيد مثبطة لفيروس إلتهاب الانف والقصبة البقرى المعدى. و قد امتدت فترة بقاء مستوى و 36 32الحماية لعيارية الاجسام المضادة المعادلة للفيروس فى السيرم الى 24 و أسبوع بعد الحقن للقاحات المثبطة لفيروس إلتهاب الانف والقصبة البقرى المعدى و206 على التوالى. وقد تم ISA 25 , 50 باستخدام ممتزجات زيت المونتانيد استنتاج أن اللقاح المثبط لفيروس إلتهاب الانف والقصبة البقرى المعدى باستخدام 206 أعطى مستوى الحماية لعيارية الاجسام المضادة ISAممتزج زيت المونتانيد المعادلة للفيروس فى السيرم بأسرع معدل للظهور و أطول معدل للاستمرار.

Field evaluation of egg yolk antibodies in prevention and treatment of enteric colibacillosis in calves.

Germine, S.S.[1]; Ebied, M.H.[2]; Ibrahim, F.K.[1]; Mettias, K.N.[1] and Daoud, A.M.[1]

[1] Veterinary Serum and Vaccine Research Institute, Abbassia , Cairo, Egypt.
[2]Faculty of Veterinary Medicine, Department of Animal medicine, Benha University-Kalubia, Egypt.

Abstract

Neonatal calf diarrhoea remains one of the most important causes of calf mortality and a major disease problem facing livestock that leads to great economic losses not only from calf mortality and treatment costs, but also from losses in future growth and production. E. coli is one of the most lethal bacterial agents causing neonatal calf diarrhoea (calf scour). So, tins study throws the lights on the field use of egg yolk as passive immunization against E. coli K99 infection and also can be used in treatment of calves suffering from colibacillosis in addition to preventive action. There is a grateful increase in ELISA antibody titre in cows' dams sera of different groups at the 14th day (1/6000-118000) and began to decrease gradually till reached (114500) at day of parturition, also there is a sharply increase of the serum antibody in offspring of vaccinated cows after ingestion of colostrum. So, administration of scour vaccine (Rotavec corona) to pregnant cow dams help transfer scour protection to calf via antibody reaches colostrum.

Introduction

Enterotoxigenic Escherichia coli (ETEC) IS the mam cause of diarrhoea affecting calves less than one week old, the losses caused by colibacillosis due to infection with ETEC are measured not only by deaths of affected calves but also by losses of weight gains (1).

The present· work was carried out to evaluate the protective potentials of egg yolk antibodies against E. coli K99 diarrhoea in new born calves.

Material and Methods

Material:

Hens and Animals:

A total number of (40) laying red hens (Red Bovans) located at Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, of about five months old age were included in the present study. The hens were immunized with inactivated three different E. coli K99 vaccine, as a way to obtain hyperimmune egg yolk (Ig Y).

Pregnant cows and their offsprings:

A total number of 30 pregnant Fresian cows at the 7th month of pregnancy and their offsprings (of one day old) were used to evaluate the protective clinical effects of prepared egg yolk containing Ig Y antibodies against E. coli K99 antigen after injection of 3 vaccines (Rota vec - corona vaccine, Scour-Guard vaccine and Entero-3 vaccine). All those cows and their calves were located in a private dairy farm at Wadi El-Natroun govemorate.

Samples:

A. Serum samples:

They were prepared from the ollected blood of:

1. Hens:

Blood samples were collected for separation of serum from wing vein before and at different periods after immunization with E. coli K99 vaccine.

2. Pregnant cows and their Calves:

a. Serum samples were taken from pregnant cows before and till time of parturition day.

b. Serum samples were taken from calves before and after suckling or drinking of egg yolk weekly till the end of 60 days.

B. Colostrum samples:

They were taken from recently parturition cows ill a clean sterile container on day of parturition.

C. Egg yolk samples:

Eggs were collected before and at different periods after immunization of hens with inactivated E. coli K99 vaccine (three previous vaccines) and control group. Egg yolks were separated and collected in clean dry screw capped bottles and preserved till use.

D. coli antigen for ELISA test:

The K99 pilus antigen used for ELISA test was prepared following the method reported by (2). Strains were isolated from diarrhoeic calves after inoculation on Minca medium and tested for K99 antigen with Quevet coli check K99 kit - a rapid immunoassay for detection of K99 in animals faeces according to instructions. Laboratories Quelabinc, Batch No. 643/51B (Quebec) Montreal, Canada (3).

Reagents:

1. Rabbit antibovine IgG conjugated with horse Radish Peroxidase (HRP) (Sigma-USA). It was diluted (1:2000) and used according to the instructions given by producer. It was used in ELISA test on calves sera.
2. Sheep anti-chicken IgG (H and L) conjugated with Horse Radish peroxidase (England). It was diluted (1:2000) and used according to the instructions given by the producer and it was used in ELISA for chicken sera and egg yolk.

Vaccines:

i. Rota vec corona ®:

Combined inactivated bovine Rota VlfUS, E. coli K99 and bovine coronavirus vaccine. It was supplied by Schering - Plough Animal health, USA. It was used as two ml intramuscular for 7 months pregnant cow once without booster dose. Also, it was also used to immunize hens for production ofhyperimmune egg yolk.

ii. Scourgaurd ®:

It was supplied by Pfizer Company, USA. Batch Number A130046. It was used as two ml intramuscular (IIM) only for pregnant cow, two months before calving. Boostering after two weeks from the first inoculation. Also, it was used also to immunize hens for production ofhyperimmune egg yolk.

iii. Entero-3 ®:

Inactivated polyvalent Entero-3 vaccme contains Rota and Corona viruses as well as E. coli K99 strains with alum hydragel as adjuvant. It was used for vaccination of pregnant cow dams 2 months before delivery (four ml

IIM) and boostered after 2 weeks. Also, it was used to immunize hens for production ofhyperimmune egg yolk.

Material used for ELISA technique:

1- Phosphate buffer saline (PBS).
2- Carbonate bicarbonate buffer (Coating buffer).
3- Blocking buffer.
4- Dilution buffer.
5- Washing buffer.
6- Preparation of substrate.
7- Stopping solution.

Ouevet coli check K99 kit:

Rapid immunoassay for the detection of K99 in animal faeces used according to instructions (3).

Methods:

(1) Design of hens inoculation with different vaccines:

A total number of 40 laying hens (5 months old) were divided into (4) groups each of 10 hens as shown in the following table:

1. First group:

Each bird immunized intramuscularly at different sites of the breast with 1 ml of Rota vec corona vaccine.

2. Second group:

Each bird immunized intramuscularly at different sites of the breast with 1 ml of Scour-guard vaccine.

3. Third group:

Each bird immunized intramuscularly at different sites of the breast with 1 ml of Entero-3 vaccine.

4. Fourth group:

It was kept as negative control without any injections. Inoculation was done according to the method of (4) and (5).

Scheme of inoculation of hens by three different vaccines

Vaccinated Groups	Inoculation weeks				
	0	6	14	22	30
Group (1) Rota vec corona	+	+	+	+	+
Group (2) Scour -Guard	+	+	+	+	+
Group (3) Entero-3	+	+	+	+	+
Group (4) Control -ve	-	-	-	-	-

+ : Inoculation time

B. Samples:

1- Blood samples for serum separation and eggs of inoculated hens were collected just before vaccination and every week till one month from the last inoculation.
2- Serum sample of each hen was separated, aliquoted in small capped tube and freezed at -20°C till testing.
3- Egg yolks were separated, weighed, processed, pooled and then stored at -20°C till testing.

(2) Determination of specific antibody titre against E. coli (K99) in chicken serum, egg yolk, pregnant cow dams and their offspring sera by Enzyme Linked Immunosorbent Assay (ELISA):

This test was done for determination of specific antibodies titre against bovine E. coli K99 vaccine (previous 3 different vaccines) in serum and pooled egg yolk sample according to (6).

1. Preparation of E. coli K99 Antigen:

It was performed by inoculation of Minca Vitox agar media (PH 7.5) according to (7) with E. coli K99+ strain. Two colonies and a portion of growth in an area of confluence were tested 'per isolate by the test kit. Positive isolates were furtherly grown on minca media, reidentified and harvested. Extraction of E. coli K99 antigen was done according to methods of (3). Purification was checked by polyacrylamide gel electrophoresis and the used antigen gave one band at a molecular weight of 18.5 kDa.

(3) Immunization of pregnant cow dams against E. coli K99 infection:

Thirty pregnant cow dams 2 months before parturition were divided into two main groups (15 cows for each group):

1. Group one:

Fifteen cows were divided into three subgroups each of (5) cows:

a. First subgroup:

It was vaccinated by Rota vec corona vaccine as 2 ml intramuscular without booster dose according to instruction of manufacturer.

b. Second subgroup:

It was vaccinated by Scour-Guard vaccine as 2 ml intramuscular and boostered after 2 weeks.

c. Third subgroup:

It was vaccinated by Entero-3 vaccme as 4 ml intramuscular and boostered after 2 weeks.

2. Group Two:

The other 2nd group (15 cow) was kept as control negative for vaccinated cow dams (we well use their offsprings for determination of the efficacy of prepared Ig Y in protection against E. coli infection.

A. Field evaluation of egg yolk in newly born calves as source of passive immunization against E. coli 1(99 infection:

All calves under experimental study were clinically observed till the period of experiment and these animals were below 30 days age.

The first three groups, each group of 4 newly born calves of non-vaccinated dams were deprived from colostral antibodies and received milk mixed with egg yolk (20 ml yolk/calf) mixed with (1.5 - 2.0 kg milk) twice/day for 21 days then calves fed on milk only according to their body weight (normal feeding schedule in the farm).

1. First group:

Four newly born calves suckled Ig Y produced by hens inoculated with Rota vec corona vaccine.

2. Second group:

Four newly born calves suckled IgY produced by hens inoculated with Scour-Guard vaccine.

3. Third group:

Four newly born calves suckled IgY produced by hens inoculated with Entero- 3 vaccine.

4. Fourth group:

The last group (3 newly born calves) kept as negative control (suckled their mother colostrums) deprived from E. coli K99 antibody.

B. Field evaluation of egg yolk:

* All calves groups were kept under observation for morbidity and mortality, general health conditions and undesirable symptoms or clinical manifestation releated to E. Coli infection.

* Calves groups of control groups who showed positive result severe diarrhoea against E. coli K99 were taken IgY two times daily (every 12 hours as one egg yolk/day) for 21 days and these calves were kept under observation and faecal samples were taken and examine by Quevet E. coli K99 check kit (their age was below 30 days) according to methods of (4) and (8).

Results

1. Determination (measurement) of mean antibody titre against E. coli K 99 infection estimated by ELISA in serum and egg yolk of different groups of hens:

From the three different groups of vaccinated hens sera and egg yolk were collected, pooled, page then antibody titres were measured by ELISA. Results are shown in table (1), Fig. (1) revealed that: Antibody titre of hens in sera and egg yolk table (1):

(1) Hens of first group: that received Rotavec corona vaccine showed mild increase of antibody titre against E. coli K99 in senun (1/1120) and egg yolk (1/950) after 6 weeks of vaccine inoculation then increased gradually reaching in senun (1/7200) and egg yolk (1/6450) after (14) weeks (second booster dose) and then increased till reached their maximum level at 22 weeks in senun (1113150) and egg yolk (1111450) and then remain stable till the end of the experiment.

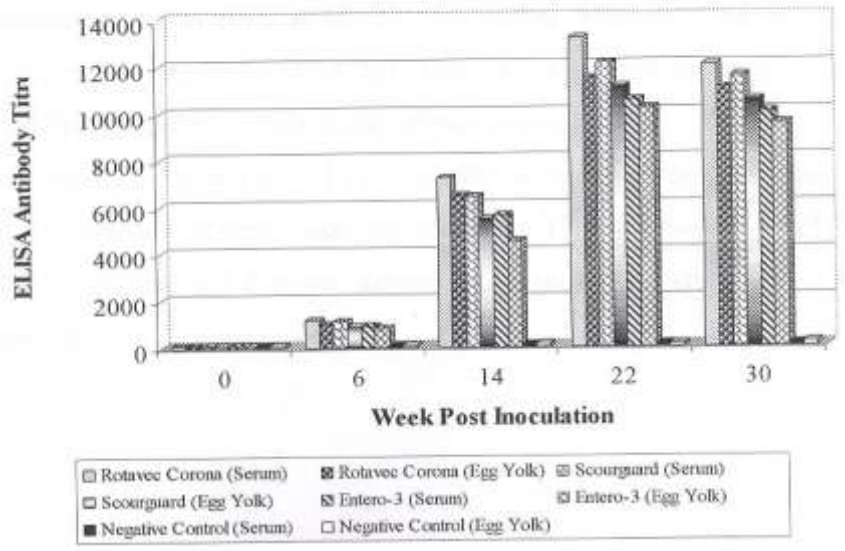
(2) Hens of second group: that received scour guard vaccine showed mild increase of antibody titre in senun (1/1100) and egg yolk (1/850) after 6 weeks of vaccine inoculation then increased gradually reaching in senun (1/6400) and in egg yolk (1/5440) after 14 weeks post inoculation reached their maximum level at 22 weeks in serum (1112100) and egg yolk (1111100) and then remained stable Table (1), Fig. (1).
(3) Hens of third group: that received Entero-3 vaccme showed mild increase of antibody titre in senun (1/900) and egg yolk (1/800) after 6 weeks of inoculation then increased gradually reaching in senun (1/5600) and in egg yolk (1/4500) after 14 weeks of inoculation and reached their maximum level at 22 weeks in senun (1/10520) and egg yolk (1110200) and remained stable till the end of the experiment (Table 1, Fig. 1).

Table (1): Correlation between titers of E. coli K99 antibody in sera and yolk of different groups of hens.

Time of Inoculation (Weeks)	Group (1)		Group (2)		Group (3)		Group (4)	
	Serum	Egg yolk	Serum	Egg yolk	Serum	Egg yolk	Serum	Egg yolk
0	100	80	60	60	80	60	60	80
6	1120	950	1100	850	900	800	80	80
14	7200	6450	6400	5440	5600	4500	80	110
22	13150	11450	12100	11100	10520	10200	125	80
30	12000	11000	11500	10500	10000	9500	80	160

Group (1): Rota vec corona. Group (2): Scour-Guard. Group (3): Entero-3. Group (4): Negative control.
The correlation between serum and egg yolk was similar.

Fig. (1): Correlation between titres of E. coli K99 antibody in sera and yolk of different groups of hens



2. Field evaluation of vaccines against E. coli K99 pregnant cows and their offsprings:

Determination of mean ELISA antibody titre against E. coli K99 infection in cow dams sera of different group:

Group (1):

The results in group one revealed as in table (2) and Fig. (2) that at (0) day before vaccination, antibody titre was > 50'0 then increased gradually after vaccination and reached 1/8000 at (14) days then began to decrease gradually till reached 1/6000 at time of parturition.

Group (2):

Our results showed that in table (2) and Fig. (2) that at (0) day before vaccination, antibody titre was > 500 then increased gradually after vaccination and reached 117000 at (14) days, then began to decrease gradually till day of parturition and reached 1/5000.

Group (3):

The results of group three tabulated in Table (2) andFig. (2) documented that at (0) day before vaccination, antibody titre was> 600 then increased gradually at 14 days (booster dose) and began to decrease gradually till reached 1/4500 at day of parturition.

Also, in Table (3) and Fig. (3) the main anti-E. coli K99 antibody titre in colostrum of vaccinated cow reached 1117000, 1114000, and 1112500 for groups who received Rotavec corona, scourguard and entero-3 vaccines, respectively.

Table (2): Mean ELISA antibody titre against E. coli K99 infection in vaccinated cow dams sera.

Days	Vaccinated cows			
	Group (1)	Group (2)	Group (3)	Control -ve
0	> 500	> 500	> 600	1000
7	7000	2000	1500	1000
14 *	8000	7000 *	6000 *	1000
21	7500	6500	5500	500
28	7000	6200	5000	400
35	6800	6000	4800	300
42	6500	5500	4750	200
49	6300	5300	4600	150
Parturition day	6000	5000	4500	> 50

Booster dose for both group (2 and 3) only

Table (3): Mean ELISA antibody titre against E. coli K99 of colostrums

Time	Group (1)	Group (2)	Group (3)	Group (4)
Parturition day	17000	14000	12500	> 500

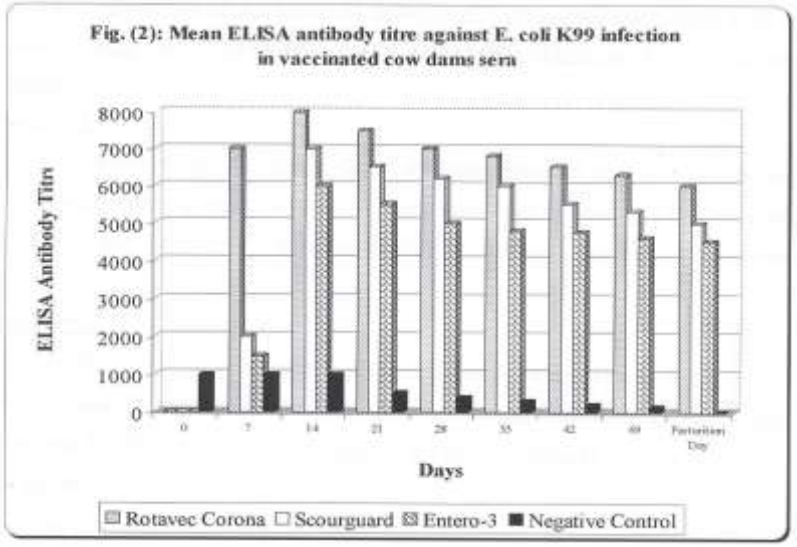


Fig. (2): Mean ELISA antibody titre against E. coli K99 infection in vaccinated cow dams sera
ELISA Antibody Titre
8000
7000
6000
5000
4000
3000
2000
1000
0
0
7
14
21
28
35
42
49
Parturition Day
Days
Rotavec Corona
Scourguard
Entero-3
Negative Control

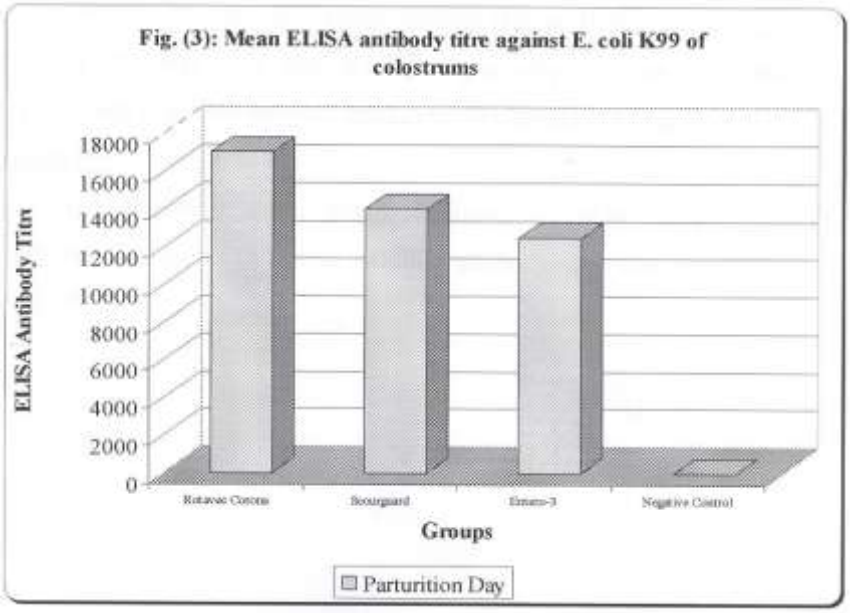


Fig. (3): Mean ELISA antibody titre against E. coli K99 of colostrums
ELISA Antibody Titre
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Rotavec Corona
Scourguard
Entero-3
Negative Control
Groups
Parturition Day

3. Determination of mean ELISA antibody titre against E. coli 1(99 in calves sera of vaccinated dams (received colostrum) in different groups:

Group (1):

Regarding to the first group, the results showed that at 0 day before suckling, antibody titre was> 50 then increased gradually after suckling and reached 116000 at 1 day and began to decrease gradually till reached 11460 at 60 days (Table 4, Fig. 4).

Group (2):

The results of second group cleared that in table (4) and Fig. (4), at 0 day before suckling, antibody titre was> 50 then increased gradually and reached 114550 at 1 day and began to decrease gradually till reached 11290 at 60 days.

Group (3):

Our results for third group proved that at 0 day before suckling, antibody titre was> 50 then increased gradually till reached 114490 at 1 day and then began to decrease gradually till reached 11245 at 60 days.

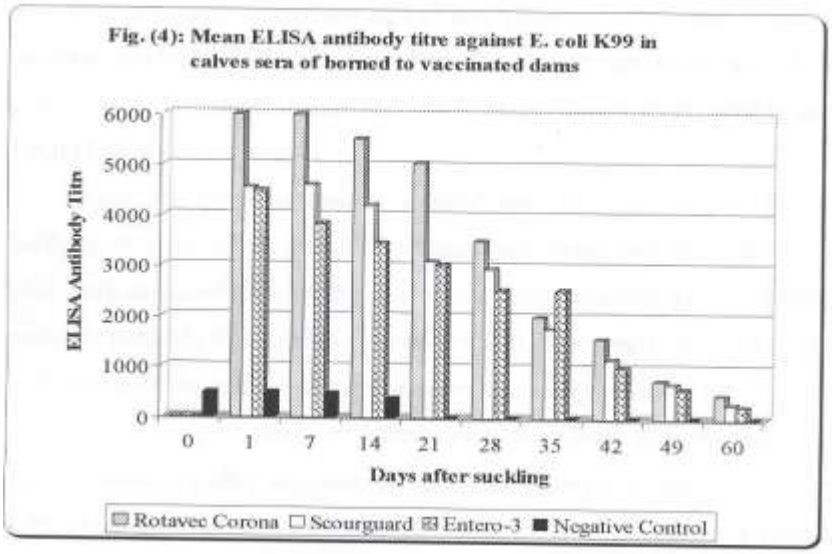
Group (4):

The results of forth group tabulated in Table (4) and Fig. (4) illustrated that at 0 day before suckling antibody titre was 11490 and at 1 day was 11495.

Table (4): Mean ELISA antibody titre against E. coli K99 in calves sera of born to vaccinated dams.

Time	Vaccinated cows			
	(1)	(2)	(3)	-ve Control
0 day before suckling	> 50	> 50	> 50	490
1 day after suckling	6000	4550	4490	495
7 days	6000	4600	3840	480
14 days	5520	4200	3450	390
21 days	5040	3090	3010	> 250
28 days	3500	2940	2520	> 200
35 days	1995	1760	2520	> 100
42 days	1560	1150	995	> 50
49 days	750	680	580	> 50
60 days	460	290	245	> 50

The antibody titres in calves sera in group four (-ve control) whose take egg yolk only not detected because it does not absorb from the small intestine.



Discussion

The current study aimed to compare the different inoculation by three different vaccines against E. coli K99 infection (Rotavec corona vaccine, Scourgaurd vaccine and Entero-3 vaccine) for obtaining the highest concentration of anti-E. coli antibodies IgY.

In Table (1) and Fig. (1) showed that there is a similarity in serum and egg yolk of different groups of hens and these results are in agreement with (9) and (10).

So, it could be recommended to inject hens by Rotavec corona vaccine and booster injection every two months in order to obtain stable steady titre all the season of laying hens (7) and (11).

Concerning with the detectable antibody titre against E. coli K99 infection in cow dams sera of different groups (Table 2, Fig. 2), it is clear that there is a gradual increase at 14th day (1/6000-118000) and began to

decrease gradually till reached (1/4500) at day of parturition and these results are in coordinated with that obtained by (12) and (13).

Also, the previous results revealed that there are an increase in antibody level of all groups of vaccinated cow dams and their off springs little more in animals who received Rotavec corona vaccine as they showed excellent antibody titre against E. coli K99 with only single dose of vaccine as it seen to be more economic and less stress on pregnant cow dam.

In offspring of their vaccinated cows, the serum antibody titre was sharply increased after ingestion of colostnun where the main anti-E. coli K99 antibody titre in colostnun of vaccinated cow reached 1117000, 1/14000, 1112500 for groups who received Rotavec corona, Scourgaurd and Entero-3 vaccines respectively (Table 3, Fig. 3).

In this field, many authors have reported the importance of colostnun as the only effective tool for preventing calf diseases in the first days of life (their critical period) (1) and (11).

In Table (4) and Fig. (4) clarify that there is no detection of titres in calves sera in group four (-ve control) whose take egg yolk only because it does not absorb from the small intestine. These results are in parallel with that obtained by (5) and (8).

Control negative calves deprived colostnun and who showed scour due to E. coli infection designed by Quevet E. coli K99 check kit who received Ig Y as two egg per day with milk show rapid recovery of diarrhoea per 2-3 days. No undesirable symptoms, normal body weight gain. These results were supported by previous work of (4), (5) and (8).

By the end of the result of this study, it is advisable to use egg yolk as a tool for prevention and treatment of enteric colibacillosis in calves where the use of immunoglobulin IgY technology is less costly, non-invasive, fast sample and high efficient to produce polyclonal antibodies (Poison, 1980 and Stack, 1996).

It could be concluded that:

1. Sound management practices including vaccination (the best by Rotavec corona vaccine) and calves receive adequately colostnun within the first 2-6 hours of life can greatly reduce the incidence of scour.
2. Also administration of scour vaccine as (Rotavec corona, Scour-guard, and Entero-3 vaccines) to pregnant cow dams help transfer scour protection to calf via antibody reach colostrum.
3. Egg yolk IgY can be used in treatment of calves showing E. coli K99 infection in addition to preventive action.

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Effect of Interferon-Alpha on Bovine Rotavirus.

Abuelyazeed A. Elsheik[1], Shimaa M.G. Mansour[1] and Mohamed E.M. Mohamed[2]

[1]Department of Virology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.
[2]Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

Abstract

Bovine Rotavirus (BRV) is a major cause of neonatal calf diarrhea throughout the world. The effects of different concentration of interferon (IFN) (10, 100, 500 and 1000 U/ ml) on Rotavirus were studied. The results showed that IFN decreased virus cytopathic effect, virus replication, virus titer and virus RNA. The effect of IFN on the virus was dose dependent manner and the maximum effect was seen at the concentrations 1000 U/ml. In conclusion, IFN could be used as inhibitor of BRV.

Introduction

Group A rotaviruses are segmented double-stranded RNA viruses that cause severe dehydrating diarrhea in human and animals worldwide (1). The viruses replicate primarily in mature enterocytes of the small intestine, but viremia and systemic infections are well documented in both humans and animals (2). Bovine-like rotavirus strains had been isolated from symptomatic infants in Italy (3) and neonates in India (4). Genetic analysis identified the later strains as likely natural reassortants between human and bovine rotaviruses. Typical rotavirus types in human are G1-4 and G9 combined to P[8] or P[4]. Strains with common bovine G types, such as G8 and G10, have been identified in children with diarrhea suggesting a zoonotic transmission (5, 6, and 7). Interferons are cytokines that were discovered over 50 years ago for their ability to interfere with viral replication. They are broad-spectrum, antiviral agents. An important signal is the accumulation of double-stranded RNA, a product of RNA and DNA virus replication (8, 9, and 10).

The best-characterized IFN-inducible cellular components are PKR and the 2'–5' oligoadenylate synthetases. Other factors may be involved, especially molecules that regulate the cell cycle or cell death and thereby limit virus replication (11, 12).

IFN response plays an important role in protection against rotavirus natural disease. Early studies showed that levels of type I and II interferon are elevated

in rotavirus-infected children and animals (13, 14, 15, and 16). Interferons also inhibited rotavirus infection in human intestinal HT-29 and CaCo-2 cells when the cells were treated 24 h or more prior to infection (17). Therefore this study was designed to demonstrate the effect of interferon on Rotavirus replication post-infection.

Material and Methods

1-Cells and Viruses:

Rhesus monkey kidney fetal (MA104) cells were obtained from American Type Culture Collection (ATCC). Bovine Rotavirus (NCDV) was obtained from Department of Veterinary Sciences, South Dakota State University, USA. The virus was propagated in MA104 cells. The titer of stock rotavirus was determined by plaque assay in MA104 cells and expressed as PFU per milliliter as described previously (18).

2- Recombinant human IFN-alpha (Sigma):

It was prepared according to manufacturer's instruction and stored at –80^{o}C in small aliquots.

3- Effect of IFN on BRV cytopathic effect:

Confluent monolayers of MA104 distributed in 24 wells cell culture plates were infected with trypsin-activated BRV at a multiplicity of infection (MOI) of 4000 PFU/ 106 cells. After adsorption, the cultures were washed with GKN and refed with serum-free MEM containing different concentration of interferon (10, 100, 500, or 1000 U/ml) and 0.5 µg/ml trypsin. Treated non-infected and non-treated infected cells were maintained for control. The plates were incubated at 37oC in 5% CO2 incubator for 3 days with daily observation for the development of specific BRV CPE.

4- Effect of IFN on BRV replication:

Confluent monolayers of MA104 were infected with 4000 PFU/ 106 cells of BRV. After adsorption, the inoculum was removed and the cells were overlaid with 2x media containing 0.8% seaplaque agarose and different concentration of interferon and 5 µg/ml trypsin. Controls were carried out on a mock infected cultures supplemented with DMEM or interferon as well as

virus infected cultures. The cultures were examined after 5 days of incubation at 37oC in 5% CO2 condition by fixation with 10% formol saline and staining with crystal violet 0.1%. (18)

5- Effect of IFN on BRV titer:

MA104 cells, grown in 24 wells plates, were infected with trypsin-treated BRV at MOI of 4000 PFU/ 106 cells for 1 h at 37oC. The infected cells then were treated with 10, 100, 500, or 1000 U/ml of IFN. The cells were incubated for 72 h at 37oC. At time intervals of 72h pi, both supernatants and monolayers were harvested, submitted to three cycles of freezing/thawing. The viral titers were determined using plaque assay.

6- Real-time quantitative PCR (qRT-PCR):

Total RNA was extracted from BRV infected MA104 cells, 72h pi, using QIAampR viral RNA Mini kit (Qiagen) according to the manufacturer's instruction. The extracted dsRNA of BRV were denatured by heating at 95oC for 5 min and then chilled on ice for 5 min. The cDNA was generated by reverse transcription using random hexamers with SuperScript III RT (Invitrogen). Real-time PCRs were carried out using SYBR green PCR master mix (Roche, Molecular Biochemicals) and primers VP6-Fw1: 5` GGATGTCCTGTACTCCTTGTCAAAA 3` and rev1: 5` TCCAGTTTGGAACTCATTTCC 3`. Each sample was analyzed by PCR, in duplicate wells. Negative and positive controls were included. Thermal cycling conditions: 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and 1 min at 60°C. Reactions were run in Stratagene MX3000 real-time PCR. The specificity of the reactions was determined by melting curve analysis of the amplicons. Real-time fluorescence measurements were taken and a threshold cycle (CT) value for each sample was calculated by determining the end point at which the fluorescence exceeds a threshold limit. (19).

Results

Effect of IFN on cytopathic effect of BRV:

When the MA104 monolayers were treated with IFN after infection with BRV, interferon had a clear effect on the intensity or progression of CPE in comparison to infected non-treated cultures with dose dependent manner.

Low concentrations of IFN (10, 100U) had no effect on the rate of cytopathic effect of BRV,while high concentrations were able to reduce the intensity of BRV cytopathic effect (500, 1000U). Intact cell monolayers were observed in wells incubated with 1000 U (Figure 1).

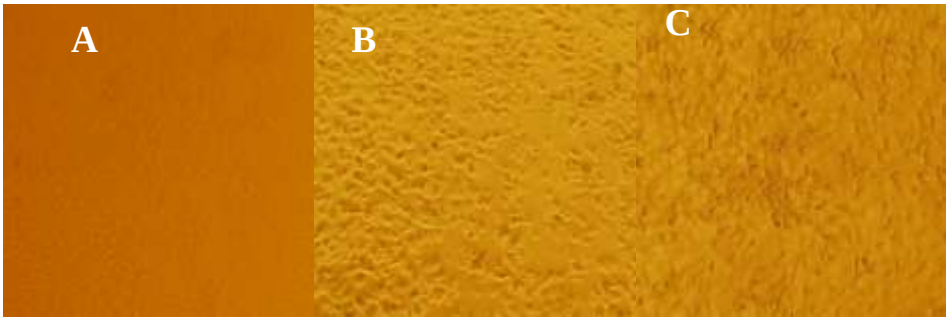

Figure 1: Effect of interferon on cytopathic effect of BRV: A- Normal cells showing confluent monolayer cell sheath. B- Virus infected cells showing complete rounding and lysis of MA104 monolayer sheet. C- Virus infected treated cells with 1000 U interferon showing rounding and aggregation of cells. Inverted microscope X 100.

Effect of IFN on plaques of BRV:

It was observed that after 5 days of incubation at 37oC, there was a dose-dependent decrease in the number of plaques with respect to the concentration of IFN added. Low concentrations of IFN (10, 100U) didn't reduce the number of BRV plaques; while high concentrations (500, 1000U) were able to reduce the number of plaques (Table 1 and Figure 2).

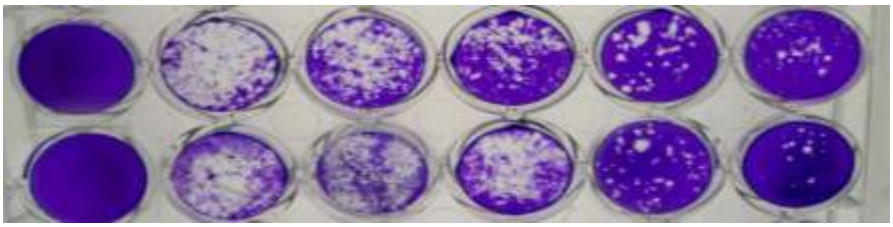

Figure 2: Inhibition of BRV replication by interferon: The effect was determined by treatment of MA104 cells with different concentration of IFN post infection using plaque assay: there is difference in number of plaques with dose-dependent decrease.

Table 1: Effect of interferon on plaque number of BRV.

	Virus control	**Interferon concentration**			
		10	**100**	**500**	**1000**
Number of plaques	4000 PFU	4000	4000	46.3 ± 4.37	18.6 ± 1.76
Log 10 PFU	3.6	3.6	3.6	1.7	1.3
Reduction Log 10 PFU	-	0	0	1.9	2.3

The table displays the mean values of number of plaques ± the standard errors from three experiments.
** Reduction in plaques was determined by Log 10 PFU in virus control – Log 10 PFU of treated cells with different concentration of interferon.*

Effect of interferon on titer of BRV: IFN-alpha treatment decreased BRV yields and the virus inhibition was dose dependent. There was significant decrease in virus titer in supernatant by 1.3 and 2.3 logs when the MA104 cells were treated with 100 and 500U IFN respectively.

There was no virus detected in supernatant when the cells treated with 1000U IFN. Also it was observed that IFN had the ability to decrease virus titer in cell associated by 0.9 and 1.5 in virus infected and treated cells with 500 and 1000U IFN respectively (Table 2 and Figure 3).

Table 2: Effect of IFN-alpha on titer of BRV.

	Virus control	Interferon concentration			
		10	100	500	1000
Supernatant	4.6	4.5	3.3	2.3	0
Cell associated	5.5	5.5	5.3	4.6	4

The table represents the virus titers (Log 10 PFU/ml).

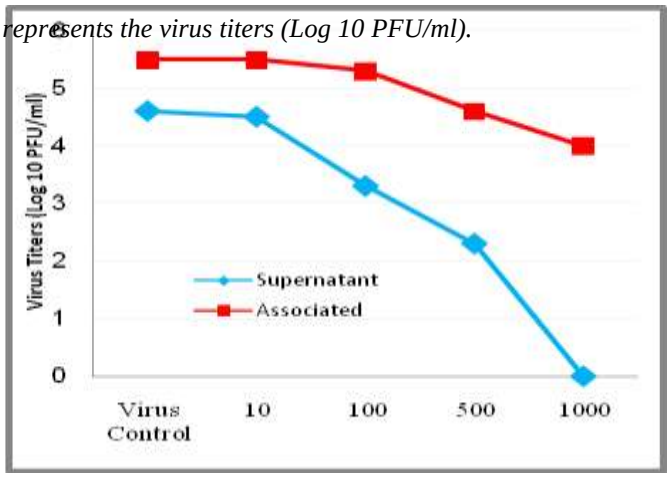

Figure 3: Effect of interferon on titer of BRV

Effect of IFN on Rotavirus RNA:

The viral RNA was extracted from both BRV infected treated and infected non treated cells 72 h post-infection. At concentrations 500 and 1000 U of IFN, there was decrease in viral RNA. While, little difference in amount of RNA in virus infected not treated and virus infected treated cells with 10 and 100 U of IFN, data not shown.

Discussion

Rotaviruses, a genus of the *Reoviridae* family, are classified into seven groups (A–G). It causes world-wide acute viral gastroenteritis in young animals and children **(20).** Rotavirus-induced immuneresponses, especially the T and B cell responses, have been extensively characterized; however, little is known about innate immune mechanisms involved in the control of rotavirus infection.

To test the effect of IFN on rotavirus replication post infection, the MA104 cells were treated with different concentration of IFN. It was observed that IFN had a clear effect on the intensity or progression of CPE, plaque number and virus titer of BRV in comparison to infected non-treated cultures with dose dependent manner. As seen in Fig1, 2 and 3, and in table 1 and 2, MA104 cells were effectively protected, in a concentration-dependent manner, against BRV infection by IFN. To study the antiviral role of IFN on RNA production level of BRV. The most advanced and accurate method; real time quantitative RT-PCR was done. With this approach, viral RNA level in BRV infected cells was decreased.

The action of IFN on rotavirus was previously tested on MA104 cells by incubation of cells with IFN at 37°C overnight before inoculation with BRV **(21)** and IFN inhibited the virus replication**.** Similar results were recorded by **(22)** who reported that IFN inhibit rotavirus replication and the inhibition was dependent on the IFN concentration. IFN was used to successfully treat rotavirus diarrhea in bovine and porcine models **(23, 24).** Exogenous IFN is effective in preventing and treating biliary and liver disease in RRV-infected newborn mice **(25).** Using IFN signaling-deficient mouse models, oral RRV infection in suckling mice could result in a prolonged disseminated infection in multiple extra-intestinal organs, including liver, bile duct, pancreas, and mesenteric lymph nodes and a lethal systemic disease **(25).**

On the other hand, most wild type rotavirus strains reported to efficiently suppress IFN responses. In suckling mice, IFNs appear to have little if any effect on the course of diarrhea or virus shedding during rotavirus infection **(26, 27).** It has recently been shown that rotavirus nonstructural

protein NSP1 can interact with interferon regulatory factors 3 and 7 (IRF3 and IRF7) and enhance their degradation **(28, 29).** The mammalian rotaviruses have evolved specific mechanisms to evade the Type I IFN antiviral response. Rotavirus likely represses the IFN response by at least 4 mechanisms. Bovine rotavirus replication in primary ECK cells is extremely resistant to interferon **(30).** These results suggest that repression of the IFN response is dependent on both the rotavirus strain and the cell type. The role for IFN is virus strain-specific **(31).**

In conclusion, it appears that IFN plays an important role in limiting infection of rotavirus; however, this effect varies significantly among concentration of IFN. Further studies on the effect of interferon on BRV at different time in-vivo (lab animal and natural host), on rotavirus protein expression need to be done.

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تأثير الأنترفيرون على فيروس الروتا البقرى

ابواليزيد عبدالقادر الشيخ[1]، شيماء محمد جلال محمد منصور[1]، محمد السيد محمد[2]
قسم الفيرولوجيا- كلية الطب البيطرى- جامعة الزقازيق- الزقازيق- مصـــــــر[1]
* قسم الأمراض المشتركة- كلية الطب البيطرى- جامعة الزقازيق- الزقازيق- مصـــــــر[2]

الملخص العربى

يعتبر فيروس الروتا البقرى من أهم العوامل المسببة للاسهال فى العجول حديثة الولادة فى جميع أنحاء العالم. لقد اجري هذا البحث لدراسة تأثير الانترفيرون بالتركيزات 10، 100، 500 و 1000 وحدة لكل ملى على تكاثر فيروس الروتا. وأظهرت النتائج ان الانترفيرون يؤثر على نسبة التأثير الضار لفيروس الروتا على الخلايا وكذلك على عيارية الفيروس والحمض النووى. وكان تأثير الإنترفيرون على الفيروس بطريقة تعتمد على الجرعة، حيث ان التأثير كان ملحوظا عند التركيز 1000 وحدة لكل ملى. يمكن استخدام الانترفيرون كمثبط لتكاثر فيروس الروتا.

Genotoxic Effect of Sodium Metabisulphite as Food Additives in Albino Rats.

Samar S. Ibrahim[1]; Bakery H. H. [1]; El-Shawarby R. M. [1] and Abuo Salem M. E[1].

[1]Department of Forensic Medicine & Toxicology. Faculty of Vet. Med. Benha University

Abstract

This study is designed to investigate the genotoxic effect of Sodium metabisulphite which is used as antimicrobial substance in foods. Sixty rats classified to three groups. First group used as control; second and third group was given 1/20 and 1/10 LD50 of SMB respectively (orally via stomach tube). Serum samples used for detection of liver and kidney functions. Bone marrow was used for genotoxic effect. Histopathological examination was performed in liver and kidney tissue. Results showed increase level of serum Alanine aminotransferase (ALT), Aspartat aminotransferase (ALT), urea and creatnine. The recorded structural chromosomal aberrations were acentromeric, deletion, break, fragment, ring and sticky, while the numerical chromosomal aberrations were hyperploids and polyploidy. It can be concluded that SMB have genotoxic effect on albino rats.

Introduction

Many methods were improved to store the food for a long period, so many of chemical substances are used for the preservation of food as antimicrobial agents. It was reported that certain food additives substances, especially antimicrobial agents are genotoxic in different test systems. However, there are a lot of food preservatives whose their genotoxic effects are unknown. **[24].** Sulphites are compounds that contain the sulphite ion SO_3^{-2}. They are often used as preservatives in wines (2,000 mg/L) to prevent spoilage and oxidation, dried fruits (2,000 mg/kg), dried potato products (400 mg/ kg), biscuits and chocolate (50 mg/kg), jam (50 mg/kg), and sausage and salami (450 mg/kg). **[33].** Sodium Meta Bisulphite (SMB) used as food preservatives and is usually noted as E223. SMB acts as an antimicrobial agent which inhibits the growth of fungi and bacteria so keeping food fresh and safe. The maximum dose for using SMB is 450 mg/lor 450mg/kg **[29]** and is 300mg/l or 300mg/kg **[30].** Recent studies have reported that SMB can induce chromosomal aberrations and sister chromatid exchanges in human lymphocytes **[24]** , which are similar to the effect of sodium bisulphite; sulfite; sulfur dioxide (SO_2) and SO2

derivatives in vivo **[16]**. These studies suggested that SMB has cytotoxic and genotoxic effect similar to SO_2 derivatives. Bisulphite induced the chromosomal aberrations, sister chromatid exchanges and formation of micronuclei in human lymphocytes **[17]**, enhanced guanine phosphoribosyl transferase (GPT) mutation in Chinese hamster ovary AS52 cells **[18].** In normal individuals, the amount of sulfites present in serum is low with reported levels of 4–5 nmol/L **[13]**. This is probably due to the presence of sulfite oxidase **[6]**, a mitochondrial enzyme that catalyzes the oxidation of sulphite ion to which is excreted in the urine **[31]**. Hepatic oxidation of exogenous sulfite is limited **[7]**. That is to say the liver metabolizes a constant fraction of sulfite, but a limited amount will pass through the organ and enter the systemic circulation **[8]**. Sulfites can also undergo a nonenzymatic reaction with disulfide bonds generating glutathione *S*-sulfonate **[9]**, causing an increase in intracellular glutathione (GSH) levels **[15]**. *S*-Sulfonate can be detected at low concentrations in the urine of healthy individuals, but are excreted in large amounts in sulfite oxidase deficient patients **[32]**. Our study aimed to investigate the genotoxic affect of sodium metabisulphite on albino rats.

Materials and Methods

Experimental animals:

Sixty apparently healthy male albino rats (western strain) weighted 130 - 150 g were obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The animals housed in stainless steal wire bottom cages and kept under constant environmental conditions and fed on fresh standard pellet and given tap water throughout the study. All animals were acclimatized for 1 week before the beginning of the experiment.

Tested substance:

According to safety data sheet Issued 25/ 9/ 2002 revision No 1 Sodium metabisulphite SMB (sodium pyrosulphite or sodium disulfite) is white crystalline powder with rotten egg odor; stable on normal condition and soluble in water and in most organic solvents. Its molecular weight is 190.1. with chemical formula Na2S2O5. Median lethal dose (LD_{50}) for acute oral toxicity is reported to be 1131 mg/kg for male rats **[19].**

Experimental design:

In this study 60 male albino rats (western strain) were divided in to three groups each one contain 20 rats. First group was kept as control; while second and

third groups were given 1/20 and 1/10 LD50 of SMB respectively (orally by stomach tube) daily for 2 months.

Sampling:-

At the end of 1st and 2nd month, ten rats were sacrificed from each group and samples were collected.

Serum samples: - whole blood collected in clean dry centrifuge tubes, allowed to stand for one hour at room temperature till clotted and centrifuged at 3000rpm for fifteen minutes for serum separation, and kept at -20C till biochemical analysis. Dissect femur bone for flushing of bone marrow for genotoxic effect (chromosomal aberrations). Liver and kidney samples kept in formalin 20% for histopathological examination.

Biochemical analysis:

Serum ALT and AST were performed according to **[26]**; while serum urea was detected according to **[33]** and serum creatnine were detected according to **[12]**.

Genotoxic studies Chromosomal aberration

According to **[1]** all rats were injected intraperitoneal by colchicine 4 mg/kg bwt. After 2 hours of injection rats were scarified, then dissect femurs bone and flush bone marrow by normal saline in centrifuge tube which centrifuged at 1000 rpm for 5 minutes then decant supernatant. Add sufficient amount of hypotonic potassium chloride (KCL 0.56% solution at 37C° to each tube and incubated at room temperature for 10 minutes then centrifuged and decant the supernatant. The sediment was fixed by cold methanol: glacial acetic acid (3:1), the fixation was repeated twice time with 10 minutes interval, the last fixation for 30 minutes at 4C° then centrifuged and decant supernatant. Pellets were resuspended in fresh fixative solution. Dropping 2-3 drops of the content by paster pipette on clean cold glass slides in a distance more than 50cm length and placed on wormer slid for drying then stained with 5% geimsa stain for 15 minutes and washed by distilled water, left to dry .these slides were examined under oil immersion lens to determin structural and numerical aberration in 50 metaphase for each rat according to **[21].**

Histopathological examination:

Autopsy samples were taken from liver and kidney in different group of rat. Samples fixed in formalin solution 20%. Washing was done under tape water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome the obtained tissue

sections were collected on glass slides, deparaffined and stained by hematoxyline and eosin stains for histopathological examinations using light microscope **[3].**

Statistical analysis:

Data were analyzed for obtaining mean, standard deviation (SD) and statistical comparisons between means of different groups. The statistical analyses were done by one way ANOVA and DUNCAN test using SPSS program version 11. P value < 0.05 was assumed for statistical significance.

Results

- **Effect of sodium metabisulphite on serum Alanine aminotransferase (ALT) and Aspartat aminotransferase (AST) showed in Table (1) & Fig (1):**

AST level at 1st month was significant increase in treated groups in comparison to control group, while at 2nd month was more significant increase in treated groups in comparison to control group. ALT level at 1st month was non significant increase in treated groups in comparison to control group, while at 2nd month was more significant increase in treated groups in comparison to control group. These results were more clear at group receive large dose of SMB if compared with control group.

Table (1) level of serum (ALT) and (AST) per (U/ L) on rats received 1/10&1/20 LD50 of SMB (Mean ± SD):

	First month		Second month	
	AST	**ALT**	**AST**	**ALT**
Group I control	5.37 ± 2.4 [a]	10 ± 3.08 [b]	5.98 ±5.9 [c]	11.61 ±4.0 [c]
Group II 1/20 LD50	7.74 ± 1.2 [a]	19.1± 2.9 [b]	20.16 ±4.2 [b]	23.0 ± 4.7 [b]
Group III 1/10 LD50	9.44 ± 2.4 [a]	25.72 ± 3.9 [a]	29.98 ±3.1 [a]	30.4±4.7 [a]

Mean with different letters at the same raw differ significant (P < 0.05).

- **Effect of sodium metabisulphite on serum Urea and Creatinine showed in Table (2) & fig (2):**

At 1st month urea level was significant increase in treated groups in comparisons to control group, while at 2nd month was non significant increase in treated groups in comparisons to control group. Creatinine level was significant increase in treated group in comparisons to control group at 1st & 2nd month. These

results were more clear at group received large dose of SMB (1/10 LD50) if compared with control group.

Table (2) Effect of SMB on Urea and Creatinine per (U/L) on serum of rats:

	First month		Second month	
	Urea	Creatnine	Urea	Creatnine
Group I control	22.6 ± 1.5 ^{c}	0.57 ± 0.10 ^{b}	21.4 ± 2.4 ^{c}	0.67 ±0.13 ^{c}
Group II 1/20 LD50	24.4± 4.8 ^{b}	1.04 ± 0.21 ^{a}	28.3 ± 3.2 ^{b}	1.28 ± 0.10 ^{b}
Group III 1/10 LD50	30.6 ± 4.6 ^{a}	1.87 ±0.24 ^{a}	35.8 ± 2.5 ^{a}	3.01 ±0.26 ^{a}

Mean with different letters at the same raw differ significant ($P < 0.05$).

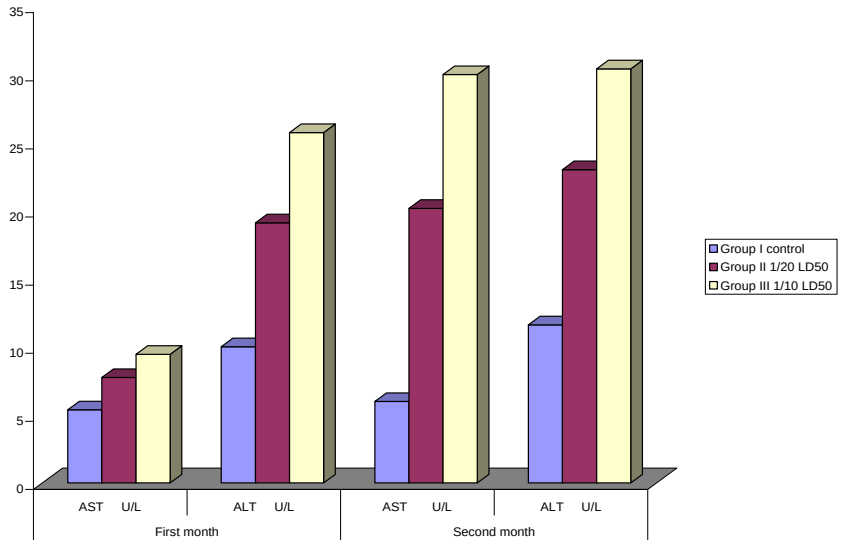

Fig (1) level of serum (ALT) and (AST) on rats.

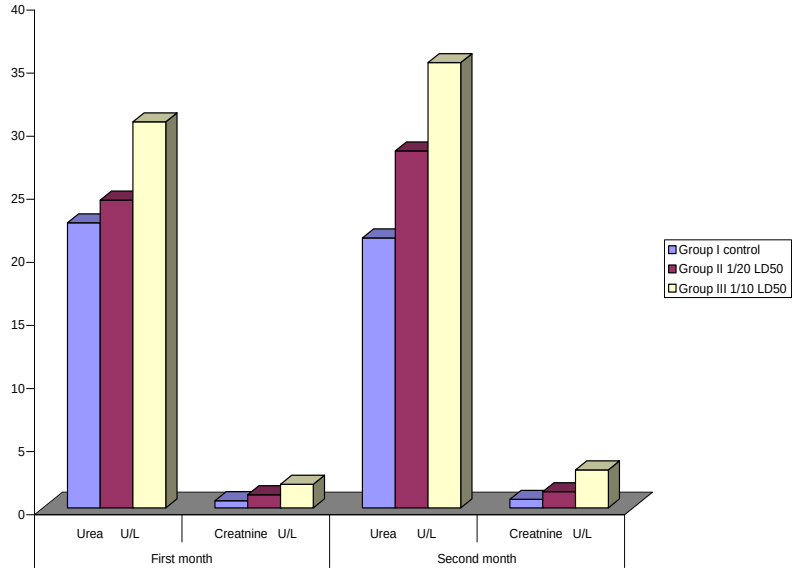

Fig (2) level of serum Urea and Creatinine on rats.

- **Effect of sodium metabisulphite on chromosomal aberration on albino rats were showed in Table (3, 4):**

At 1st month there was significant increase on structural aberrations (acentromeric, dicentric, break, fragment, deletion, sticky and ring) and numerical aberrations (hyperploids and polyploidy) in treated groups if compared with control group, while at 2nd moth there was more significant increase in structural and numerical aberrations in treated groups if compared with control group. These results were more pronounced at group received large dose of SMB (1/10 LD_{50}) if compared with control group fig (3, 4, 5, 6, 7, 8).

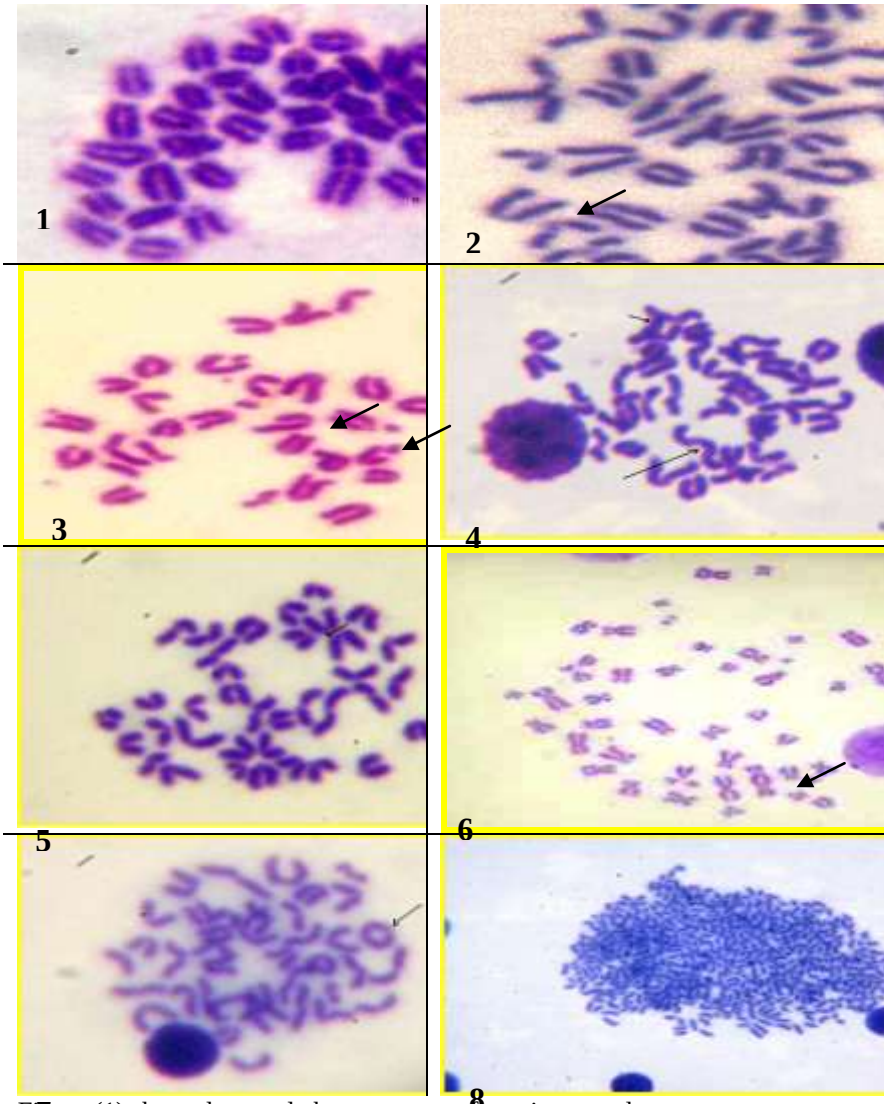

Figure (1) showed normal chromosomes of rats in control group.
Figure (2, 4, 5,8) showed break, stickiness, centric fusion and polyploidy on chromosome on rats treated by 1/10 LD50 of SMB.
Figure (3, 6, 7) showed deletion, fragment, dicentric and ring chromosome in rats treated by 1\20 LD50 of SMB

Table (3) Effect of SMB on chromosomal aberrations of Rats were given 1/20 and 1/10 LD50 of SMB in comparison to control (First month) (mean± SD) :

Groups	Examined cells	Structural aberration							Total %	Numerical aberrations		Total %
		Acentromeric	Di centric	Break	Fragment	Deletion	Sticky	Ring		Poly	Hyper	
Group I control	250	0.89 ± 0.55 c	0.95 ± 0.68 c	1.5 ± 1.0 c	2.07 ± 1.05 c	1.74 ± 0.71 c	0.8 ± 0.37 c	2.0 ± 0.55 c	3.98	1.4 ± 0.4 c	1.6 ± 0.89 b	1.20
Group II 1/20 LD50	250	1.96 ± 0.3 2 b	2.0 ± 0.32 b	11.6 ± 1.08 b	8.4 ± 1.03 b	4.7 ± 0.55 b	1.4 ± 0.51 b	3.8 ± 3.14 b	13.54	4.2 ± 2.75 b	2.0 ± 0.32 b	2.48
Group III 1/10 LD50	250	3.67 ± 0.68 a	4.06 ± 0.24 a	15.4 ± 1.02 a	9.02 ± 0.63 a	6.94 ± 0.75 a	3.4 ± 0.24 a	4.7 ± 0.20 a	18.88	10.8 ± 0.80 a	4.5 ± 0.89 a	6.12

Mean with different letters at the same raw differ significant (P < 0.05).

Table (4) Effect of SMB on chromosomal aberrations of Rats were given 1/20 and 1/10 LD50 of SMB in comparison to control (second month) (mean± SD):

Groups	Examined cells	Structural aberration							Total %	Numerical aberrations		Total %
		Acentromeric	Di centric	Break	Fragment	Deletion	Sticky	Ring		Poly	Hyper	
Group I control	250	1.5 ± 0.47 c	1.08 ± 0.32 c	1.8 ± 0.99 c	2.60 ± 1.05 c	0.8 ± 0.41 c	0.8 ± 0.87 c	2.0 ± 0.25 c	4.23	1.8 ± 0.87 c	1.5 ± 0.54 c	1.32
Group II 1/20 LD50	250	2.05 ± 0.87 b	3.4 ± 0.32 b	16.09 ± 2.5 b	10.24 ± 4.6 b	5.7 ± 0.65 b	2.50 ± 0.51 b	3.8 ± 3.14 b	17.51	5.2 ± 0.8 b	4.2 ± 0.32 b	3.76
Group III 1/10 LD50	250	4.98 ± 1.5 a	5.07 ± 1.09 a	20.4 ± 3.8 a	13.50 ± 1.83 a	8.70 ± 4.6 a	4.95 ± 1.05 a	5.46 ± 0.47 a	25.22	11.64 ± 0.48 a	6.80 ± 2.65 a	7.37

Mean with different letters at the same raw differ significant (P < 0.05)

- **Histopathological Examination:**

The microscopical examination of the organs of treated rats administered 1/10 LD_{50} SMB revealed histopathological changes of the examined organs differ from control group.

- **4 weeks post sodium metabisulphite administration:-**

Liver of treated rat showed interstitial edema rich in fibrin network and mixed with multiple focal areas of hemorrhage, congestion of the portal

vain and periportal edema accompanied by hyperplasia of the billiary epithelium and formation of newly bile ductules. Furthermore congestion of the central vein and fatty change of the hepatocyte evidence by clear, variable size inter cytoplasm vacuoles. Fig (11). The examined Kidneys revealed interstitial hemorrhage and edema admixed with moderate number of inflammatory cells, multiple focal area of hemorrhage in the medulla, homogenous eosinopilic cast in the lumen of some renal convoluted tubules, congestion of the interstitial blood vessel with perivascular hemorrhage. Moreover dilatation of the lumen of some renal tubules. Fig (9).

- **8 weeks post sodium metabisulphite administration:**

The liver of treated rats showed severs congestion of the portal vein and perivascular edema admixed with lymphocytic cellular infiltration, vascular and hydropic degeneration of the hepatocyte, portal areas expanded by fibro plastic cell proliferation and edema admixed with fibrin and inflammatory cells and mild hyperplasia of the biliary epithelium fig (12).

The examined kidneys revealed homogenous eosinopilic cellular cast in the lumen of some renal convoluted tubules lined by attenuated epithelial cells. Furthermore congestion of the critical blood vessels, vascular and hydropic degeneration of the lining epithelium of renal tubules, fatty change of tubular epithelial cells represented by clear well delineated cytoplasmic vacuoles. Occasionally displaced the nucleus of the periphery of the cell .fig (10)

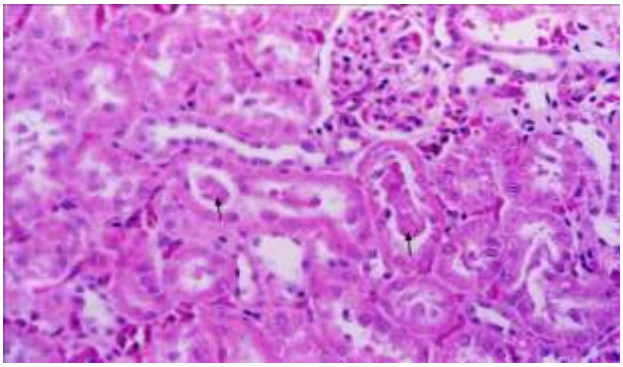

Fig. 9: Kidney of rat administered 1\20 LD50 of sodium meta sulphite for 30 day showing homogenous eosinophilic casts (arrow) in the lumen of some renal convoluted tubules. H&E stain x 400.

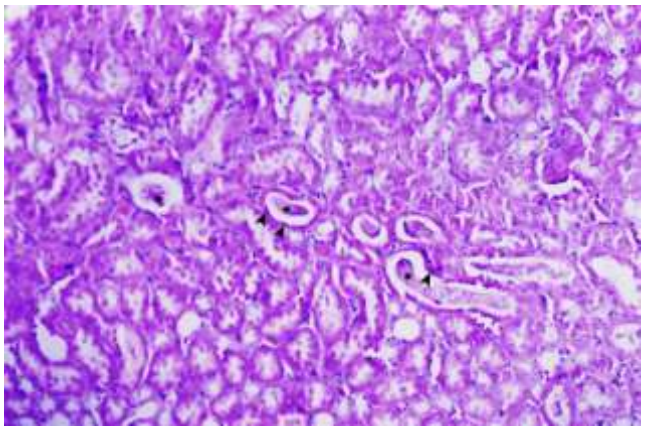

Fig. 10: Kidney of rat administered 1\10 LD50 of sodium meta sulphite for 60 day showing homogenous eosinophilic cellular casts (asterisk) in the lumen of some renal convoluted tubules lined by attenuated epithelial cells (arrow head). H&E stain x 200.

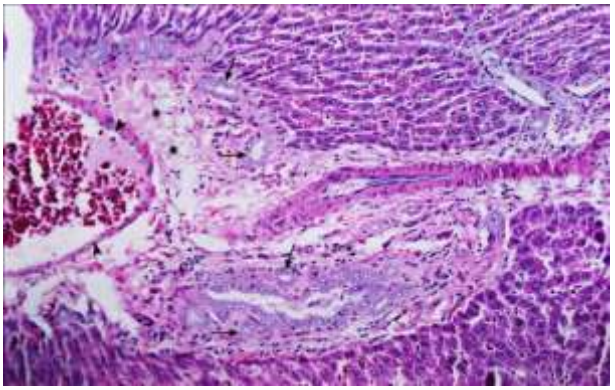

Fig. 11 Liver of rat administered 1\20 LD50 of sodium meta sulphite for 30 day showing congestion of the portal vein (arrow head) and periportal oedema (asterisk) accompanied by hyperplasia of the billiary epithelium and formation of newly formed bile ductules (arrow). H&E stain x 200.

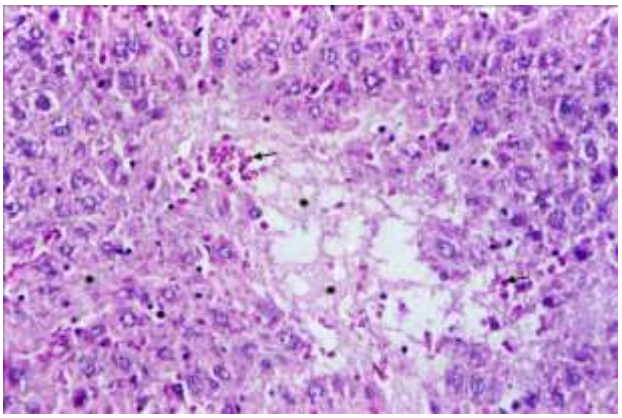

Fig.12: Liver of rat administered 1\10 LD50 of sodium meta sulphite for 60 days showing interstitial oedema (asterisk) rich in fibrin network and admixed with multiple focal areas of hemorrhages (arrow). H&E stain x 200.

Discussion

Sodium metabisulphite is most commonly used as a preservative in food products, such as biscuit, chocolate, sausage, dried fruit and vegetables. It has cytotoxic and genotoxic effect similar to SO_2 derivatives.

Concerning to the effect of sodium metabisulphite on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level Table (1) revealed significant increase on ALT and AST level at 1st month and highly significant increase at 2nd month of experiment that more clear at treated groups compared to control group, our result similar to result obtained by [27]. The significant increase on ALT and AST activities throughout the experimental period is directly related to progressive liver damage and necrosis leading to liberation of these enzymes or due to extensive break down of body tissue [10]. Furthermore may be due to free radicals generating during thiol autoxidation (Thiol and oxygen radicals) which may be the primary sources of oxidants that may contribute to the sodium metabisulphite induced hepatitis [22].

Regarding to the effect of sodium metabisulphite on serum urea and creatnine levels were detected. Table (2) revealed significant increase on serum urea and creatnine levels of treated rat compared with control group. Our result of urea and creatnine level agreed with result recorded by [2] in rat. This elevation may be attributed to severe renal affection and chronic renal failure as S-adenosyle sulfate significantly increased renal dysfunction [26]. These

result confirmed by pathological lesions of kidney (congestion, degeneration and lymphocytic aggregation) which impaired urea excretion and increase its level in blood [20]. Furthermore increase in protein catabolism considered other cause of increase serum urea level [8].

Regarding to the effect of sodium metabisulphite on the chromosomes Table (3 and 4) revealed significant increase on structural and numerical aberrations on treated groups compared with control one, our data agreed with result of [14] in rats and[24] in cultured human lymphocytes. These result attributed to sodium metabisulphite convert to sodium bisulphite and sulphur dioxide. Bisulphite cause deamination of cytosine in both double or single stranded DNA and RNA [4, 5]. The deamination of cytosine caused base pair substation mutation. On other hand bisulphite induce the GC-AT and AT-GC transition like nitrous acid (HNO_2) which caused the deamination in cytosine and adenine [23]. Bisulphite react with 5-hydroxymethyle cytosine instead of normal deamination process gave cytosine-5-methyle sulfonate [11]. Furthermore [16] reported that SO_2 is aclastogenic and genotoxic agent and inhibits the mitosis and increase chromosomal aberrations frequency of the bone marrow cells of rats [15].

The conclusion we draw from the results is that, SMB most likely has a genotoxic risk. For this reason, it is necessary to be careful when using it in food as an antimicrobial substance and it is necessary to find new safe substances alternative to SMB.

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الملخص العربى

التأثير السام على الجينات لميتا بيسلفيت الصوديوم كإضافات غذائية للفئران البيضاء.

سمر صاير ابراهيم [1] وحاتم حسين بكرى[1] ورجب محمود الشواربى[1] و محمد السيد ابوسالم[1]

1 قسم الطب الشرعى والسموم - كلية الطب البيطرى بمشتهر جامعة بنها –القليوبية -مصر

تم تصميم هذه الدراسة لمعرفة التأثير السام على الجينات لميتا بيسلفيت الصوديوم والذى يستخدم كمادة ضد الميكروبات فى الاغذية. قسمت ستون فأرا الى ثلاث مجموعات. استخدمت الاولى كمجموعة حاكمة والثانية والثالثة اعطيت 20/1 و 10/1 جرعة مميتة بنسبة 50% لميتا بيسلفيت الصوديوم على الترتيب بالفم من خلال استخدام انبوب اللى المعدى. تم استخدام عينات المصل لتقدير وظائف الكبد والكلى. تم استخدام النخاع العظمى لتحديد التأثير السام على الجينات. تم اجراء الفحص الهستوباثولوجى على انسجة الكبد والكلية. أظهرت النتائج زيادة مستوى انزيمات Alanine aminotransferase (ALT), Aspartat aminotransferase (ALT) وكذلك اليوريا والكرياتينين فى عينات مصل الفئران. وكانت التشوهات الكروموسومية الهيكلية المسجلة عبارة عن اختلاف القسيم المركزي والمسح والكسر والتجزىء والاستدارة واللزوجة بينما شملت التشوهات الرقمية للكروموسومات فرط الصيغة الصبغية و تعدد الصيغ الصبغية hyperploids and polyploidy. تم استنتاج التأثير السام لميتا بيسلفيت الصوديوم على الجينات فى الفئران البيضاء.

Biochemical Alterations in Experimental Hepatic Stress.

Abd EL Maksoud. H., Omayma. A. R. Abou Zeid, Afaf Desoky. A., Omnia. M. Abdel Hamid, Sa1em, R. Salem.

Faculty of Veterinary Medicine, Department of Biochemistry,
Benha University-Kalubia Egypt.

Abstract

The current investigation has been conducted to investigate the influence of Ethanol on hepatic antioxidant enzymes system in ethanol treated rats. Serum ALT, AST, Total Billirubin, GGT and Alkaline phosphatase are increased significantly in plasma ethanol treated rats compared to controls. Ethanol significantly decreased the superoxide dismutase, catalase, and glutathione content while an increases of malondialdehyde (MDA) levels were estimated in the hepatic tissue.

This effect was induced by a treatment with ethanol for 4 weeks in rats by decreased antioxidant status which induced hepatotoxicity.

Introduction

Alcohol is the most frequently abused psychomotor drug throughout the world and has been known in all civilizations since ancient times (1). Alcoholism is associated with numerous degenerative and inflammatory disorders affecting many organs including liver, brain, heart, kidney, skeletal muscle and pancreas (2). Alcohol liver disease, a common consequence of prolonged and heavy alcohol intake, is a leading health problem after the cardiovascular disease, cancer and AIDS. The consumption of alcoholic beverages is a common feature of the modem way of life and alcoholism ranks as a major health problem today (3). Alcohol consumption is associated with a number of changes in cell function and the oxidant-antioxidant system. Chronic ethanol (Et OH) intake is known to cause direct and indirect toxic effects to mammals and humans by the effect of its by products such as acetaldehyde and acetate (4). Acetaldehyde, a primary metabolic product of alcohol in the liver, appears to be a key generator of free radicals (5).

During metabolism of alcohol via microsomal enzyme oxidizing system (MEOS) pathway, ethanol can increase liver concentration of cytochrome P450 2El (CYP2El) up to ten folds this induction is responsible for oxidative damage in hepatocytes (6). Much of the direct cell damage during alcoholic liver disease is believed to be caused by free radicals (7). High levels of NADH in mitochondria can cause an increase in the number of superoxide

(02·-) free radicals leaked form oxidative phosphorylation leading to the formation of hydroxyl radicals (OH·), lipid peroxidation and damage to mitochondrial DNA These free radicals in high amounts can diminish or impair the antioxidant homeostasis and leads to hepatic tissue damage (1).

Material and Methods

Material:

a-Experimental Animals:

A total of 40 adult male albino rats, average body weight 200-250 gm were used in the experimental investigation of this study. Rats were obtained from laboratory animals' research center, faculty of veterinary medicine, Moshtohor, Benha University. Animals were housed in separate metal cages in the biochemistry department two week before starting of the experiment to accommodate them to the experiment environmental condition in the laboratory (average temp. 25°C±2 and average relative humidity 50%±5); fresh and clean drinking water was supplied ad-labium through specific nipple. Rats were kept at constant environmental and nutritional conditions throughout the period of experiment.

Rats were randomly divided into two main groups as follows:

Group I: (Control group)

Comprised of ten (10) rats, were fed ordinary diet and not receive drugs, served as control for experimental group.

Group 11: Ethanol group (EtOH - group).

Included thirty (30) rats were fed on normal diet and received of absolute EtOH orally at the dose of 2.0 g/kg (2.54 mllkg) body weight (2.54 mllkg) via oro gastric tube for 4 weeks. According to (1), (EL Gomhoria Co.), and served as experimental group.

Sampling

Blood samples:

After overnight fasting, blood samples were collected by vein puncture from all animals group (control and experiment groups) four times along the duration of experiment at 1, 2, 3 and 4 weeks from the onset of Ethanol treatment (Et).

Blood samples were collected in clean and dry sterile tubes and left in refrigerator for some time to clot, then centrifuged at 3000 r.p.m. for 15 minutes at room temperature. Serum was separated using clean, dry sterile tubes, sterilized pipettes and clear serum was received then subjected freshly

for determination the following parameters.

Aspartate-AminoTransferase (AST), Alanine-AminoTransferase (ALT), Gamma- Glutamate Transferase, Total Billiruin, Alkaline Phosphatase, Malondialdehyde (MDA) Superoxidedismutase (SOD), Glutathione (GSH) and Catalase (CAT).

Tissue Samples::

Preparation of hepatic tissue homogenates sampling After 24 h of the last treatment and collection of blood samples rats were scarificed: livers tissue were washed with ice cold saline then immediately excised and kept stored in deep freezer and at -20°C until used. At the time of assay, 1.0 g of hepatic tissue of each one was homogenized in 10 ml of distilled water using electrical homogenizer and centrifuged at 3000 r.pm for 15 minutes at room temperature to get of cell debris. The resulting supernatant was taken and used for determination of parameters.

Results

Chronic intake of ethanol is associated with marked alterations in antioxidant defense system of mammals and leads to oxidative stress. All the antioxidant enzymes evaluated are significantly decreased by ethanol administration. Also increased all liver enzymes than control group as table (12) & fig (1-2).

Table I: Effects of Ethanol on ALT, AST, GGT, Total Billirubin and Alkaline Phosphatase levels of treated rats.

Parameter	ALT	AST	GGT	Total BiIi.	Alk. phos
Control group X ±S.E	55.03±8.7	41.9± 3.2	38.5± 2.4	0.93 ± 2.5	30.47± 1.2
Liver stress group X ±S.E	*** 140±9.9	*** 130.4± 8.2	*** 119.6 ±11	* 1.52±0.38	*** 110.4±2.9

Data are presented as mean ± S.E	S.E= Standard Error
Non - significant difference at p > 0.05	* : significant difference at p< 0.05
**highly significant difference at p <00 I	*** Very Highly significant Difference at p < 0.00 I

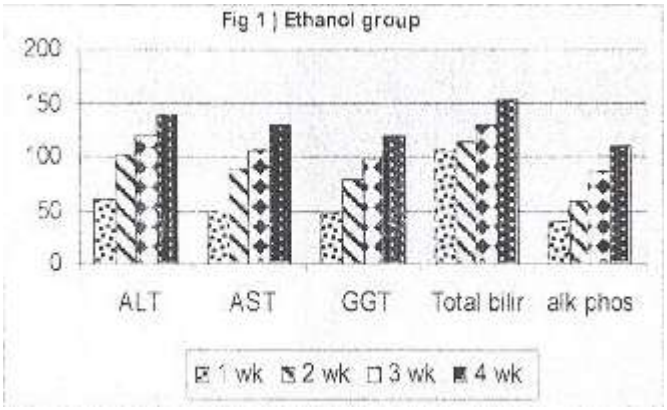

Figure (1): Mean values of serum ALT, AST, GGT, Total Bilirubin and Alkaline Phosphatase concentration of liver stress male rats.

Table 2: Effects of Ethanol on the levels of MDA ,GSH and antioxidant enzymes (SOD, CAT)of treated rats.

Parameter	SOD	GSH	CAT	MDA
Control group X±S.E	35.1 ±2.48	45.2±5.19	60.8±8.19	58.3 ± 0.19
Liver stress group X± S.E	19.6 ±1.12**	21.9±2.32**	3.91 ±4.3***	104.8±0 12**

Data are presented as mean ± S.E	S.E= Standard Error
Non - significant difference at p > 0.05	* : significant difference at p< 0.05
**highly significant difference at p <00 I	*** Very Highly significant Difference at p < 0.00 I

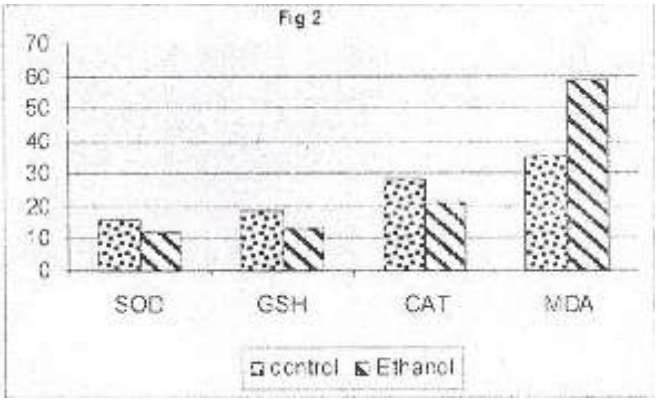

Figure (2) : Mean value of level SOD, GSH, CAT and MDA concentration of tissue liver stress rats and their control.

Discussion

Liver is the major target of ethanol toxicity and the role of oxidative stress in the pathogenesis of alcohol related diseases, particularly in liver, has been repeatedly confirmed (7)

Alcohol is the most frequently abused psychomotor drug throughout the world and has been known in all civilizations since ancient times (8) Alcoholism is associated with numerous degenerative and inflammatory disorders affecting many organs including liver, brain, heart, kidney, skeletal muscle and pancreas (2). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP, total bilirubin, GGT, Thus, the obtained results of our study go in agreement with (9;10; 6;11) this fact that including the deleterious effect of alcohol on liver function.

Alcohol liver disease, a common consequence of prolonged and heavy alcohol intake, is a leading health problem after the cardiovascular Disease, cancer and AIDS. The consumption of alcoholic beverages is a common feature of the modern way of life and alcoholism ranks as a major health problem today (3; 4).

Alcohol consumption is associated with a number of changes in cell function and the oxidant-antioxidant system (5). Chronic ethanol (Eta H) intake is known to cause direct and indirect toxic effects to mammals and humans by the effect of its byproducts such as acetaldehyde and acetate.

Acetaldehyde, a primary metabolic product of alcohol in the liver, appears to be a key generator of free radicals. During metabolism of alcohol via microsomal enzyme oxidizing system (MEOS) pathway, ethanol can increase liver concentration of cytochrome P450 2E1 (CVP2E1) up to ten folds. This induction is responsible for oxidative damage in hepatocytes (12). Much of the direct cell damage during alcoholic liver disease is believed to be caused by free radicals. High levels of NADH in mitochondria can cause an increase in the number of superoxide (020-) free radicals leaked form oxidative phosphorylation leading to the formation of hydroxyl radicals (OHo), lipid peroxidation and damage to mitochondrial DNA (13). These free radicals in high amounts can diminish or impair the antioxidant homeostasis and leads to hepatic tissue damage.

The direct effect of ethanol consumption in body is achieved by the formation of free radicals, which react with various cellular components and cause damage to the tissues. Among other antioxidant enzymes, SOD is considered as front line of defense against the potentially cytotoxic free radicals that cause oxidative stress. In the present study the rats which received 2.0 g of EtOH for a period of 4 weeks showed a significant decrease in the hepatic SOD activity. Similar decrease in SOD activity in plasma and liver (14) and in hepatic tissue (15) has also been reported during EtOH intoxication.

The over-production of superoxide radicals due to EtOH intoxication implies low activity of SOD under ethanol induced oxidati ve stress in the hepatic tissue. The significant decrease in SOD activity due to ethanol indicates inefficient scavenging of reactive oxygen species (ROS) which might be implicated to oxidative inactivation of enzymes (16). These compounds may be responsible to scavenge the superoxide anion radicals and thereby maintain the high activity of SOD even in alcoholics.

We also found that the administration of EtOH has considerably decreased liver CAT activity Ethanol enhances the Production of oxygen derived free radicals and decreases the CAT activity in the hepatic tissue. (17).

Reported a significant decrease in CAT activity with 4 g/kg EtOH treatment for a period of 50 days in rats. (5) reported a significant decrease in CAT activity the hepatic tissue of rats treated with 2 g/kg EtOH for a period of 4 weeks.

The decreased CAT activity with EtOH treatment indicates inefficient scavenging of hydrogen peroxide due to oxidative inactivation of enzyme (IS) the groups of rats which received ginger for a period of 4 weeks showed significant elevation in CAT activity in the hepatic tissues which indicates the antioxidant property of dietary ginger. This beneficial result indicates further evidence for the hepatoprotective effect of dietary ginger The present study showed that also the activity of GSH-Px was significantly decreased in

EtOH treated rats, which may disturb the glutathione homeostasis in the liver cells and ultimately leads to the damage of hepatocytes. Decrease in GSH-Px activity may be implicated to either free radical dependant inactivation of enzyme (19) or depletion of its co-substrate ie, GSH and NADPH in the ethanol treatments (20) The reduced GSH-Px activity may also be due to reduced availability of GSH as observed in the current investigation.

The activity of hepatic GR was significantly decreased with ethanol treatment in the rats. (14) Also reported similar decrease in GR activity with Et OH treatment (1.6 g/kg) in hepatic tissue of rats. Also (5) in their alcohol dose dependent studies found a significant decrease in GR activity in the hepatic tissue of rats. The decrease in GR activity after ethanol intoxication reflects the impaired conversion of glutathione of oxidized form to reduced form (20) thus alters the GSHlGSSG ratio. The increase in GSH/GSSG ratio in the liver of EtOH fed rats and inhibition of GR activity are indicative of ethanol induced oxidative stress in the liver (21) leading to the decreased antioxidant enzyme capacity The present results also showed significant elevation in MDA during EtOH intoxication in the hepatic tissue of rats. Recently (22) reported increased lipid peroxidation with ethanol in their dose dependant studies.

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الملخص العربى

التغيرات الكيميائية الحيوية فى الاجهاد الكبدى المحدث تجريبيا

حسين عبدالمقصود واميمة أبوزيد و عفاف دسوقى وأمنية عبدالحميد وسالم سالم

قسم الكيمياء الحيوية، كلية الطب البيطرى، جامعة بنها

أظهرت هذه الدراسة التى أجريت على ذكور الفئران التى أحدث فيها اجهاد كبدى بحدوث تغيرات كيميائية وحيوية فى وظائف وانزيمات الكبد المختلفة.

ونستطيع ان نستخلص من نتائج الدراسة ان الاجهاد المحدث فى الكبد قد احدث الاتى: زيادة فى تركيز المالوندايالدهيد وايضا حدث ارتفاع فى بعض الانزيمات مثل انزيم اللانين امينوترانسفيريز و انزيم اسبرتيت امينوترانسفيريز وانزيم الفوسفاتيز القاعدى والبيلروبين الكلى وجاما جلوتاميت ترانسفسيريز. كما اظهرت النتائج احداث نقص فى تركيز انزيم سوبر اوكسيد ديسميوتاز وانزيم جلوتاثيون ريدكتاز وانزيم كتاليز.

Microscopic Observations On The Lung Of Quail (Coturnix Coturnix): Pre-Hatching Studies

EL-Zoghby, I. M. [1]; Bakery, H. H. [2]; Metwally, M. A. [3]; EL-Bealawy. M. A. [4] and Eed, R. M. [4]

[1]Faculty of Veterinary Medicine, Departments of [1]Histology and Cytology, [2]Forensic Medicine and Toxicology and [3]Anatomy and Embryology, Benha University-Kalubia Egypt.
[4] Animal Health Research Institute, Department of Biochemistry, Dokki-Giza, Egypt.

Abstract

This work was carried out on 90 quail embryos to illustrate the anatomical and histological changes in the lung tissue during the prehatching period (16 days in 9uail). The lung buds appeared as small ridge -like protuberances on the ventrolateral aspects of the foregut at the end of the 3rd embryonic day (ED). At the 4th ED, the lung buds fused in the ventral mid line and subsequently divided into left and right lung primordia. During the 5th ED, the lung primordium exhibited a saccular shape: At the 6th ED, the primitive lung tissue changed from a saccular to be a wedge-shaped. The primitive lung was formed from mesenchymal tissue embedded in it some tubular structures together with small blood vessels. At the 7th ED, the epithelium of the mesobronchus was formed of pseudostratified columnar cells. At the 8th ED, the primitive lung appeared to be circumscribed. At the periphery of the lung, the parabronchi appeared as solid clusters of epithelial cells and parabronchi started their origin from the tips of secondary bronchi. During the 9th ED the parabronchi increased greatly and some of which appeared luminized. The 10th ED was characterized by appearance of cartilaginous plates in the wall of the mesobronchus at its entrance to the lung. During the 12th ED, the lung appeared to be filled with star-shaped parabronchi. At the 14th ED, the lung appeared to be filled with lung lobules. Each lobule was formed from parabronchial lumen at the center and numerous outpouchings. Before hatching, the wall of the mesobronchus contained well-developed intraepithelial glands and goblet cells.

Introduction

Quails are newly introduced species in poultry industry in Egypt. Quail's meat is an ideal food for human consumption **(1)**.The respiratory system represents the most extensive surface across which the body is exposed to the

external environment. The lung is the most important organ of the respiratory system (**2**). The birds do not possess alveoli blind as terminal portion **(3)**. The lack of blind – ended terminal units, provides in avian lungs a basis For continuous circulation of air through the rich anastomosing air capillaries, in contrast to the alternating inflow and outflow that occurs in mammalian lung (**4**). So the avian (parabronchial) lung differs in certain quintessential ways from the reptilian (alveolar) and the mammalian (Bronchalveolar): (**5**); **(6)**; **(7)**; **(8)**, and **(9**). To our knowledge, there is no previous study about the microscopic anatomy of the development of the lung in quails. Therefore, the main object of this study was to describe the structural pattern and developmental stages of the quail's lung at pre- hatching periods.

Materials and Methods

The present work was conducted on 90 healthy normal eggs collected from quail's farm, Faculty of Veterinary Medicine, Moshtohor, Benha University: After collection, the eggs were washed in combined detergent and antiseptic solution at room temperature, then dried, fumigated and stored at 14-15c (58-60 Fr-and 80% relative humidity (**10**). The eggs were incubated at 99 F for 14 days, and then transported to a hatcher at 92 F until hatching. During the incubation period (16 days in quail), the whole embryos were taken from the 3rd day of incubation to the 11th day, whereas from the 12th day until the 16th day, the embryos were opened and lungs were obtained. The specimens were fixed in 10% neutral formalin then dehydrated and embedded in paraffin. Sections of 5-6 urn in thickness were obtained and stained with Haematoxylen and Eosin, Crossman's modification (**11**) and combination between Alcian blue method and Periodic acid Schiff technique (PAS). These stains were done according to the methods given by **(12**). The stained sections were examined and photographed.

Results

The development of the lung of quail (Coturnix coturnix) was traced from the 3rd embryonic day (EO) until the 16th day.

The 3rd embryonic day(ED) stage:

During the 3rd embryonic day, the respiratory system appeared as an invagination of the epithelial (endodermal) lining of the ventral aspect of the foregut (Fig.1) to form the primitive lung bud. This epithelial lining was of

simple columnar cells characterized by prominent basally situated nuclei and vacuolated cytoplasm (Fig.2).

The 4th ED stage:

The developing lung buds appeared in the median plane (Fig.3).The lung buds appeared during its course as an endodermal tube formed from lining epithelium surrounded by mesenchymal tissue (Fig.4). The lung buds fused in the ventral med line (Fig.5) forming a unitary bud that subsequently divided into rather saccular left and right lung primordia (Fig.6). The lining epithelium was of high columnar cells (Fig. 7) rested on a well distinct PAS + ve basement membrane. The mesenchymal tissue surrounding the epithelium was arranged into an inner loose layer and an outer dense one. Growing RBCs were found singly between the mesenchymal cells and some of the mesenchymal cells showed mitotic activity (Fig.7).

The 5th ED stage:

The lung primordium exhibited a saccular shape and was growing and extending caudally, slightly caudal to the developing liver and heart, and dorsally related to the primitive vertebral column and the mesonephros (Fig.8). The cells of the outer mesenchymal layer were differentiated into flat to cuboidal cells covering the lung primordium externally. An epithelial cell cord was appeared and extended deep into the surrounding mesenchymal tissue and started canalization (Fig.8).

The 6th ED stage:

The lung tissue grossly changed from a saccular to a wedge - shaped form (Fig. 9). The tubular structures appeared including; primitive mesobronchus, primitive secondary bronchi together with solid parabronchi. The secondary bronchi appeared at its origin surrounding with non- cellular thin layer which not found around the primitive solid parabronchi (Fig. 10). The primitive mesobronchus appeared branching at different levels due to secondary bronchi sprouted off from its wall (Fig.ll). This formative mesobronchus was lined with high columnar epithelium resting on a well distinct PAS + ve basal lamina (Fig.l2) which was dissociated at the point of origin of secondary bronchi. The epithelium changed gradually to became cuboidal toward their ends (Fig. 13).

The 7th ED stage:

The secondary bronchi increased in number and extended toward the periphery of the lung (Fig.l4). The wall of the mesobronchus was lined with pseudo stratified columnar epithelium. The nuclei were arranged in several levels. Some cells contained large vacuoles and acquired goblet shape. The lining epithelium of the secondary bronchi varied from low columnar to high cuboidal cells that rested on a clear basal lamina. These cells characterized by possessing cytoplasmic extensions towards the lumen, growing blood vessels were found in different parts of the lung particularly between the secondary bronchi (Fig. 15) and small blood vessels were scattered in other parts of the mesenchymal tissue (Fig. 16).

The 8th ED stage:

The lung appeared circumscribed and closely related to the vertebral column dorsally and the liver and heart ventrally. The secondary bronchi were still growing and branching. The dorsal border of the lung became irregular due to indentations of the ribs. The mesenchymal tissue was formed mainly of flattened mesenchymal cells. Growing blood vessels were increased in number and scattered in the mesenchymal tissue. At the periphery of the lung, parabronchi were appeared as solid clusters of epithelial cells and originated from the tips of secondary bronchi (Fig.17).

The 9th ED stage:

The primary bronchus appeared to be differentiated in its caudal portion to intrapulmonary primary bronchus or mesobronchus from which secondary bronchi aroused (Fig. 18). The lung tissue was formed mainly of dividing secondary bronchi with parabronchi budding from their tips. Parabronchi were in the form of solid clusters of epithelial cells and others appeared lumenized (Fig. 19). The parabronchi at the periphery of the lung appeared as simple hollow tubes with widened lumen. The lining epithelium became less elaborate and the cell decreased in height until it became low columnar or cuboidal as the lumen widened. In between the developing parabronchi growing blood vessels were randomly scattered in the mesenchymal tissue (Fig.19). The lining epithelium of the mesobronchus was still pseudo stratified columnar ciliated epithelium rested on well distinct basement membrane. Beneath the epithelium, a narrow dense mesenchymal layer followed by a wider looser one, then a thin layer of smooth muscle fibers

longitudinally oriented. Then an adventitia contained aggregations of large round or polyhedral cells at the points of ramification of secondary bronchi.

The 10th and 11th ED stage:

The embryonic lung situated more dorsally and pressed against the vertebrae and the proximal one third of the developing ribs that made impressions in the lung tissue (Fig.20). This stage was characterized by appearance of cartilaginous plates in the wall of the mesobronchus at its entrance to the lung (Fig.21). The parabronchi appeared at the periphery of the lung. The wall of parabronchus was lined with columnar epithelium. The epithelium was surrounded by mesenchymal cells that were differentiated into myoblasts. Some of the epithelial cells of parabronchial wall appeared taller than others, while other cells appeared to be about to sink into the surrounding mesenchyme. The number of blood capillaries and blood vessels were greatly increased around the developing parabronchi (Fig.22).

The 12th and 13th ED stage:

The wall of the mesobronchus was lined with cuboidal cells at the areas of cartilage and pseudo stratified columnar ciliated epithelium at the areas without cartilage (Fig.23). There were few layers of fibroblast cells under the epithelium or the epithelium rested directly on the perichondrium. The atria stared appearance as solid outpunching from the parabronchial wall into the surrounding mesenchymal tissue then started canalization producing depressions (atrial- pits) in the luminal outline of the parabronchi. These outpouchings increased in number so that a series of diverticulae aroused from the central parabronchial lumen (Fig. 24).

The 14th ED stage:

The epithelial lining of the mesobronchus showed circumscribed groups of epithelial cells projected externally together with the submucosa, in the form of mucosal folds).The apical part of some other cells contained PAS +ve materials (Fig.25). Other cells had ciliated apical surface. At the end of this stage some epithelial cells appeared alcian blue + ve and these were the first goblet cells to appear (Fig.25). The lung lobules were uniformly distributed in all the lung tissue (Fig.26). Each lobule was separated from the neighboring ones by interlobular septa. The parabronchial lumens were forming the centers of the lung lobules. The wall of the parabronchi formed of lamina epithelialis, muscular layer and adventitia (Fig.27). The epithelial lining was of cuboidal

epithelial cells. At the end of this day, the parabronchial lumen appeared irregular in cross section. The irregularity was due to several outpunching from the lumen that invaded the underlying tissue forming the atria and the appearance of prominent club-shaped projections or inters atrial septa into the lumen of parabronchi. The club- shaped projections were formed of head and stalk.

The 15th and 16th ED stage:

The mesobronchus was the same as the previous stage except there were developed PAS + ve and Alcian blue + ve intra epithelial glands. The lamina epithelialis was formed from pseudo stratified columnar ciliated epithelial cells with intraepithelial mucous glands and goblet cells. The secondary bronchi at their origin were lined with low pseudo stratified epithelium. The atria were formed conspicuous due to an increase in the length of the club- shaped projections (Fig. 28, 29). The parabronchial exchange tissue mantle and the blood capillaries were well formed and these two structural elements were separated by the interstitium (Fig.28). The club-shaped projections appeared well - developed and each was formed from a head and stalk. The head was occupied by smooth muscle cells; the atrial muscles. The stalk appeared as a septum; inter atrial septum and was formed of thin fibrous connective tissue containing fibroblasts (Fig. 30, 31). During this stage, the parabronchial unit was formed from conspicuous atrium and well developed infundibulae (Fig. 32).

Legends of Figures:

Fig. (1): Photomicrograph of 3 days old quail embryo foregut showing: Lung bud (Lb) appeared as invagination from the ventral aspect ofthe foregut (Fg). H & E. XI00.

Fig. (2): Photomicrograph of lung bud (Lb) of 3 days old quail embryo; appeared as ridge- like protuberance on the ventro - lateral aspect of the fore gut (Fg). H & E. X 400.

Fig. (3): Photomicrograph of L.S. of 4th day quail embryo showing: Lung bud as endodermal tube (1) right and left lung buds (2,3), notochord (N) and liver (L). H & E. X 40.

Fig. (4): Photomicrograph of lung buds of 4th day quail embryo; appeared as endodermal tube (1) surrounded by loose mesenchyme (Lm) then dense mesenchyme (Dm) and left and right lung buds (2,3). H & E. X 100.

Fig. (5): Photomicrograph of 4th day quail embryo showing fusion of the left and right lung buds (Lb), epithelium (E), mesenchymal tissue (M). H & E. X 400.

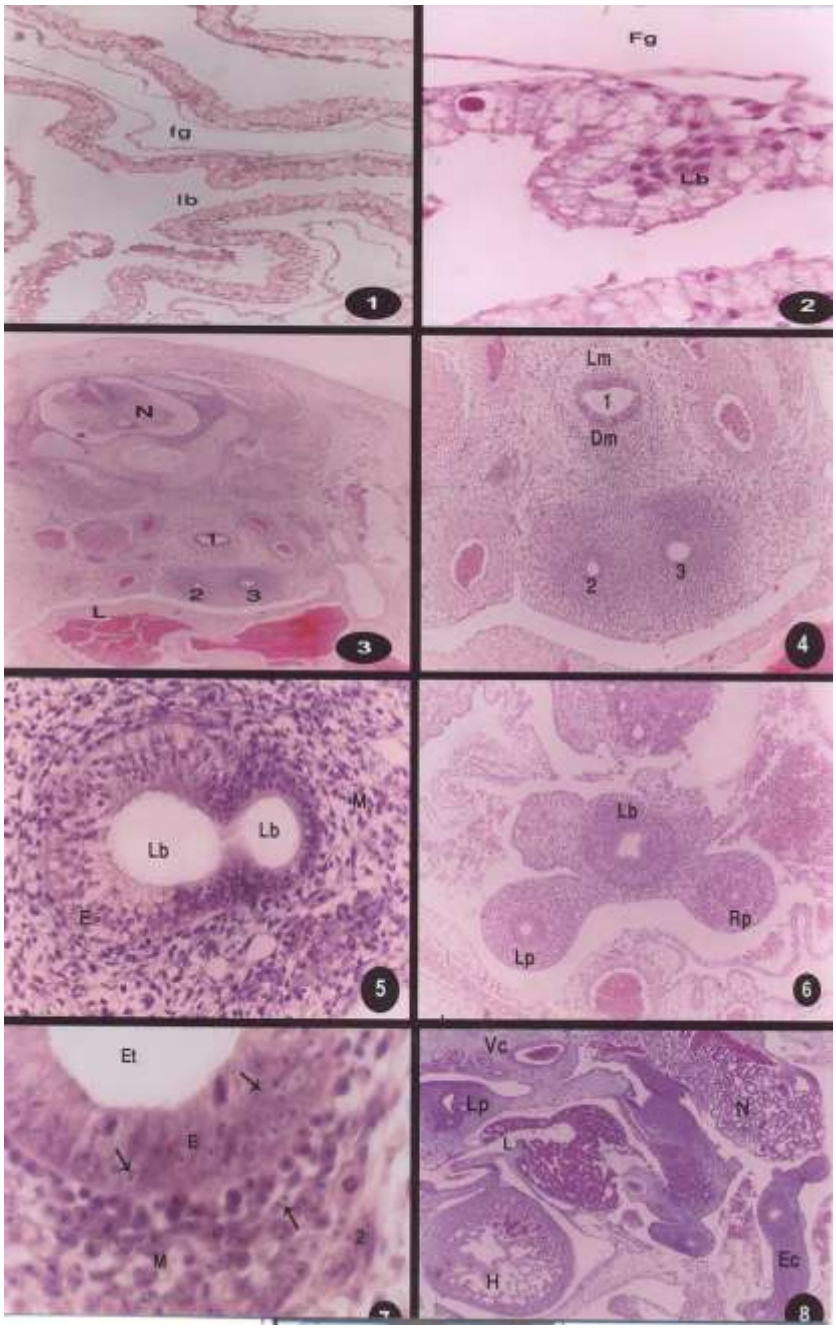
Fig. (6): Photomicrograph of 4th day quail embryo showing unitary lung bud (Lb) divided into left and right lung primordia (Lp & Rp). H & E. X 150.

Fig. (7): Photomicrograph of 4th day quail embryo showing Endodermal tube (Et), Lining epithelium (E) mitotic activity (arrows), mesenchymal tissue (M) and growing RBCs (2). H&E.X 1000.

Fig. (8): Photomicrograph of L.S. of 5th quail embryo showing: Epithelial cell cord (Ec), lung primordium (Lp) Liver (L), heart (H), mesonephros (N) and primitive vertebral column (Vc). H&E. X40.

Fig. (9): Photomicrograph of L.S. of 6th quail embryo showing: wedge- shaped lung tissue (Lt) and mesobronchus (Mb) related dorsally to mesonephros (N) and primitive vertebral column (Vc) and ventrally to liver (L), heart (H) and primitive stomach (St). AL- PAS method. X 40.

Fig. (10): Photomicrograph of 6th day quail embryo showing Primitive lung (Lt); formed from mesenchymal tissue (Mt), mesobronchus (1), secondary bronchi (S), parabronchi (P) and a cellular layer (arrow). H & E. X ISO.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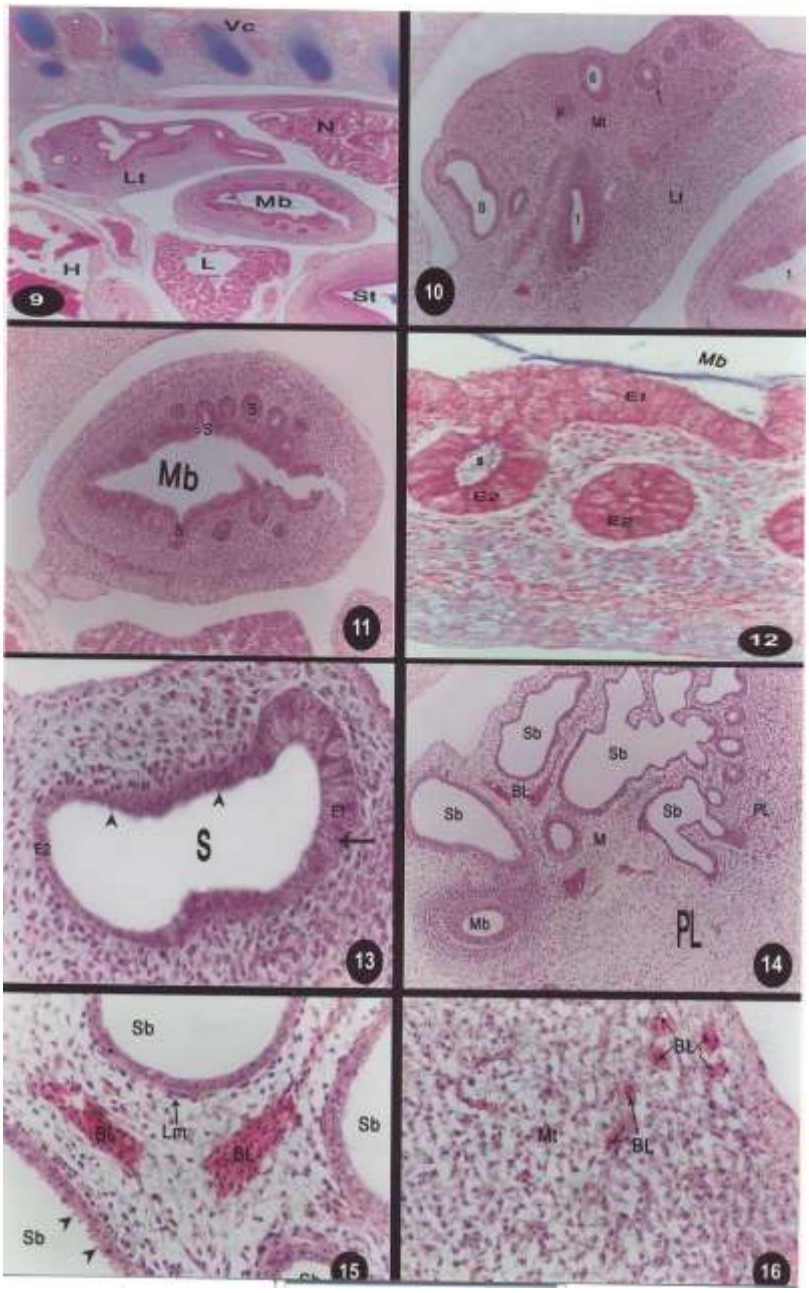
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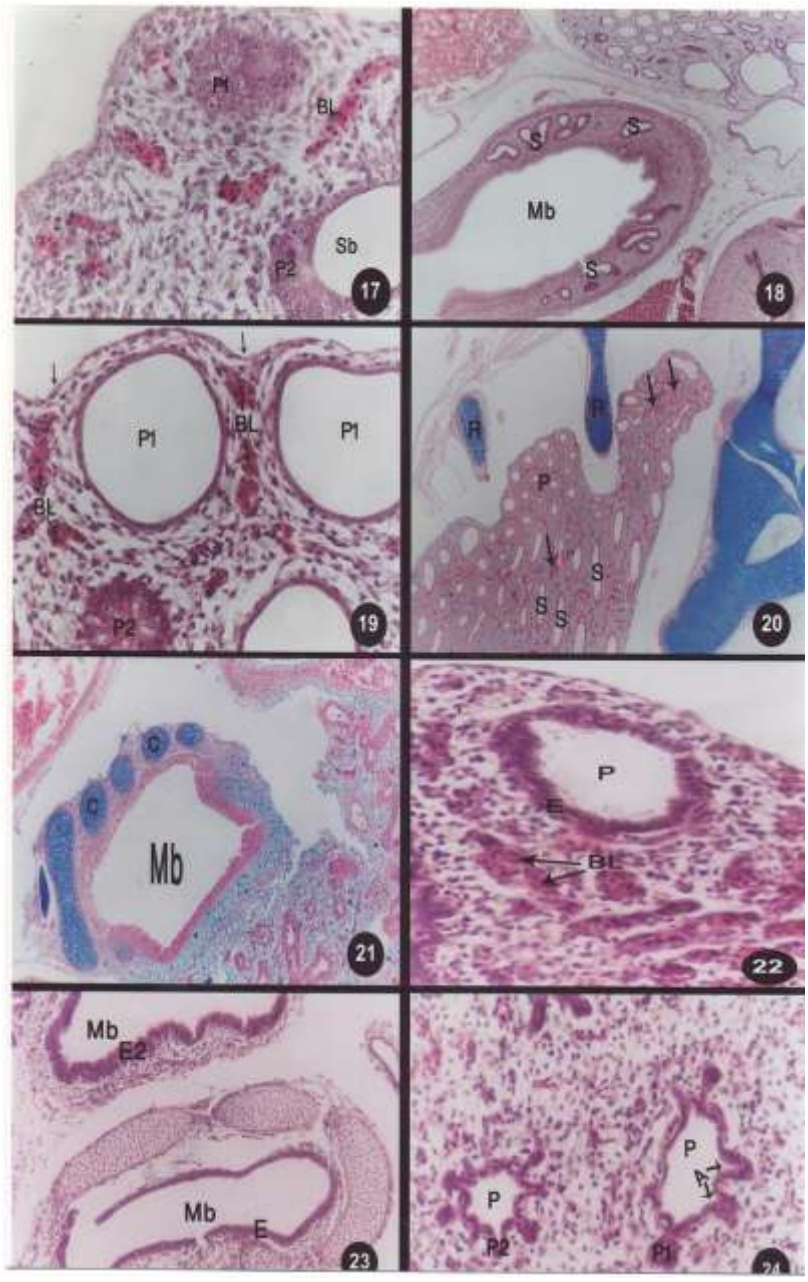
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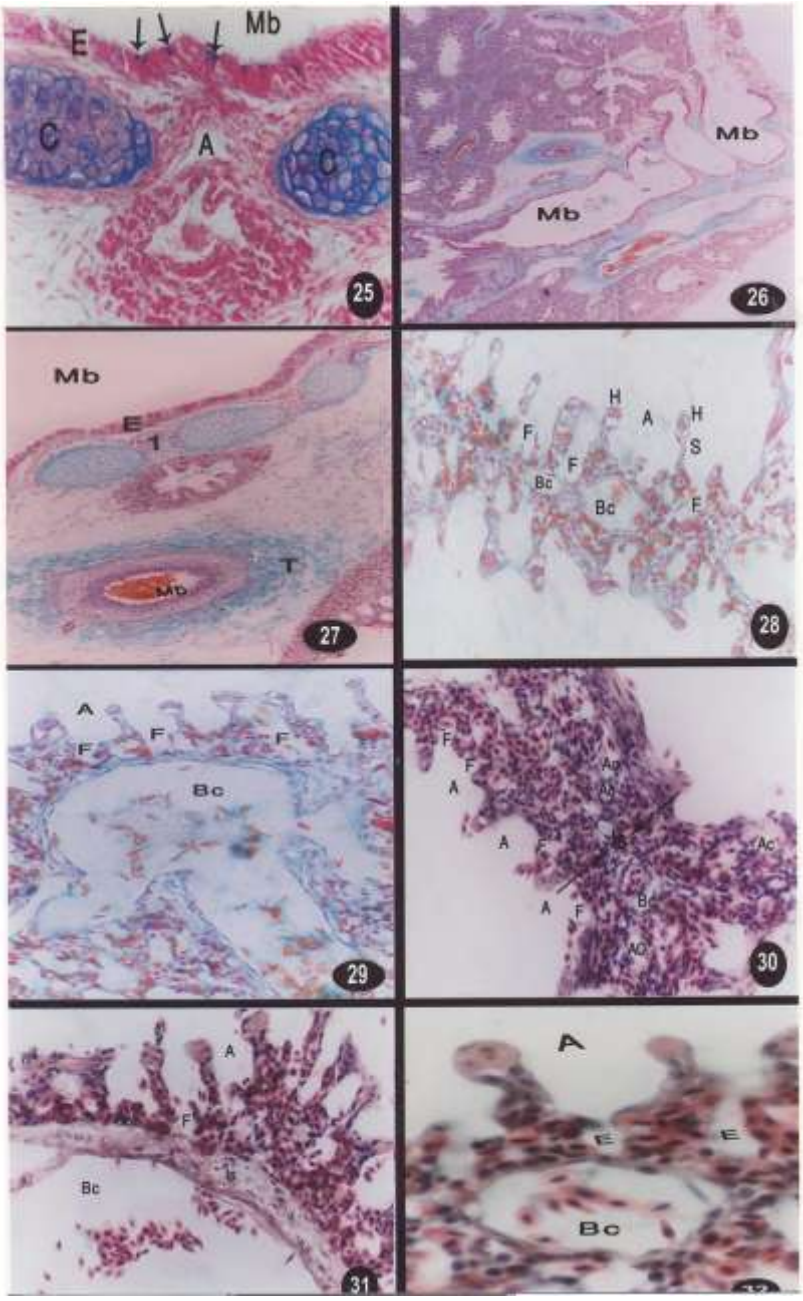
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Fig

. (11): Photomicrograph of 6th day quail embryo showing Primitive mesobronchus (Mb), with secondary bronchi (S) sprouted offfrom its wall at different levels. H & E. X 150.

Fig. (12): Photomicrograph of 6th day quail embryo showing PAS + ve basal membrane underlined the epithelium (El) of mesobronchus (Mb), and investing the epithelium (E2) of secondary bronchi (S). AL- PAS method. X 400.

Fig. (13): Photomicrograph of 6th day quail embryo showing Simple columnar epithelium (El) of secondary bronchus (S), changed gradually into cuboidal epithelium (E2), cells showing mitotic activity (arrows) and some cells showing cytoplasmic extensions (arrow's head). H &E. X 400.

Fig. (14): Photomicrograph of 7th day quail embryo showing Primitive lung (PL), mesobronchus (Mb) and numerous secondary bronchi (Sb) embedded in mesenchymal tissue (M) contained blood vessels (BL). H & E. X 150.

Fig. (15): Photomicrograph of 7th day quail embryo showing secondary bronchus (Sb) lined with low columnar to high cuboidal epithelium with cytoplasmic processes (arrowheads) surround by a cellular layer (arrow) and loose mesenchymal tissue (Lm) containing blood vessels (BL).H & E. X 400.

Fig. (16): Photomicrograph of 7th day quail embryo showing lung mesenchymal tissue (Mt) with growing blood vessels (BL) scattered in the mesenchymal tissue. H & E. X 400.

Fig. (17): Photomicrograph of lung periphery of 8th day quail embryo showing: solid parabronchus (PI) and parabronchus (P2) originating from secondary bronchus (Sb) and growing blood vessels (BL). H & E. X 400.

Fig. (18): Photomicrograph of L.S. of 9th day quail embryo showing: primitive mesobronchus, (Mb) and secondary bronchi (S) originated from its wall. H &E. X 40.

Fig. (19): Photomicrograph of 9th day quail embryo showing parabronchi (PI) at the periphery of the lung as hollow tubes, solid parabronchi (P2), small blood vessels (BL) and flattened mesothelial cells (arrows). H & E. X 400.

Fig. (20): Photomicrograph of L.S. of 10th day quail embryo showing: rib (R) impressed in dorsal aspect of the lung, the lung filled with secondary bronchi (S) and parabronchi (P) and scattered blood vessels (arrow). AL - PAS method. X 40.

Fig. (21): Photomicrograph of 10th day quail embryo showing mesobronchus (Mb) contained cartilaginous plates (C). AL - PAS method. X 150.

Fig. (22): Photomicrograph of lung periphery of l Oth day quail embryo, contained parabronchus (P) lined by columnar epithelium (E), and surround by numerous small blood vessels (BL). H & E. X 400. -

Fig. (23): Photomicrograph of 12th day quail embryo showing Mesobronchus (Mb), simple cuboidal epithelium (E) above the cartilage and pseudostratified epithelium (E2) in the wall without cartilage. H & E. X 150.

Fig. (24): Photomicrograph of 13th day quail embryo showing growing parabronchi (P) contained epithelial outsourcings (solid PI and canalized P2) which produced depressions or atrial pit (A) in the wall. H & E. X 400.

Fig. (25): Photomicrograph of 14th day quail embryo showing mesobronchus (Mb) with cartilage (C), cuboidal epithelium (E) above the cartilage, Alcian blue+ ve cells (arrow) and inter cartilaginous area (A). AL - PAS method. X 400.

Fig. (26): Photomicrograph of L.S. of 14th day quail embryo showing: the mesobronchus (M b) extended through the lung until reached the periphery of the lung also the lobulation of the lung. Crossman's trichrome stain. X 40.

Fig. (27): Photomicrograph of 14th day quail embryo showing mesobronchus (Mb), columnar epithelium (E), inter cartilaginous area (1) rich in collagenous fibers and adventitia (T) rich in collagenous fibers. Crossman 's trichrome stain. X 150.

Fig (28): Photomicrograph of parabronchial units of 15th day quail embryo. Atria (A) are separated by inter atrial septa formed from head (H) and stalk (S) and infundibulae (F) adjacent to inter lobular blood capillaries (Be). Crossman's trichrome stain. X 200.

Fig (29): Photomicrograph of parabronchial units of 15th day quail embryo formed from atria (A), infundibulae (F) adhered directly to interlobular blood capillary (Be). Crossman's trichrome stain. X 400.

Fig. (30): Photomicrograph of parabronchial units of 15th day quail embryo, formed form atria (A), infundibulae (F) and thick interstitium (Is) separating them from the inter lobular blood capillary (Bc). H & E. X 400.

Fig. (31): Photomicrograph of lung tissue of 16th day quail embryo showing: outpouchings formed from atrial zone (A) and infundibular zone (F) and thick inter lobular septa (Is) contained blood capillaries (Be) and air spaces (Ac). H & E. X 400.

Fig. (32): Photomicrograph of parabronchial units of 16th day quail embryo showing: atrium (A), infundibulum (E), blood capillary (Be), head (H) and stalk (S). H & E. X 1000.

Discussion

The present work could identify the lung buds in the quail embryos of 3 days old. In chick embryo, (13); (14); (15) and (16) were the first to observe them on the third day. In duck embryos, (17) described their appearance at Late of the 5th ED and during the 6th day of incubation. In agreement with (17) in duck, the present results showed that the difference in the time of appearance of the lung buds may be due to difference in the time length of incubation period, which is 16 days in quail, 21 days in chicken and 28 days in the duck. Our results agreed also with those obtained by (15) and (18) in the first appearance of the lung buds as a pair of small ridge -like protuberances on the ventro lateral aspect of the foregut.

The present results disagreed with (19), (20); (21) and (17); in that the loosening of the mesenchyme was accompanied by the formation of the blood vessels and that it was due to the increased hydration of intercellular substance. The above-mentioned authors supported and dued these findings by the presence of thin walled blood vessels that led to diffusion of fluids to the surrounding mesenchyme which resulted in its loosening. The disagreement of the present work could be supported and explained by the presence of the loose mesenchymal tissue surrounding the endodermal tube before the first appearance of blood vessels in this mesenchyme during this age.

Our results in the quail embryo revealed that after the lung primordium have formed the lung tissue; the endodermal tube in the primitive lung was still surrounded by two layers of mesenchymal tissue (inner loose and outer denser layers) originated from the previous inner loose one . These results were similar to those obtained by. (21) in buffalo, (22) in Camel, and (23) in rabbit.

The present study revealed that as the primitive lungs developed and increased in size, they exhibited a saccular shape at the 5th ED, then became separated from the vertebral column by the mesonophros and changed from a saccular to wedge shape forms at the 6th ED. At the 7th ED they assumed an ovoid shape and reached their definitive topographic location in the coelomic cavity. In chick embryo, (15) and (9) reported more or less similar results. The lungs grossly changed from a saccular to a wedge shape form and reached their definitive topographical locations in the coelomic cavity on the 6th day of chick embryo. They also reported that the lung had assumed an ovoid shape by the day 6.5 of chick embryo (9), (15), and (16) have reported that the lungs settled onto the ribs and began to attach and gradually sink into them on day 7.5 where each lung assumed an ovoid shape. They noticed deep costal sulci on the dorsal aspects of the lung on day 8 of chick embryogenesis. Although the quails have incubation period shorter by 5 days than the chick,

our result revealed that the lungs were assumed an ovoid shape on the 8th day quail embryos and the deep costal sulci produced by the ribs were evident at the 10th ED.

Our results revealed appearance of growing RBCs found singly between the mesenchymal cells at the 4th ED. This was in accord with (18) in chick who stated that blood cells formed from undifferentiated mesenchymal (stromal) cells from day 5 of chick embryogenesis. The resent work revealed that these cells appeared in clusters of growing RBCs at the 5th ED. Each cluster was formed from 1-2 growing RBCs surrounded by elongated mesenchymal cells that attached to each other to form the endothelium of the blood vessel. These results was in accord with (19), in duck at 7-8 days of incubation and (18) who stated that by day 8, blood cells were widely dispersed in the lung of the developing chick's embryo.

About the start - formation of the intrapulmonary primary bronchus or mesobronchus, our results agreed with (15) in the appearance of an epithelial cell cord at the 5th ED (present results). This epithelial cell cord extended in a cranio - caudal direction, then extended deep into the developing lung and canalized to form the primitive mesobronchus. Also agreed with (9) in that the mesobronchus is the focal point of air way (bronchial system) development in the avian lung. The present findings showed that the primitive mesobronchus and the secondary bronchi at their origin were lined with high columnar epithelium rested on a well distinct PAS + ve basement membrane, which was dissociated at the point of origin of secondary bronchi (24) in duck reported similar result. It was investigated that an extracellular matrix molecule was commonly found in the basal lamina along the sides of the developing bronchi but not in the budding points, this interpret the previously mentioned PAS reaction in the basement membrane (25).

Our results showed that at the 10th ED the parabronchial lumen started to loss its smooth regular appearance due to start of atrial formation. This was in accord with (15) and (16) who reported that from the 10th embryonic day in chick, the epithelium that lined the parabronchial lumen and the adjacent mesenchymal cells underwent drastic reorganization through massive differentiation, migration and apoptosis (programmed cell death) as they transformed into atria, infundibulae and air capillaries; structural elements that came to constitute virtually all the parabronchial gas exchanges tissue mantle.

The present study reported that the atria started formation as solid outpouchetings from the parabronchial wall into the surrounding mesenchymal tissue then stared canalization producing depressions (atrial pits) in the luminal outlines of the parabronchi. These outpouchings increased in number so that a

series of diverticulae aroused from the central parabronchial lumen. Similar results were obtained by (26) who reported that the atrium started appearance as an outpouching from the tertiary bronchi due to invading epithelium to the underling mesenchyme. They added that the outpouchings were initially formed by elongation of each epithelial cell, there after migration of the whole cell into the mesenchyme took place. Also in agreement with (15), (16) and (26) in chick in that, firstly the outpouchings were solid cords of cells that eventually canalized so that a series of diverticulae aroused from the central parabronchiallumen.

During the 15th-16th day old quail embryo, the infundibulae appeared projected from the bottom of conspicuous atria. This was in accord with (16) who stated that, forming as diverticulae from the parabronchiallumen, atria gave rise to infundibulae (small air passages) and air capillaries on day 16 and 18 of chick embryogenesis. Also in agreement with (26), (27) (28) at the 17th day old chick, our results revealed at the 15th and 16th day old quail embryo that the outpouchings could be divided into two zones, inner and outer ones. The inner zone formed of the cuboidal cells nearest the lumen and was covering the groups of smooth muscle cells adjacent to the lumen. The outer zone consisted of more flattened cells that form the lining of infundibulae.

In agreement with (29) in quail and (26), (18) in chick, our findings revealed that each individual outpouching was separated by mesenchymal elements that included undifferentiated cells, blood capillaries, fibroblasts and smooth muscle cells. Our results agreed also in that these mesenchymal elements formed the core of the stalks, the boundaries of the atria, infundibulae and so these respiratory structural elements could constitute a separated units called parabronchial units.

At 16th day quail embryo, our results revealed that the air capillaries, the last chambers of the parabronchial unit were not conspicuous at this stage or even absent in some areas of parabronchi, while in some other areas were appeared unconnected with the infundibulae. Hence, the parabronchial units were formed from conscious atria and well developed infundibulae, structural elements, that constituted the pre-hatching parabronchial exchange tissue mantle. These fmdings were in accord with (29) in quail but disagreed with (5), (26), (27) and (15), (16), (18), (28) in chick embryos and (17) in duck embryos who concluded that the parabronchial unit was formed from conspicuous atria and well developed infundibulae and air capillaries before hatching.

Our results on 16th day quail embryo showed that the parabronchial units and the blood capillaries appeared well formed. The interstitium (formed of thin fibrous elements and interstitial cells) separating the above two structural elements were thinned. The well-developed infundibulae with their attenuated epithelium lining were closely interrelated with the attenuated

endothelium of the well-developed blood capillaries. So the quail parabronchial lung became capable of gas exchange by the end of incubation period. Similar results were obtained by (5), (26), (27) and (15), (16), (18) and (28) at 21 days old chick embryo and (17) at 26 days duck embryos.

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الملخص العربى

دراسات مجهرية على تطور الرئة فى السمان قبل الفقس

ايهاب محمود عبد العال الزغبى[1] وحاتم حسين بكرى[2] و محمد عطية متولى[3] و معروف عابدين البعلاوى[4] و رضا محمد عيد [4]

[1]قسم الانسجة والخلايا، كلية الطب البيطرى، جامعة بنها
[2]قسم الطب الشرعى والسموم، كلية الطب البيطرى، جامعة بنها
[3]قسم الانسجة والخلايا، كلية الطب البيطرى، جامعة بنها
[4] قسم الكيمياء الحيوية، معهد بحوث صحة الحيوان

أجري هذه الدراسة على عدد 90 من أجنة السمان فى الاعمار المختلفة من مرحلة ما قبل الفقس حيث تم دراسة التغيرات التشريحية والنسيجية فى الرئة. تم حفظ وتثبت العينات و اعداد الشرائح والصبغ بالصبغات المختلفة و تم فحصها بواسطة الميكروسكوب الضوئى .بدأ ظهور الرئة فى اليوم الثالث للتحضين على هيئة نتوء مكون من نسيج طلائى عمودى ثم تحول الى انبوب مبطن بنسيج طلائى محاط بنسيج ميزنشيمى. بدأ ظهور الشعبة الهوائية الرئوية فى اليوم السادس ثم ظهرت الشعيبات الرئوية الثانوية متفرعة من الشعبة الرئوية الرئيسية وكانت مبطنة عند بدايتها بنسيج طلائى عمودى ثم تحولت الى نسيج مكعب وقد ظهرت بعض الاوعية الدموية. فى اليوم التاسع بدأت الشعيبات الرئوية الثانوية فى التفرع لتعطى الانبوبات جار الشعبية. فى اليوم العاشر بدأ النمو الكامل للشعب الهوائية الرئيسية وكانت مبطنة عند بنسيج طلائى عمودى كاذب مهدب كما بدأ ظهور الغضاريف والنسيج العضلى. فى اليوم الثانى عشر بدأت الانبوبات جار الشعبية فى تكوين الردهات الهوائية. فى اليوم الرابع عشر بدأ ظهور الغدد المخاطية داخل النسيج الطلائى للشعب الهوائية الرئيسية كما بدأ ظهور الحواجز بين الردهات الهوائية كما بدأت الردهات الهوائية فى التفرع لتعطى مداخل الشعيرات الهوائية وكانت مبطنة بخلايا حرشفية بسيطة.

Public Health Importance of Zoonotic Salmonellosis.

El-Newishy,A,A. [1] and Sylvia. O. Ahmed[2]

[1]Zoonoses Department, Faculty of Veterinary Medicine, Benha University
[2]Dept. of Animal Hyg. and Zoonoses, Faculty of Veterinary Medicine, Assuit University

Abstract

Salmonella is an important zoonotic pathogen and it's prevalence in the animals acts as a continuous threat to man. The present study was carried out to report the isolation along with the serotypes, phage types and antibiogram pattern of Salmonella among man, poultry and cattle. A total of 300 samples from diarrheic livestock and humans were processed for the isolation of Salmonella. Representative isolates of S. typhimurium and S. enteritidis were phage typed. Ninety five isolates of Salmonella enterica belonging to 5 serotypes- S. typhimurium, S. enteritidis, S. gallinarum, S. Paratyphi B and S. Bareilly were obtained with an overall prevalence rate of 14.40 percent. S. typhimurium isolates were distributed among four phages- DT003, DT004, DT096 and DT193 and all the S. Enteritidis isolates belonged to a single phage type, PT13a/7. Our findings showed that three of the five serovars as well as some of the phage types of these serovars were shared by animals and humans indicating the zoonotic potential of the organism. Thus, it is imperative that salmonellosis control measures adopted for humans should give adequate importance to its control in the animals particularly their products. All the isolates were subjected to antibiogram studies against 8 antimicrobials which revealed that cephalexin (Cp, 30), ciprofloxacin (Cf, 30), enrofloxacin (Ex, 10), gentamicin (Gm, 30), were most effective, whereas, doxycycline (Do, 10), ampicillin (Ap, 25), amoxycillin (Am, 10), and tetracycline (Tc, 30) were relatively less effective.

Introduction

Food of animal origin can be the vehicle for transmission of salmonellae to man, meat and meat products which may be contaminated by human excreta at any step in the chain of processing, meat handling from raw material to the preparation of meat and meat products (1). Zoonotic Salmonella is the cause of the food-borne salmonellosis pandemic in humans, in part because it has the unique ability to contaminate poultry meat. The incidence of Salmonella food poisoning in the United States in 1988 was estimated to be between 840,000 and 4 million (2). Enteric Salmonella infection is a global problem both in man

and animals, and has been attributed to be the most important bacterial etiology for enteric infections worldwide. Salmonellosis is endemic in Egypt and its importance, as potential zoonoses needs. Therefore this investigation was planned out to throw some light on prevalence of enteric Salmonella infection among humans and various livestock and poultry in Egypt with their phage typing and also antimicrobial sensitivity test of the isolated Salmonella strains (3).

Material and Methods

Collection and processing of samples: one hundred stool samples from patients with diarrhea were collected from diagnostic laboratories in healthy insurance hospital in benha city. As well as one hundred each, cloacal swabs from diarrheic poultry and rectal swabs from diarrheic cattle from various farms in the same locality were collected aseptically in sterile test tubes and immediately brought to the laboratory for processing.

Bacterial culturing:

The following bacteriological media were used: brilliant green agar (BBL), MacConkey agar (BBL) for direct plating of specimens, Selenite-F broth (BBL) (4 and 5).

Biochemical identification of isolates:

The biochemical identification of isolates made on the basis of the following tests: glucose metabolism negative; production of indole negative, Methyl red reaction positive (MR) and Voges Proskaur test (VP) negative and positive utilization of Citrate and H2S production and hydrolysis of urea negative (6).

Phage typing:

Phage typing was performed in accordance with the methods of Dutch Phage typing system (7 and 8).

Antimicrobial susceptibility testing:

The disk diffusion method was used for susceptibility testing made according to (9). eight drugs were routinely used to test gram-negative enteric bacteria: Antibiotic sensitivity test: In vitro susceptibility of the organisms to various antimicrobial agents was determined by the disc diffusion technique. The antimicrobial agents (concentration in mg) used were: ampicillin (Ap, 25), amoxycillin (Am, 10), cephalexin (Cp, 30), ciprofloxacin (Cf, 30), doxycycline (Do, 10), enrofloxacin (Ex, 10), gentamicin (Gm, 30), tetracycline (Tc, 30). Results were recorded in tables (9).

Results

Table (1): The percentage of Serotypes of Salmonella species isolated from human:

Source	*Number of Samples*	*Number of Positive for Salmonella (%)*	*Serotypes of Salmonella Isolated*	
			Serotypes	*Number of Isolates*
Human (Stool Samples)	*100*	*23 (23%)*	*S. typhimurium*	*10*
			S. enteritidis	*11*
			S. paratyphi B	*2*

Table (2): The percentage of Serotypes of Salmonella species isolated from poultry:

Source	*Number of samples*	*Number of positive for salmonella (%)*	*Serotypes of Salmonella isolated*	
			Serotypes	*Number of isolates*
Poultry (cloacal swabs)	*100*	*34 (34%)*	*S. typhimurium*	*12*
			S. gallinarum	*12*
			S.enteritidis	*8*
			S. paratyphi B	*2*

Table (3): The percentage of Serotypes of Salmonella species isolated from cattle:

Source	*Number of samples*	*Number of positive for Salmonella (%)*	*Serotypes of Salmonella isolated*	
			Serotypes	*Number of isolates*
Cattle (rectal swabs)	*100*	*13 (13%)*	*S. typhimurium*	*5*
			S.enteritidis	*6*
			S. bareilly	*2*

Table (4): The numbers and percentage of phage typable isolated from Human stools:

Serotype	*Source*	*Number of isolates Phage type*	*phage typed*	*Number of isolates*	*Phage untypable*
S. typhimurium	*human*	*6*	*DT003*	*5*	
			DT096	*1*	*11*
S. enteritidis	*human*	*6*	*PT13a/7*	*6*	

Table (5): The numbers and percentage of phage typable isolated from poultry:

Serotype	*Source*	*Number of isolates Phage type*	*Phage typed*	*Number of isolates*	*Phage untypable*
S. typhimurium	*Poultry*	*12*	*DT003*	*6*	
			DT004	*4*	
			DT193	*2*	*19*
S. enteritidis	*Poultry*	*3*	*PT13a/7*	*3*	

Table (6): The numbers and percentage of phage typable isolated from cattle:

Serotype	*Source*	*Number of isolates Phage type*	*phage typed*	*Number of isolates*	*phage untypable*
S. typhimurium	*cattle*	*3*	*DT193*	*3*	
					6
S. enteritidis	*cattle*	*4*	*PT13a/7*	*4*	

Table (7): Summarized results of antimicrobial sensitivity test of isolates:

Antimicrobic agent	*Disc potency*	*Inhibited zone*	*Results*
Gentamicin (Gm)	*(30 µg)*	*12 or less*	*S*
enrofloxacin (Ex)	*(10 µg)*	*13 or less*	*S*
Ciprofloxacin (Cf)	*(30 µg)*	*14 or less*	*S*
Cephalexin (Cp)	*(30 µg)*	*14 or less*	*S*
Ampicillin (Ap)	*(25 µg)*	*20 or less*	*R*
Amoxicillin (Am)	*(10 µg)*	*19 or less*	*R*
Tetracycline(Tc)	*(30 µg)*	*14 or less*	*R*
Doxycycline (Do)	*(10 µg)*	*14 or less*	*R*

S=Sensitive R= Resistant

Discussion

There are more than 2500 Salmonella serovars distributed throughout the world; some of these viz., S. typhi, S. gallinarum, S. dublin and S. choleraesuis are host specific, the majority are non adapted and can cause infection in man and animals alike. In the present study, 70 (70%) Salmonella isolates belonging to S serovars - S. typhimurium, S. Enteritidis, S. gallinarum, S. paratyphi B and S. bareilly were isolated from 300 samples from diarrheic cases in humans, poultry and cattle (Table 1,2 and 3).

In this study, 23 (23%) Salmonella were recovered from 100 human stool samples examined. The potential hazard of the organism can be viewed from the fact that virtually one in every five stool samples collected from diarrheic patients was positive for Salmonella. All the salmonellae isolated from humans in the present study, S. enteritidis(11%), S.typhimurium (10%) and S. paratyphi B (2%) were non host-specific and their role in human salmonellosis has been well established(10). Poultry is known to be the largest single reservoir of Salmonella (11), (Table 1).

In the present investigation, 34 (34%) of the 100 cloacal swab samples from diarrheic birds showed presence of Salmonella. The isolation rate was higher than that usually reported and could be attributed to the fact that the swabs from only the diarrheic birds were processed in our study. Four serovars, S. typhimurium (12%), S. gallinarum (12%), S. enteritidis (8%) and S. paratyphi B (2%) were recorded. S. gallinarum, the causative agent of fowl typhoid, is the most prevalent host-adapted Salmonella strain in poultry. S. typhimurium and S. enteritidis are not only involved in severe outbreaks of avian salmonellosis and economic losses to the poultry industry(12 and 13), these serovars also pose a definite zoonotic hazard as poultry are known to be the major transmitters of non host-adapted salmonellosis in humans(14), (Table 2).

A total of 13 (13%) Salmonella isolates were recovered from cattle diarrheic rectal swabs comprising S. enteritidis (6%), S. typhimurium (5%), and S. bareilly (2%). S. bareilly, isolated from cattle in the study has also been reported to be involved in salmonellosis outbreaks in the children18. One of the characteristic features observed during the study was that human as well as the livestock and poultry in the region shared most of the serovars indicating the potential hazard of interspecies sharing of these organisms. It has been reported that livestock and their products can contribute to the total Salmonella infection in humans(15). Involvement of these serovars emphasizes the need to control their transmission from one generation to the next and horizontal spread within the herds/flocks as well as interspecies transmission (16), (Table 3).

The phage typing results of the representative isolates of Salmonella entrica revealed that of the 21 S. typhimurium isolates, Five were belonged to phage type DT193, Eleven to DT003, and Four to DT004. Though phage type DT003 was found to be present in both human and poultry isolates, phage types DT193 were found in poultry and cattle. Presence of phage type DT003 in poultry has also been reported earlier (17). The sharing of phage types among various species indicated the interspecies transmission of organism (18-19 and 20) and re-emphasized the need to control salmonellosis at every step. All the 13 isolates of S. enteritidis (six from humans, three from poultry and four from cattle) were found to belong to a single phage type PT 13a/7. The presence of this phage type among different species was also reported by other workers (21,23 and 24), (Table 4,5 and 6).

In recent years, antibiotic resistance in Salmonella has assumed alarming proportions worldwide (25). Monitoring drug resistance pattern among the isolates not only gives vital clues to the clinician regarding therapeutic regime to be adopted against individual cases, but also an important tool to devise a comprehensive chemoprophylactic and chemotherapeutic drug schedule on herd basis within a geographical area. In the present study, highest number of isolates showed resistance against doxycycline (58; 61.05 %), followed by ampicillin (49; 51.57%), amoxycillin (43; 45.26%) and tetracycline (42; 44.21%) cephalexin (24; 25.26%), ciprofloxacin (8; 8.4%), gentamicin (6; 6.3%),and enrofloxacin (2; 2.1%) (Table 7). All the human isolates were resistant to at least one of the 8 antibiotics tested. This could be due to the wide and varied use of different antibiotics by human patients with simultaneous evolution of newer antibiotics that have precipitated into pathogens of multiple drug resistance. Moreover, the presence of antibiotic residues in foods of animal origin may result in increased drug resistance amongst human isolates (25).

From this work we can concluded that to avoid contamination of poultry and cattle carcasses with such pathogens, food handlers must be free from diseases that may be transmitted by foods, should have medical certificate and subjected to periodical medical examination. Proper examination of the poultry and cattle at the farms & at the slaughter houses in both antemortem and postmortem examinations. Personal hygiene, good sanitation and application of good hygienic conditions at the slaughterhouses are also recommended.

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الملخص العربى

الاهمية الصحية العامة للسالمونيلا المشتركة

عادل عبد العزيز النويشى[1] و سيلفيا أحمد [2]

[1]قسم الامراض المشتركة كلية الطب البيطرى جامعة بنها
[2]قسم صحة الحيوان والامراض المشتركة كلية الطب البيطرى جامعة أسيوط

السالمونيلا من الميكروبات المشتركة المهمة و إنتشارُها في الحيواناتِ يجعلها تهديدا مستمرا للانسان. الدراسة الحالية أجريت لعزل أنواع وأنماط للسالمونيلا بين الانسان والدواجنِ والماشيةِ. أخذت 300 عينة مِنْ الماشيةِ المصابة بالأسهال و الأنسان لعزل السالمونيلا. وقد تم عْزلُ سالمونيلا typhimurium .و سالمونيلا enteritidisوذلك بأستخدام لاقمات البكتيريا. خمسة وتسعون عترةَعْزلُت مِنْ سالمونيلا enterica يَعُودُ إلى 5 أنواع سيرولوجية سالمونيلا typhimurium . ، سالمونيلا enteritidis . ، سالمونيلاgallinarum . ، سالمونيلا Paratyphi B و سالمونيلا Bareilly بنسبةِ إنتشارِ عامّةِ 40.14 بالمائة. بينما سالمونيلا typhimuriumعْزلُت وُزّعَت بين أربعة أنواع من لاقمات البكتيريا دي تي 003، دي تي 004، دي تي 096 ودي تي 193 وبينما كانت كُلّ سالمونيلا Enteritidis المعْزولُة خاصة بلاقمات البكتيريا نوع، بي تي 13 .7/ a بأستخدام بعض مِنْ الأنواع لاقمات البكتيريا أظهرت النتائجَ بأنّ ثلاثة مِنْ الخمسة أنواع سيرولوجية كَانتْ مشتركةَ مع الحيواناتِ والبشرِ والذى يُشير إلى إمكانيةِ حدوث العدوى التبادلية لهذا الميكروب. و لهذا، فمن الضروريُ للسيطرةِ على مرض السالمونيلا فى الأنسان يَجِبُ أَنْ يَعطي أهميةَ كافيةَ إلى للسيطرِه علية في الحيواناتِ وخصوصاً مُنتَجاتِهم. خضعَت العترات المعزولة إلى دراسة تاثير 8 مضادات ميكروبية والذي كَشفَ بأنّ الأكثر فاعلية ، السيفالوكسين (Cp,30)و سيبروفلوكساسين (Cp,30) وأنتروفلوكساسين (Ex,10) و الجنتاميسين (Gm,30).بينما كان الدوكسيسيكلين (Do,10) ,الأمبسيلين (Ap,25) و الأمكيسيلين (Am,10) و التتراسيكلين(Tc,30) كَانتْ نسبياً أقل فعّالية.

Efficacy of Ramectine Against Internal and External Parasites in Sheep.

Attia, T.A. [1]

[1]Department of Pharmacology, Faculty of Veterinary Medicine, Menofia University, Sadat Branch.

Abstract

Efficacy of Ramectin administered via parenteral routes against natural infections with internal and external parasites were tested in sheep. Ramectin is a new combination of ivermectin (1%) and rafoxanide (12.5%), recently introduced in many markets around the world as a broad spectrum parasiticide. The efficacy of Ramectin (1ml per 50 kg. body weight) was tested in sheep for control of natural infestation by internal parasites (Gastrointestinal nematodes and liver flukes) and external parasites (mange and ticks) in Egypt. The drug showed excellent efficacy against mature and immature form of round worms [Trichostrongylus, Ostertagia and Haemonchus species larvae] as well as Trichuris species after 7 days post treatment. Its eradication effect was delayed for another week (14th d.p.t.) Cooperia and Nematodirus species larvae. With mean efficacy reach 80-90 % after 7 day post treatment (d.p.t). Increased to 100 % after 14 (d.p.t). Very good efficacy (100%) for the drug was recorded toward the previously diagnosed Fasciola eggs, the eggs which disappeared completely from the feces of treated animals at the day 21 post treatment inward. Two doses of ramectin with one week interval succeeded in eradication of mange accompanied with marked improvement in mange infected skin areas in the form of disappearance of scales during the third week post treatment. The effect of Ramectin on blood picture and some enzyme activity was also investigated.

Introduction

Control of the parasites using chemical preparations still as an important part in the efforts applied to induce fast eradications of different parasites and they still remained as one of the easily available means of control during the last decades. Frequent use of suppressive drugs may lead to widespread anthelmintic resistance among animal parasites (1). Recommendations for slowing the spread of resistance thus conserving the efficacy of broad spectrum anthelmintics, have been outlined and include the use of anthelmintic

combination (2),(3). Ramectin injectable solution is a new patent preparation of New pharma Research Center AB Sweden, composed of Ivermectin (1 %) and Rafoxanide (12.5 %). It is produced under license by AVICO Jordan for the treatment of internal and external parasites in cattle, buffaloes, camels, sheep and goats. Ivermectin is macrocyclic lactones exhibit a broad spectrum activity against gastrointestinal and lung nematodes (4) as well as external parasites of domestic animals (5). Since 1981, ivermectin has been released in over 60 countries for use in cattle, sheep, goats, horses, sheep, reindeer and humans (6). Ivermectin is highly effective against adult, developing and hypobiotic larvae of most gastrointestinal nematodes, lung worms and many arthropods (7), (8). Ivermectin has been shown to cause paralysis of the parasites by affecting amminobutyric acid mediated signals between nerves and muscle, (9).

On the other hand, Rafoxanide is one of halogenated salicylanilides group which includes closantel, niclosamide and oxyclosanide, (10). All these members are synthesized compounds, having a potent antiparasitic activity against liver fluke and blood sucking nematodes in sheep and cattle (11), (12). Rafoxanide is used extensively for controlling of Haemonchus, and Fasciola species infection in sheep and cattle, also for Oestrus ovis in sheep (12). Its fasciolicidal effect resulted from uncoupling oxidative phosphorylation and interfering with ATP production which includes adults, (6 and 12 week-old) flukes as this activity will be increased as the flukes become mature and move from liver parenchyma to bile duct. (5).

The efficacy of the Ramectin was tested previously in camels (13) and the pharmacokinetics of Ivermectin associated with rafoxanide (Ramectin) was also studies in calves, sheep (14)

The present study aimed to evaluate the efficacy of the new formulation Ivermectin in combination with Rafoxanide (Ramectin) in treatment of natural parasitic infections (gastro-intestinal nematode, liver fluke, ticks and mites) in sheep. The effect of Ramectin on blood picture and some enzymes activities was also investigated.

Material and Methods

1-Drugs and dose of administration:-

Ramectin injectable solution was obtained from Arab Veterinary Industrial Company (AVICO), Amman, Jordan. Each ml containing10mg Ivermectin plus125mg Rafoxanide. The drug was injected subcutaneously at a dose of 1.0 ml per each 50 Kg. B. W., (equivalent to 2.5 mg Rafoxanide plus 200 µg Ivermectin per kg b.wt.)

2-Animals:

After examination to a number of 120 apparently healthy sheep (25-60 kg body weight) at El-Fayoum Governorate, Egypt, eighty she sheep naturally infected by the target parasites were selected. These infected animals include:
- Forty five naturally infected by nematodes eggs. They were randomly divided into two groups, one treated with the drug (35 animals) and the other (10 animals) used as control non-treated.
- Ten animals naturally infested with *Fasciola* were treated.
- Twenty five animals have high to moderate level of mange infection. They were allocated into two groups, as twenty animals will be treated and 5 animals left as control.

The animals were housed in their owner's houses along the duration of the trial without control measures on the floor level. Each animal received his normal food and water as usual.

3-Collection and examination of samples:

The animals were collected in the local veterinary clinic associated with their owners. All animals were identified, rectal fecal samples were collected from all animals at days, -7, Zero, 7^{th}, 14^{th}, 21^{st}, 28^{th} and 35^{th} day post-treatment (P.T.). Total number of different *G.I.P.* eggs/gm. of feces were calculated in each time using the Mc-Master technique **(15),** while *Fasciola* infection was diagnosed using "fluke finder" technique **(16)**. The animals were considered positive if one Fasciola per gram was recorded. Cultivation of the collected fecal samples was done using the modified Baermann technique and the detected larvae were identified **(17).** The larvae were counted relatively for each individual animal where the mean number per animal in each group was calculated.

Efficacy of the drug was calculated according to the disappearance of the target eggs from the feces of the treated animals before and after treatment according to the equation **(18):-** Drug efficacy % = a-b/a X 100, where,
a= mean number (of EPG or of ticks or mite) recorded at zero day.
b= mean number (of EPG or of ticks or mite) recorded at day of observation.

4-Testing the efficacy of the drug against mange infection:

Using separate sheet, the diameter and site of the lesion allover the animal body were recorded for each animal. Separate skin scraping was collected from the periphery of the lesion from each animal at the previously mentioned days of tick's observations. Samples were treated with Sodium hydroxide 10% solution where the mite types were identified according to **(15).**

The mean number of mite per microscopic field was calculated in each case.The clearance rate was calculated mathematically as before.

The clearance of the animal from mite considered as the guide for the drug efficacy. The pre-treatment infection of each animal was considered as control for its condition post treatment.

For mange, animals were treated with Ramectin with two doses with one week interval at a rate of 1ml per 50 kg body weight. The animals in the three experiments were remained under observation during the first 3 hours after medication where any abnormalities at the site of application or in the general health condition of the animals were recorded.

5-The effect of Ramectin on blood picture and on the liver and kidney function:

Two blood samples were collected from five sheep (one with heparin for hematological study and the second for biochemical study) just before injection with Ramectin. Blood samples were taken gain 2, 7 and 15 days post treatment

- **Biochemical analysis :**

Serum samples were obtained by centrifugaticn of the blood samples collected in non heparinized centrifuge tubes at 3000 r.p.m for 15 minutes. Serum samples were assayed for the activities of ALT, AST enzymes as well as urea and creatinine levels.

1- Liver functions tests :

A-Determination of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) :

Serum alanine aminotransferase (ALT) was estimated by the colorimetric method **(19)** using kits obtained from Diamond Diagnostic Company.

B- Determination of Serum creatinine and urea :

Serum creatinine was estimated according to the method of **(20),** while urea levels were measured according to the method of **(21).**

- Hematological studies :

1-Total erythrocytic count :

Total red blood cells were counted under the microscope by using improved double Neubaur haemocytometer **(22).**

2-Packed cell Volume (PCV):

The microhematocrite method **(23)** was used in determination of PCV.

3-Hemoglobin content:

The method used for Hb contents determination was adopted by **(24).**

4-Total leukocytic count :

The total leukocytic count was carried out by **(22)** using double Neubour hemocytometer.

Results

1- Efficacy of Ramectin against Gastro-intestinal Nématodes.

Results in table (1) showed that the treated animals were divided into 3 groups according to the mean number of eggs gram feces (E.P.G.) as high (1000-1500 E.P.G.) in group (1), moderate (600 to less than 1000 E.P.G) in group 2 and low (250-500 E.P.G.) in group 3, while representing animals for all level of infection was included in the control group.

Treatment with single dose of ramectin injection induced excellent activity in the form of complete elimination of mature and immature form of round worms [*Trichostrongylus, Ostertagia* and *Haemonchus* species larvae] as well as *Trichuris* species after 7 days post treatment , while its eradication effect was delayed for another week (14^{th} d.p.t.) toward elimination of *Cooperia* and *Nematodirus* species larvae from the feces of this treated groups, with mean efficacy reach 80-90 %(table 3), after 7 day post treatment (d.p.t), increased to 100 % after 15 (d. p.t). This marked efficacy was continuing till 35^{th} day post treatment, (end of the observation period) in comparison with the control non treated animals.

Table (1): Effect of RAMECTIN injection in treatment of sheep naturally infested with Nematode & *Fasciola* .

Treated groups	Mean number of Gastro-intestinal nematode eggs / gram/animal days post treatment										
	0			7		14		21	28	35	60
	No./ group	Low-high	Mean No.	No. of still positive animals	Efficacy %	No. of still positive animals	Efficacy %	Efficacy %	Efficacy %	Efficacy %	Efficacy %
Group I (Sheep of high level of Nematodes)	15	1000-1500	1100	2	86.6%	0.0	100%	100%	100%	100%	No. of samples collected for G.I.N.
Group II (Sheep of moderate level of Nematodes)	**10	600- less than 1000	700	2	80%						
Group III (Sheep of low level of Nematodes)	**10	250-500	350	1	90%						
Control Group (Sheep of different egg count)	**10	250-1200	550	10	--	10	Animal still infected by variable numbers of eggs / gram feces				
Fasciola infected sheep	10	2-4	2.9	6	40%	4	60%	0/100%	0/100%	1/100%	90/100%

** Two animals in these groups have *Trichuris* species eggs (300 E.P.G.), and another two have 1-3 *Paramphistomum* E.P.G.

2- Efficacy of Ramectin against Fasciola infection;-

Marked efficacy for Ramectin was observed in comparing animal fecal data before and after 3 weeks post treatment, (table 1). The drug efficacy was increased from 40 % at 7th day increased to 60% at 14th day and . to 100% at 21st and 30 days post treatment., where complete eradication for the previously diagnosed *Fasciola* eggs was recorded. No Fasciolla eggs could be diagnosed in these animals again at 60th day Post treatment.

3- Efficacy of Ramectin in treatment of sheep mange :

The data in table (2) cleared that two doses of Ramectan produce marked effect in treatment of *Sarcoptic species* mite infection in sheep. The scales was drop down and the hair start to develop during 3-4 weeks post treatment in animals of moderate to milled infection in comparison with the control non treated sheep. Cases of severe infection all over the body (group 3) needed another dose for complete eradication of the parasite.

Table (2): Efficacy of RAMECTIN injection in treatment of sheep naturally infested with Sarcoptic mange.

Animal groups	No. of animals	*Description of lesion on the body days post inoculation*						
		0.	7		14		28	60
		Status of infection	Observation	Efficacy %	Observation	Efficacy %	Efficacy %	Efficacy %
G (1)	7	Lesion on nose & ear	Smooth skin without scales	moderate	Normal growing of the hair	good	good	good
G- (2)	6	Lesion in head & shoulders	Smooth skin without scales	moderate	Normal growing of the hair	good	good	good
G-(3)	7	Wide Lesion all over the body	Smooth skin without scales	moderate	improved	Improved	Improved	good
Control	6	Different level of lesion	Lesion still present	--	Lesion still present		Lesion still present	Lesion still present

Local tolerance:

The drug did not cause any digestive disturbances or even local reaction at site of injection for the treated animal at any time post treatment.

Discussion

The present study demonstrated the high efficacy of Ramectin at a dose of 0.2 mg kg^{-1}ivermectin in association with rafoxanide at a dose of 2.5 mg kg^{-1}, against common gastointistinal nematodes (G.I.N.) liver flukes as well as some

external parasites of sheep. Single treatment of Ramectin (1ml per 50 kg. body weights) eliminated all of the previously diagnosed G.I.N. eggs and larvae in feces with high sensitivity for *Trichostrongylus, Ostertagia, Haemonchus* and *Trichuris* species than that of *Cooperia* and *Nematodirus* species which disappeared after 14 days P.T. in comparison with the control non treated animals. This marked efficacy was continued till 35th day post treatment, where no G.I.N. eggs could be detected at examination of the collected fecal samples. Similar results were previously reported for Ramectin in camels **(13).** In addition, it was reported that Ivermectin concentrations following subcutaneous administration of ivermectin alone or in combination with rafoxanide in sheep was quantified until 11 and 20 days post administration, respectively, indicating that ivermectin was extended in serum after combination with rafoxanide **(14)**. Ivermectin paralyze and kill the parasite through interrupting their nerve impulses by enhances binding of their neurotransmitter (GABA) to special receptors at nerve junctions **(25).** In addition, rafoxanide is used extensively for controlling of *Haemonchus*, and *other blood sucking nematodes* infestation in animals **(5).** Littel delay in sensitivity of *Cooperia* and *Nematodirus* species toward Ivermectine was recorded previously by **(7) and (26).**

Concerning to effect of the drug on Fasciola infection, the single treatment with Ivermectin/ rafoxanide combination (Ramectin) revealed a very high efficacy (100%) for the drug was recorded toward the previously diagnosed *Fasciola* eggs, from the 21st day post treatment inward. This delay in clearance of feces from Fasciola eggs was described previously by **(27)** who mentioned that the disappearance of *Fasciola* eggs from feces of treated animals was related to original number of stored eggs in gall bladder than the number of living flukes available where some egg still shed in feces after eradication of their adult worms. Also prolongation to duration of efficacy may be due to the persistent concentration of rafoxanide given subcutaneously which may probably due to its prolonged half-life in the plasma of treated sheep. In this respect, **(28)** were agreement with findings obtained in sheep treated with rafoxanide. They found that, the efficacy of rafoxanide against 6-week-old flock (7.5 mg kg^{-1}) appeared to be similarly effective to a dose rate of 2.5mg^{-1} against 10 wee-old-fluke (86 %and 88% respectively). They concluded that, this putative efficacy against immature flukes may be due to rafoxanide persisting in plasma (105 days) and affecting the mature flukes when they reach the bile ducts.

Concerning the efficacy of ramectin against mange, marked improvement was recorded on mange infected skin areas in treated sheep. In addition, all scales were disappeared during the second week post treatment and hairs start to develop again at the end of the third week post treatment. Furthermore,

some sheep were given another dose which completely eliminates the mites. It was worthily to mention that heavy infected animal need more accurate estimation for the recommended dose or application of another treatment. This finding was similar to that obtained previously by ivermectin in sheep **(29).**

Sucutaneous injection with Rmectin in sheep with the recommended dosage level (1ml/50 kg. body weight) did not cause any digestive disturbances or even local reaction at site of injection for the treated animal at any time post treatment.

For conclusion, using of Ramectin injection was effective in controlling internal parasite as round worms, Fasciola as well as external parasites such as mites and ticks when given at a single subcutaneous dose of I ml per 50 kg body weight equivalent to 200 ug ivermectin and 2.5 mg rafoxanide per kg body weight.

It is recommended that administration of the drug should be given as two dose regimen with one week interval to obtained full efficacy against mange mites. Strategic use of this combination would ensure that incoming larvae from pasture are killed before establishment. This, in turn, would lead to reduced pasture contamination and increased production efficiency of sheep. The common measurements of the drug withdrawal time must be associated to application of the drugs on sheep also.

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Some Factors Affecting Economic and Productive Efficiency of Broiler Production Farms

Iman.R. Kamel[1], Bakry, H. H. [2] and Atallah, S. T.[3]

[1]Faculty of Veterinary Medicine, Department of Animal Wealth, Benha University.
[2]Faculty of Veterinary Medicine, Department of Forensic Medicine and Toxicology, Benha University.
[3]Faculty of Veterinary Medicine, Department of Animal Husbandry and Animal Wealth, Alexandria University.

Abstract

This study was carried-out during the period extended from year 2007 to year 2009 on (200) random cycles of broiler farms. Different localities were the areas of research which include Kaliobia, Dakahlia, Damietta and Sharkia governerate. The data were collected from health and production records and also, by using the structured questionnaire method in case of no farm records. The aim of this study is to determine and highlight on the most important factors affecting the efficiency of such farms and their profitability under Egyptian conditions. The most important localities and breeds within the localities, ranged from 545.02 to 685.59 LE / 100 broilers in Damietta and El.-Kaliobia provinces; respectively, and rangedfrom 521.41 to 788.31 LE /100 broilers for Hubbard and Ross breeds; respectively. The densities and breeds within the densities, as ranged from 611.11 to 589.54 LE / 100 broilers for medium and high density; respectively, and ranged from 481.33 to 644.23 LE / 100 broilers for Cobb and Ross breeds; respectively, Among different infection pattern. The lower net incomein infection pattern observed in coccidiosis infected birds (495.23 LEIl 00) broilers. While, the control birds achieved net income by 658.97 /100 broilers.

Introduction

Poultry industry is a major economic benefit to several areas in the world **(1)**. Intensive broiler production now exceeds 2 X 1010 bird worldwide, but it attracts accusations of poor welfare **(2)**. The economic and productive efficiency of broiler farms depend upon selected breed **(3)**, season of rearing **(4)**, housing and hygienic status of the farm **(5)**, **(6), (7)**; size of operation, diseases and mortalities **(4)**, localities, feed and its efficient utilization **(8), (9)** and veterinary managment **(10)**. Poultry production has the following advantages over the other livestock; as poultry are good converters of feed into useable protein in the form of meat and eggs, the production cost per unit is relatively low to other types of livestock and the return to

investment is high, thus farmers need just a small amount of capital to start a poultry project, poultry meat is very tender thus its palatability and acceptability to consumers are very high, it has a short production cycle (pay back period) through which capital is not tied down over a long period **(11)**. Costs of production and returns are the two major concerns in poultry sector. The problems of how much the broiler cost and how much they gain are becoming the most important formula in poultry economics. So, poultry enterprises can be made more profitable if critical standard limits for cost of production are determined and given close attention **(12)**. Poultry farms have been increasing during recent years which lead to the development of the poultry industry and its requirements **(13)**. So, the aim of this study is to determine and highlight on the most important factors affecting the efficiency of such farms and their profitability under Egyptian conditions.

Material and Methods

This study was carried-out during the period extended from year 2007 to year 2009 on 200 random cycles of broiler farms. Different localities were the areas of research which include Kaliobia, Dakahlia, Damietta and Sharkia governerate. The data were collected from health and production record and also, by using the structured questionnaires.

A- Data collected about broilers farms:

The data were collected for 3 different broiler breeds (Hubbard, Cobb, Ross) according to the implied methods **(10)** and **(14)**.

These data were classified into:

A.1. Production traits and resources:

That included, breed type, number of brooded day old chick, year and season of fattening cycles, amount of starter, grower, and finisher rations consumed, mortality percentage and its causes, marketing age, average body weight of bird at marketing and housing system.

A.2. Production costs: Which include both fixed and variable costs.

A.3. Production returns:

It included the returns from total live body weight sales and litter sales according to the prices during the years of the study.

B- Data analysis:

The data were collected, arranged, summarized and then analyzed statistically using the computer program **(15)**. The analytical design was multifactorial (nested) design.

C- Analysis of broiler production and the factors affecting it:

All the production parameters affecting broiler production including their costs and returns were calculated on a 100 birds basis at marketing age to overcome the variation in the numbers of broilers of the different farms. Then the analyses were done to determine the effect of the following interactions on the calculated broiler parameters: locality and breed, density and breed and different disease infection pattern and breed.

D- D- Economical analysis:

The economical analysis used depend upon the economical evaluation of locality and breed, density and breed, diseases and breed through the evaluation of returns and costs and net profit of the farms **(10)**.

Results and Discussion

1- Effect of different localities and / breeds within localities on total feed consumption (Kg) / 100 broilers, and constituents of veterinary management costs (LE) /100 broilers:

Table (1) indicated that, there was a significant locality effect (P<O.O 1) on total ration consumed, where it ranged from 296.55 to 380.33 kg / 100 broilers in Sharkia and Damietta provinces; respectively, and ranged from 270 to 400 kg / 100 broilers for Cobb and Hubbard breeds; respectively.

The above mentioned results agreed with those of (10), (14), (16). They reported that, the ration consumption in different growing stages and the total feed consumption and feed conversion ratio differed significantly (P<0.01) among the breeds and localities due to the differences in environmental conditions.

Table (1), explained that, the highly significant effect (P<0.01) of the different localities and breeds within localities on the values of drugs, as the drugs value's ranged from 29.31 to 132.56 LE / 100 broilers in EL-Sharkia and EL-Kaliobia provinces; respectively.

Table (I): Mean ± SE of total feed consumption (Kg) /100 broilers and the values of drugs, vaccines, disinfectants, veterinary supervision and total veterinary management (LE) / 100 broilers of different broiler breeds among different localities.

Locality	Breed	N	Total feed	Drugs	vaccines	disinfectants	veterinary supervision	Total veterinary management
			X ±S.E	*X* ±S.E	*X* ±S.E	*X* ±S.E	*X* ±S.E	X"±S.E
EL-Kaliobia	Hubbard	I	400.00' ± 139.90	98.66^{b} ±12.37	16.00' ±4.03	1.20d ±I.67	I 0.00b ± I .46	125.86' ±13.15
	Cobb	27	319.07 '±26.92	133.77' ±2.38	45.00' ±0.77	10.85^{b} ±0.32	10.00b ±0.28	I 99.62b ±2.53
	Ross	9	322.22' ±46.63	I 32.66' ±4.12	45.55^{b} ±1.34	13.77' ±0.55	13.33' ±0.48	205.33' ±4.38
	Total	37	322.02A ±3.13	132.56^{A} ±2.25	44.35^{A} ± 1.00	11.30^{A} ±0.60	10.81 A ±0.30	199.02A ±2.79
EL-Dakahlia	Hubbard	42	359.88^{c} ±21.58	99.38^{b} ±l.90	15.09^{d} ±0.62	1.29^{d} ±0.25	10.78^{b} ±0.22	I 26.55^{c} ±2.03
	Cobb	20	363.00^{c} ±3 I .28	93.49^{c} ±2.76	16.50^{d} ±0.90	1.27d ±0.374	I 1.30^{D} ±0.32	I 22.56^{c} ±2.94
	Ross	27	342.03" ±26.92	97.30^{b} ±2.38	17. 74d ±0. 77	1.22d ±0.32	1l.14D ±0.28	I 27.42c ±2.53
	Total	89	355.16^{A} ±20.03	97.43" ± 1.26	16.21 c ±0.25	1.26^{c} ±0.05	1l.0 I A ±0.08	I 25.92^{B} ± 1.28
Damietta	Hubbard	32	397.50" ±24.73	98.73^{b} ±2.18	15.18^{d} ±0.71	1.30^{d} ±0.29	10.43^{rb} ±0.25	I 25.66^{c} ±2.32
	Cobb	7	320.71 ±52.88	95.44°C ±4.67	13.57" ±l.52	1.04" ±0.63	11.28" ±0.55	121.35^{c} ±4.97
	Ross	6	358.33^{c} ±57.11	96.13°C ±5.05	15.66" ±l.64	1.53" ±0.68	11.00" ±0.59	I 24.33^{c} ±5.37
	Total	45	380.33^{A} ± 17.0 I	97.87" ±2.28	15.00^{L} ±0.42	1.29^{L} ±0.1 0 I	10.64A ±0.10	124.81 " ±2.31
EL-Sharkia	Cobb	I	270.00^{b} ± 139.90	16.00' ±12.37	16.00^{Q} ±4.03	6.00^{c} ±I.67	3.00^{Q} ±1.46	4l.00' ± 13.15
	Ross	28	297.50& ±26.44	29.78^{Q} ±2.33	23.42^{c} ±0.76	12.67' ±0.31	7.53^{c} ±0.27	73.42^{Q} ±2.48
	Total	29	296.55" ±4.91	29.3IL ±l.21	23.17" ± l.51	12.44 B ±0.49	7.37" ±0.61	72.31 L ±2.69

For breeds small letters: Means within the same column carrying different letters are significantly different at (P<0.01).

For localities capital letters: Means carrying different letters are significantly different at (P<0.01).

Also, it differed significantly (P<0.01) among different breeds within the localities, as it ranged from 16 to 133.77LE / 100 broilers for Cobb breed, while, the values ofvaccines, they differed significantly (P<0.01) among localities, as it ranged from 15 to 44.35 in Damietta and EL-Kaliobia provinces; respectively. Also they differed significantly (P<0.01) among the

different breeds within the localities, as they ranged from 13.57 to 45.55Cobb and Ross breeds; respectively. While, the values of disinfectants, as it ranged from 1.26 to 12.44 LE / 100 broilers in EL-Dakahlia and EL-Sharkia provinces; respectively. Also, the disinfectants values differed significantly (P<0.01) among the breeds within the localities, as it ranged from 1.04 to 13.77 LE /100 broilers for Cobb and Ross breeds; respectively.

Moreover, the values of veterinary supervision differed significantly (P<0.01) among the different localities, as it ranged from 7.37 to 11.01 LE / 100 broilers in EL-Sharkia and EL-Dakahlia provinces; respectively. Also, the veterinary supervision values differed significantly (P<0.01) among breeds within the localities, as it ranged from 3 to 13.33 LE / 100 broilers for Cobb and Ross breeds; respectively.

The values of total veterinary management differed significantly (P<0.01) among different localities, as it ranged from 72.31 to 199.02 LE / 100 broilers in EL-Sharkia and EL- Kaliobia provinces; respectively. Moreover, they differed significantly (P<0.01) among the different broiler breeds within the localities, as they ranged from 41.00 to 205.33 LE / 100 broilers for Cobb and Ross breeds; respectively.

The previous results showed that, the values of drugs, vaccmes, disinfectants, veterinary supervision and total veterinary management differed among different localities; this may be due to the differences in disease prevalence among different localities according to the environmental conditions, breed susceptibility to diseases and the experience of the farmer.

The above results agreed with those of **(8), (14), (16), (17)** in that, the values of veterinary inputs (drugs, vaccines, disinfectants and veterinary supervision) differed significantly (P<0.01) among different broilers breeds and localities.

2- Effect of different localities and / breeds within localities on total feed consumption(Kg)/100 broilers, and constituents of veterinary management costs (LE) /100 broilers:

The results in Table (2) illustrated that, the average marketing age significantly (P<O.Ol) differed among the different localities, as it ranged from 36.00 to 54.73days in EL-Sharkia and Damietta provinces; respectively. Moreover, it differed significantly (P<O.Ol) among the different breeds within the localities, as it ranged from 35 to 64 days for Cobb and Hubbard breeds; respectively. These may be due to the differences in bird vitality, kg market price and available feed stuff and its price.

Table (2): Means ± SE of average marketing age, total meat production(Kg), mortality%, total variable costs, total costs, total return and net return of different breeds among different localities.

Locality	Breed	N	Average marketing age	Total meat Production (Kg)	Mortality %	Total variable costs	Total costs	Total return	Net return
			X±S.E	*X* ±S.E	X±S.E	X"±S.E	*X* ±S.E	X±S.E	*X* ±S.E
EL-Kaliobia	Hubbard	I	64.00" ±5.78	17l.06" ±16.14	3.00' ±2.25	1351.53 '±422.61	1461.53" ±42l.69	2155.28" ±20 1.84	693.74" ±425.90
	Cobb	27	37.25 ±l.11	160.4Ib ±3.10	12.45' ±0.43	I I 95. 77^{d} ±8 1.33	1365.70d ±8l.15	2016.76^{c} ±38.84	651.05" ±81.96
	Ross	9	39.ll^{c} ±1.92	I 72.67' ±5.38	4.22Q ±0.75	1216.71 Q ± 140.87	I 386.77" ±140.56	2175.09' ±67.28	788.31' ±141.96
	Total	37	38.43L ±0.948	I 63.68^{A} ±2.32	10.19^{A} ±0.92	1205.04A ±9.35	1373.41 " ±8.77	2059.0 I A ±29.23	685.59^{A} ±31.11
EL-Dakahlia	Hubbard	42	45.95 '±0.89	I 53.24c ±2.49	5.30^{c} ±0.34	I 234.69^{c} ±65.2 J	136l.47Q ±65.06	1932.37' ±31.14	570.89^{Q} ±65.71
	Cobb	20	46.25° ± 1.29	152.04^{c} ±3.61	5.62c ±0.50	1241.32" ±94.49	I 370.57c ±94.29	1918.21 ±45.13	547.64Q ±95.23
	Ross	27	44. 18^{c} ±1. I I	156.67^{c} ±3.10	6.55" ±0.43	I I 84.32" ±8 1.33	1318.02 ±8l.15	1976.41 Q ±38.84	658.39·^{b} ±81.96
	Total	89	45.48" ±0.512	154.01" ±1.41	5.75" ±.18	I 220.90^{A} ±60.33	1350.34 " ±60.20	1942.55" ± 17. 75	592.21 B ±56. 70
Damietta	Hubbard	32	57.09° ± 1.02	I 57.63°C ±2.85	4.40Q ±0.39	1347.33 '±74.70	1465.30' ±74.54	1986.7J^{Q} ±35.68	521.4IQ ±75.29
	Cobb	7	50.42^{c} ±2. J 8	I 47.49^{Q} ±6.10	6.57" ±0.85	1112.8Ic ±159.73	1237.10& ±159.38	1859.64 ±76.29	622.54^{c} ± 160.97
	Ross	6	47. 16^{Q} ±2.36	153.18^{c} ±6.59	4.91Q ±0.91	1227.33^{c} ± 172.53	1350.34 ±172.15	1931.17' ±82.40	580.50^{Q} ±173.87
	Total	45	54.73^{A} ±l.50	155.46" ±2.11	4.81c ±0.22	1294.85^{A} ±52.07	1414.52A ±5l.72	1959.54" ±26.52	545.02" ±52.85
EL-Sharkia	Cobb	1	35.00& ±5.78	109.30 ±16.14	3.50 '±2.25	885.00& ±422.61	897.00' ±421.69	1378.33' ±20l.84	481.33^{c} ±425.90
	Ross	28	36.03' ±1.09	125.61 ' ±3.05	4.31° ±0.42	1005.60 ±79.86	1026.39' ±79.69	1585.18& ±38.14	558.79^{Q} ±80.48
	Total	29	36.00'" ±0.164	125.05^{L} ±4.91	4.28^{L} ±0.15	100l.44" ±15.68	1021.93^{L} ±16.18	I 578.05^{L} ±61.44	556.12B ±57.26

For breeds small letters: Means within the same column carrying different letters are significantly different at (P<0.01).
For localities capital letters: Means carrying different letters are significantly different at ((P<0.001).

This results agreed with those of (10), (12) and (16) where they observed that, the marketing age differ from breed to another and from locality to another an in Egypt commonly ranged between 45- 55 day according to the price of kilogram broiler marketed and the marginal cost of kilogram broiler sale. The total meat production differed significantly (P<0.01) among the different localities and breeds, as it ranged from 125.05 to 163.68 kg / 100

broilers in EL-Sharkia and EL-Kaliobia provinces; respectively, and ranged from 109.30 to 172.67 kg / 100 broilers for Cobb and Ross breeds; respectively. These results may be due to the differences in genetic makeup of breeds, variation in feed conversion ratio and weight at marketing and environmental conditions.

Furthermore, the localities and breeds within the localities had a significant ($P<0.01$) effect on the mortality percent, as it ranged from 4.28 to 10.19 % in EL-Sharkia and EL-Kaliobia provinces; respectively, and ranged from 3.00 to 12.45 % for Hubbard and Cobb breeds; respectively. The higher mortality percent was due to the changes in environmental conditions, disease incidence, and immune status of the bird and lower level of veterinary supervision with inexperienced farmers for the prevention and treatment of diseases among the localities.

These results agreed with (14) and (16), they concluded that, the significant ($P<0.01$) effect of locality on broiler production was due to the effect of the environmental conditions which affect on the gene expression, mortality percent and marketing age.

Also, (Table, 2) indicated that, the locality and breeds within locality had a significant ($P<0.01$) effect on the total variable costs (TVC), as they ranged from 1001.44 to 1294.85 LE / 100 broilers in EL-Sharkia and Damietta provinces; respectively, and ranged from 885.00 to 1347.33 LE /100 broilers for Cobb and Hubbard breeds; respectively.

Moreover, the total costs (TC) differed significantly ($P<0.01$) among the localities and breeds, as it ranged from 1021.93 to 1414.52 LE / 100 broilers in EL-Sharkia and Damietta provinces; respectively, and ranged from 897.00 to 1465.30 LE / 100 broilers for Cobb and Hubbard breeds; respectively.

The total return / 100 broilers differed significantly ($P<0.01$) among the different localities and breeds within the localities, as it ranged from 1578.05 to 2059.01 LE / l00 broilers in EL-Sharkia and EL-Kaliobia provinces; respectively, and ranged from 1378.33 to 2175.09 LE / 100 broilers for Cobb and Ross breeds; respectively.

While, the net return / 100 broilers differed significantly ($P<0.01$) among the localities and breeds within the localities, as it ranged from 545.02 to 685~59 in Damietta and EL-Kaliobia provinces; respectively, and ranged from 521.41 to 788.31 LE /100 broilers for Hubbard and Ross breeds; respectively.

These results agreed with (7), (16) and (18) where they, reported that, the localities and breeds within the locality affect the total returns and the total costs of broilers.

3- Effect of different densities and breeds within density on total feed consumption and constituents of veterinary management costs/100 broilers.

From Table (3) we can noticed that, there was a significant effect of the density (P<0.01) on the total ration consumed, where it was higher in high density than medium density (356.41 to 302.63 kg / 100 broilers; respectively), also it ranged from 270 to 376.46 kg /100 broilers for Cobb and Hubbard breeds; respectively.

Also, Table (3), explained the highly significant effect (P<0.01) of the different densities and the breeds within the densities on the values of drugs, as the drugs values ranged from 53.78 to 103.61 LE / 100 broilers for medium and high density; respectively. Also they differed significantly (P<0.01) among the different breeds within the densities, as it ranged from 16 to 113LE / 100 broilers for Cobb breed.

Concerning the values of vaccines, they differed significantly (P<0.01) among densities, as it ranged from 28.47 to 20.67 for medium and high density; respectively. Also they differed significantly (P<0.01) among the different breeds within the densities, as they ranged from 16 to 30.37 Cobb breeds.

Table (3): Means ± SE of total feed consumption (Kg) / 100 broilers and the values of drugs, vaccines, disinfectants, veterinary supervision and total veterinary management (LE) / 100 broilers of different broiler breeds among different among different breeds with in different densities.

Density	Breed	N	Total feed	Drugs	vaccines	Disinfectants	veterinary supervision	total veterinary management
			X·±S.E	*X±S.E*	*X* ±S.E	X"±S.E	X"±S.E	X"±S.E
Medium *(10-14* b/rn')	Cobb	1	270.00^{c} ±138.77	16.00^{Q} ±25.05	16.00^{o} ±9.61	6.00^{c} ±3.03	3.00^{c} ± 1.83	41.00Q ±34.50
	Ross	37	303.5I^{Q} ±22.81	54.81c ±4.11	28.81 '±1.58	12.94' ±0.498	8.94° ±J02	105.5 I c ±5.67
	Total	38	302.63" ±4.61	53. 78^{B} ± 7.34	28.47^{B} ±1.96	12. 76^{A} ±0.40 1	8.78^{D} ±.653	103.8ID ±9.61
High (> I 5 b/rrr')	Hubbard	75	3 76.46 '± 16.02	99.09° ±2.96	16.14^{b} ±1.1I	1.29^{b} ±0.150	10.62 '±.212	126. 16^{b} ±3.98
	Cobb	54	335.55^{c} ± 18.88	113.88 '±3.41	30J7' ± 1.30	603^{b} ±0.413	10.64' ±.250	160.94' ±4.69
	Ross	33	345.00^{b} ±24.15	97.09^{b} ±4.36	17.36^{b} ±1.67	1.28^{Q}± 0.528	11.12' ±1.20	I 26.86^{b} ±6.00
	Total	162	356.41 ±12.04	103.6IA ±I.47	20.67 ±.886	2.87D ±0.104	10.73 ±0.06	137.89^{A} ±2J9

For breeds small letters: Means within the same column carrying different letters are significantly different at (P<0.01).

For densities capital letters: Means carrying different letters are significantly different at (P<0.001).

The different densities had a significant (P<0.01) effect on the values of disinfectants, as they ranged from 12.76 to 2.87 LE / 100 broilers for medium and high density; respectively. Also, the disinfectants values differed significantly (P<0.01) among the breeds within the densities, as they ranged from densities densities 1.28 to 12.94 LE / 100 broilers for Ross breeds.

Moreover, the values of veterinary supervision differed significantly (P<0.01) among the different densities, as they ranged from 8.78 to 10.73 LE / 100 broilers for medium and high density; respectively. Also, the veterinary supervision values differed significantly (P<0.01) among the breeds within the densities, as they ranged from 3 to 11.12 LE / 100 broilers for Cobb and Ross breeds; respectively.

The values of total veterinary management differed significantly (P<0.01) among different densities, as they ranged from 103.81 to 13 7.89 LE / 100 broilers for medium and high density; respectively. Moreover, they differed significantly (P<0.01) among the breeds within the densities, as they ranged from 41.00 to 160.94 LE / 100 broilers for Cobb breeds.

4- Effect of different densities and breeds within densities on average marketing age, total meat production and mortality percent, total variable costs, total costs, total return and net return / 100 broilers:

The results in table (4) illustrated that the average marketing age significantly (P<0.001) differed among the different densities, as it ranged from 36.73 to 46.79 days for medium and high density; respectively. Moreover, it differed significantly (P<0.001) among the different breeds within the densities, as it ranged from 35 to 50.94 days for Cobb and Hubbard breeds; respectively.

The total meat production differed significantly (P<0.001) among the different densities and breeds, as it ranged from 136.33 to 155.58 kg / 100 broilers for medium and high density; respectively, and ranged from 109.30 to 156.04 kg / 100 broilers for Cobb and Ross breeds; respectively. These results may be due to the differences in genetic make up of breeds, variation in feed conversion ratio, and weight at marketing and environmental conditions. These results agreed with those of (3).

Furthermore, the densities and breeds within the densities had a significant (P<0.001) effect on the mortality percent, as it ranged from 4.26 to 6.59 % for medium and high density; respectively, and ranged from 3.50 to 9.16 % for Cobb breeds.

Also, the density and breeds within the density had a significant (P<0.001) effect on the total variable costs (TVC), as they ranged from 1'052.44 to 1238.05 LE / 100 broilers for medium and high density;

respectively, and ranged from 885.00 to 1284.31 LE / 100 broilers for Cobb and Hubbard breeds; respectively.

Table (4):): Means ± SE of average marketing age, total meat production(Kg), mortality%, total variable costs, total costs, total return and net return of different breeds among different densities.

Density	Breed	N	Average marketing age	Total meat production (Kg)	Mortality %	Total variable costs	Total costs	Total return	Net return
			X±S.E	*X* ±S.E	X±S.E	*X* ±S.E	X"±S.E	X"±S.E	X"±S.E
Medium (l0-14 b/m')	Cobb	1	35.00^{Q} ± 7.27	1 09JO^{c} ± 18.44	3.50^{c} ±2.84	885.00^{c} ±419.95	897.00^{c} ±422.46	1378J3^{c} ±32.55	481.33^{Q} ±422.82
	Ross	37	36. 78^{Q} ± 1.19	137.06^{b} ±3.03	4.28^{Q} ±0.46	1056.97" ±69.04	1114.11 " ±69.45	I 728.67' ±37.90	614.62' ±69.51
	Total	38	36.73^{D} ±0.33	136J3^{D} ±5.01	4.26^{D} ±0.12	1052.44 D ±20.49	110834B ±29.20	1719.45" ±62.80	611.IIA ±47.10
High (>15 b/ m')	Hubbard	75	50.94' ±0.84	155J5' ±2.13	4.89^{c} ±OJ2	1284JI '±48.49	1407.11' ±48.78	1958.53' ±26.62	551.42b ±48.82
	Cobb	54	42.29^{c} ±0.99	155.64' ±2.51	9.16 '±0.38	1201.85^{b} ±57.14	1350.83^{b} ±57.49	1 959.89' ±31.37	609.05^{c} ±57.53
	Ross	33	44. 72^{c} ± 1.26	156.04' ±3.21	6.25^{b} ±0.49	1 192.14c ±73.10	1323.96^{c} ±73.54	1968.19' ±40.13	644.23' ±73.60
	Total	162	46.79^{A} ±0.69	155.58^{A} ±1.10	6.59^{A} ±0.28	1238.05^{A} ±36.17	1371.41 A ±36.02	1960.95^{A} ±13.76	589.54" ±35.02

For breeds small letters: Means within the same column carrying different letters are significantly different at (P<0.01).
For densities capital letters: Means carrying different letters are significantly different at (P<0.001).

Moreover, the total costs (TC) differed significantly (P<0.001) among the densities and breeds, as it ranged from 1108.34 to 13 71.41 LE / 100 broilers for medium and high density; respectively, and ranged from 897.00 to 1407.11 LE / 100 broilers for Cobb and Hubbard breeds; respectively.

The total return / 100 broilers differed significantly (P<0.001) among the different densities and breeds within the densities, as it ranged from 1719.45 to 1960.95 LE / 100 broilers for medium and high density; respectively, and ranged from 1378.33 to 1968.19 LE / 100 broilers for Cobb and Ross breeds; respectively.

The net return / 100 broilers differed significantly (P<0.001) among the densities and breeds within the densities, as it ranged from 611.11 to 589.54 for medium and high density; respectively, and ranged from 481.33 to 644.23 LE / 100 broilers for Cobb and Ross breeds; respectively.

5- Effect of different diseases and breeds within different affections on total feed consumption and constituents of veterinary management costs / 100 broilers.

Table (5): Means ± SE of total feed consumption (Kg) / 100 broilers and the values of drugs, vaccines, disinfectants, veterinary supervision and total veterinary management (LE) / 100 broilers of different broiler breed among different breeds with in different diseases.

Disease	Breed	N	Total feed (Kg)	Drugs	Vaccines	Disinfectants	Veterinary supervision	Total veterinary management
			X- ±S.E	X·±S.E	X-±S.E	X- ±S.E	X-±S.E	X-±S.E
Control	Hubbard	27	359.25° ±26.71	98.67° ±4.91	14.74 ±1.72	1.33" ±.822	10.44" ±0.385	125.19^{c} ±6.06
	Cobb	15	274.33 ±35.84	83.65' ±6.59	17.86' ±2.31	2.35^{D} ± 1.1 0	10.80D ±0.517	114.58& ±8.14
	Ross	33	314.54i ±24.16	65.48 ±4.44	23.87° ±1.56	8.81' ±.744	9.30^{c} ±0.348	98.47" ±5.48
	Total	75	322.60^{A} ±14.92	77.09^{u} ±3.97	19.38^{c} ±.959	4.82A ±.623	10.0 I A ±0.259	1II.3Iu ±3.81
Coccidiosis	Hubbard	33	402.57' ±24.16	99 .26° ±4.44	15.42' ±1.56	1.26" ±.744	10.78" ±0.348	126.74' ±5.48
	Cobb	18	388.88^{D} ±32.71	115.55^{c} ±6.01	27.22c ±2.11	6.79' ±1.00	10.66" ±0.472	160.24^{c} ± 7.43
	Ross	28	338.92 ±26.23	88.89' ±4.82	24.85^{co} ± 1.69	7.90" ±.808	I 0.25^{D} ±0.3 78	I 31.90^{o} ±5.95
	Total	79	376.89^{A} ±17.80	99.29^{c} ±3.17	21.45" ± 1.26	4.87^{A} ±.652	10.56^{A} ±0.255	I 36.20c ±4.24
Gumboro	Hubbard	7	331.42 ±52.46	99.18° ±9.64	14.85' ±3.38	1.42b ±1.6 I	10.71 D ±O. 756	126.18' ±11.91
	Cobb	14	325.35 '±37.09	I 32.44" ±6.82	*42. W* ±2.39	7.62' ±1.14	10.14D ±0.535	I 92.35^{D} ±8.42
	Ross	7	306.42' ±52.46	99.86° ±9.64	17.85' ±3.38	0.974^{D} ±1.61	11.71" ±0.756	130.41° ±11.91
	Total	28	322.14^{A} ±22.79	115.98^{A} ±4.07	29.25^{A} ±2.93	4.4 I A ±.77 I	10.67^{A} ±0.163	160.32^{A} ±7.27
CRD	Hubbard	8	366.25^{c} ±49.07	99.74° ±9.02	15.62b ±3.16	1.20^{D} ±1.5 I	I 0.50^{D} ±0. 708	I 27.07 ±1 1.14
	Cobb	8	340.00c ±49.07	I 22.29" ±9.02	38.50" ±3.16	8.42" ±1.51	I 0.25" ±. 708	I 79 .46' ± 11.14
	Ross	2	300.00^{K} ±98.15	90.00' ±18.04	15.00' ±6.33	1. I 8^{b} ±3.02	11.00" ± 1.41	117.18& ±22.29
	Total	18	347.22A ±30.85	108.68" ±4.24	25.72" ±3.65	4.4IA ±1.l7	10.44A ±0.120	I 49.26" ±8.43

For breeds small letters: Means within the same column carrying different letters are significantly different at (P<0.01).

For diseases capital letters: Means carrying different letters are significantly different at (P<0.01). CRD (Chronic Respiratory Disease).

From Table (5) we can noticed that there was a significant effect of the diseases infections (P<0.001) on the total ration consumed, where it was high in farms infected with Gumboro (322.14 Kg) and also in control group (322.60 Kg), and the higher total feed consumption observed in farms infected with coccidiosis (376.89 Kg), also it ranged from 274.33 to 402.57 for cobb control group and Hubbard infected with coccidiosis.

These results attributed to the birds affected with coccidiosis its feed conversion decreased rapidly and so there losses in amount of feed that introduced to broilers.

Also, Table (5), explained the highly significant effect (P<0.01) of the different diseases and breeds within the diseases infection s on the values of drugs, as the drugs values ranged from 77.09 to 115.98/ 100 Control groups and Gumboro disease infected birds. Also it differed significantly (P<0.01) among the different breeds within the diseases infection, as it ranged from 65.48 to 115.55LE / 100 broilers for Ross control breeds and Cobb infected with coccidioses.

Concerning the values of vaccmes, they differed significantly (P<0.01) among diseases infection, as it ranged from 19.38 to 25.72 for control birds and CRD infected birds; respectively. Also, it differed significantly (P<0.01) among the different breeds within the diseases infection, as it ranged from 14.74 to : 1-2.14 for hubbard control group and Cobb birds infected with Gurnboro, respectively.

The different diseases infected farms had a significant (P<0.01) effect on the values of disinfectants, as it ranged from 4.41 to 4.87 for Gumboro infected farms and CRD infected farms to Coccidiosis infected farms. Also, the disinfectants values differed significantly (P<0.01) among the breeds within the infection pattern, as it ranged from 0.974 to 8.42 LE / 100 broilers for Ross birds in infected Gumboro farms and Ross control farms, respectively.

Moreover, the values of veterinary supervision differed significantly (P<0.01) among the different diseases infection pattern, as it ranged from 10.01 to 10.7 LE / 100 broilers for control birds and Gumboro infected farms.

The values of total veterinary management differed significantly (P<0.01) among different diseases infection and breed with different infection pattern, as it ranged from 111.3 to 160.32 LE / 100 broilers for control group and Gumboro infected birds. Moreover, it differed significantly (P<0.01) among the breeds within the infection pattern, as it ranged from 98.47 to 192.35 for Ross control birds and Cobb bird infected with Gumboro, respectively. These results agreed with those of (10), (12), (13) and (17) where they reported that, the diseases incidence and infection differ from breeds and locality according to the livability and the veterinary management program used to protect the birds against the infection with different diseases.

6- Effect of different diseases and breeds within the diseases infection on average marketing age, total meat production, mortality percent, total variable costs, total costs, total return and net return / 100 broilers:

Table (6): Means ± SE of of average marketing age, total meat production(Kg), mortality%, total variable costs, total costs, total return and net return 0f different breeds among different diseases

Disease	Breed	N	Average marketing age	Total meat Production (Kg)	Mortality %	Total variable costs	Total costs	Total return	Net return
			X-±S.E	X- ±S.E	X- ±S.E	X-±S.E	X-±S.E	X- ±S.E	X±S.E
Control	Hubbard	27	54.85' ± 1.43	156.5ID ±3.69	4.55° ±0.42'2	1231.57^{c} ±80.54	1351.01^{c} ±81.37	I 972.76^{d} ±46.28	62I.W ±80.10
	Cobb	15	43.00^{D} ±1.92	148 .26DC ±4. 96	5.98° ±0.566	968.63 '± I 08.05	I 096.77^{k} ±1 09.1 8	I 870.26^{h} ±62.09	773 .49° ± I 07.47
	Ross	33	38.93^{c} ±I.29	141.52^{c} ±3.34	4.84° ±0.382	1079.73& ±72.85	I 148. I 8^{J} ±73.61	I 785.56' ±41.86	637.37 ±72.45
	Total	75	45.48^{A} ±1.l4	148.27" ±2.59	4.97$^{A\text{u}}$ ±0.160	1112.17^{u} ±43.66	121 0.92^{AB} ±44. 72	I 869.89^{AB} ±32.49	658.97" ±46.21
Coccidiosis	Hubbard	33	49.09'D ±1.29	I 53.22D ±3.34	5. I 9° ±0.382	1363.41' ±72.85	1487.65^{D} ± 73.61	1931.83 ±41.86	444.18^{J} ±72.45
	Cobb	18	42.83" ±1.75	155.12" ±4.52 .	7.13° ±0.517	I 360.03" ±98.64	1505.03' ±99.66	1953.54^{c} ±56.68	448.5 Jl ±98.1 0
	Ross	28	41.42" ± 1.40	148.87°C ±3.36	4.77° ±0.414	1184.19° ± 79.09	1291.62 ±79.91	1877.05 ' ±45.44	585.43^{h} ±78.66
	Total	79	44.94^{A} ±.955	152.IIA ±2.14	5.48^{AB} ±.221	1299.12A ±53.42	1422.13^{A} ±54.16	1917.36" ±26.85	495.23^{IJ} ±50.46
Gumboro	Hubbard	7	50.42" ±2.81	167.21' ±7.26	4.42° ±0.829	1148.75^{D} ±158.17	1270.89& ±159.82	2106.91 " ±90.89	836.01' ±157.32
	Cobb	14	39.71 c ± 1.99	1 52.87^{c} ±5. 13	14.82' ±0.586	1206.83' ±111.85	1370.04^{d} ±I13.01	1923.19& ±64.27	553.14" ±1 I 1.24
	Ross	7	43.42"' ±2.81	1 52.20c ± 7.26	8.64^{D} ±0.829	1080.46 ±158.17	1214.03 '±159.82	1920 01& ±90 89	705.98^{c} ±157.32
	Total	28	43.32A ± 1.31	156.29A ±286	I 0.67" ± 1.16	1160.72L ±69.20	1306.25" ±69.83	1968.32B ±35.91	662.07^{A} ±69.15
CRD	Hubbard	8	45.87" ±2.63	149.82°C ±6.79	5. I 5° ±0.775	1254.59^{D} ±147.96	1383.34^{ca} ±149.50	1890.78^{c} ±85.02	507.44' ±147.16
	Cobb	8	43.37"' ±2.63	1 69.67"± 6. 79	9.07^{D} ±0.775	1234.92^{c} ±147.96	I 389.92^{c} ± 149.50	2133.77" ±85.02	743.85^{c} ±147.16
	Ross	2	44.00D ±5.26	158.25^{D} ±DI3.58	5.50^{o} ± 1.55	1048.51 ±295.92	1178.51 ' ±299.00	1995.53^{c} ± 170.05	816.60^{o} ±294.32
	Total	18	44.55^{A} ±2.35	1 59.58^{A} ±3.60	6.93" ±0.656	I 222.95" ±91.90	1363.5IB ±91.25	2010.37^{A} ±44.53	646.86^{c} ±93.80

For breeds small letters: Means within the same column carrying different letters are significantly different at (P<0.01).

For diseases capital letters: Means carrying different letters are significantly different at (P<0.01).

CRD (Chronic Respiratory Disease).

The results in table (6) illustrated that the average marketing age significantly (P<0.01) differed among the different infection pattern, as it ranged from 43.32 to 45.48 days for Gumboro infected farms and Control farms. Moreover, it differed significantly (P<0.01) among the different breeds within the infection pattern, as it ranged from 39.71 to 54.85 days for Cobb and Hubbard breeds; respectively.

The total meat production differed significantly (P<0.01) among the different infection pattern, as it ranged from 148.27 to 159.58 kg / 100 broilers for CRD infected birds and control groups and ranged from 14l.52 to 169.67 kg / 100 broilers for Ross control birds and cobb infected with CRD and this results may be attributed to that the farms that spread in it the CRD take a greater precautions for prevention of the infection with addition of higher amount of feed and veterinary management that achieved higher meat production. These results agreed with those of (3)

The mortality percent showed a higher level (10.67%) in Gumboro infected birds and 4.97 % in control birds. In addition to Cobb infected with Gumboro of higher mortality percent and the Hubbard control broilers of lower mortality percent 4.55 %. This results indicated that the Gumboro of higher losses disease affecting broiler production.

Also, the infection pattern and breeds within the infection pattern had a significant (P<0.01) effect on the total variable costs (TVC), as it ranged from 968.63 to 1363.41 LE / 100 broilers for cobb control birds and hubbard breeds infected with coccidiosis. Meanwhile, the higher total variable costs (1299.12 LE/100 broilers) observed in coccidiosis infection pattern and the lower total variable costs (1112.17 LE/100 broilers) observed in control birds.

Moreover, the total costs (TC) (total variable + total fixed costs) differed significantly (P<0.01) among the different infection patterns and also among different broiler breeds. As it ranged from 1210.92 0 1422.13 for control breeds and coccidiosis infection pattern. Meanwhile, the Hubbard control breeds of lower costs and coccidiosis infected hubbard breeds showed the maximum costs.

The total return I 100 broilers differed significantly (P<0.01) among the infection pattern, as it ranged from 2010.37 to 1869.89 LE / 100 broilers; respectively, and ranged from 2133.77 to 1870.26 LE / 100 broilers for Cobb breed infected with CRD and Control Cobb breeds; respectively.

The net return / 100 broilers differed significantly (P<0.01) among the different infection pattern. The lower infection pattern observed in coccidiosis and CRD infected birds (646.86 LE/I00 broilers. While, the control birds achieved net income by 658.97 /100 broilers and Gumboro infected farms achieved 662.07 LE/ 100 broilers. These results attributed to the farms infected with Gumboro diseases vaccinated and take a greater precaution against

diseases and introduce to the birds good ration that achieved a higher body weight and returns.

These results indicated that, the diseases of broiler production farms especially coccidiosis, Gumboro and CRD causes greater economic loses to broiler farms and it differed from farm to another according to the breed susceptibility and health program of the farm. This results agreed with those of (19); (20); (21); (22); (2); (23) and (24), as they reported that the most important economic diseases and problems affecting broiler industry were Gumboro, Salmonellosis, CRD, and Coccidiosis diseases, respectively.

This study concluded that, the main factors affecting broiler production under Egyptian conditions were Locality, breeds, intensity of the broilers in the house, diseases incidence and prevalance, feed , veterinary management, marketing age, total meat production, total costs, total returns and net profit.

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الملخص العربى

بعض العوامل المؤثرة على الكفاءة الاقتصادية والانتاجية لمزارع انتاج بدارى التسمين

ايمان رمضان كامل[1] وحاتم حسين بكرى[2] و سند طلعت عطا الله[3]

[1]قسم تنمية الثروة الحيوانية، كلية الطب البيطرى، جامعة بنها
[2] قسم الطب الشرعى والسموم، كلية الطب البيطرى، جامعة بنها
[3]قسم رعاية الحيوان وتنمية الثروة الحيوانية، كلية الطب البيطرى، جامعة الاسكندرية

أجرى البحث خلال الفترة من 2007-2009 على البيانات التى تم جمعها (200) دورة لبدارى التسمين من مزارع مختلفة لانتاج دواجن والتى تقع فى محافظات القليوبية والدقهلية ودمياط و الشرقية، لسلالات مختلفة من الهبرد والروس والكوب، وتم تجميع البيانات من خلال السجلات المتاحة فى بعض المزارع ومن خلال طريقة الاستبيان لبعض المزارعين ، وكان الهدف من البحث هو دراسة العوامل المؤثرة على الكفاءة الاقتصادية والانتاجية لمزارع بدارى التسمين ، وقياس اربحيتها تحت الظروف المصرية. تم تحليل النتائج احصائيا واقتصاديا باستخدام برنامج التحليل الاحصائى (SPSS.8) ، وقد اظهرت النتائج عند عمل علاقة بين الاماكن والسلالات، وجد ان افضل الاماكن من حيث العوائد والارباح/100طائر هى على الترتيب دمياط – القليوبية حيث كان صافى الربح 545.02 ، 685.59 جنيه على التوالى وكانت افضل السلالات الهبرد والروس حيث حققت 521.41 ، 788.31 جنيه على التوالى، وعند عمل علاقة بين الكثافات و السلالات وجد ان افضل الكثافات من حيث العوائد والارباح/100طائر هى على الترتيب المتوسطة – العالية، حيث كان صافى الربح 611.11 ، 589.54 جنيه على التوالى وكانت افضل السلالات الكوب والروس حيث حققت 481.33 ، 644.23 جنيه على التوالى ، وبالنسبة لمعدل الاصابات بالامراض ، كان اقل صافى ربح /100 طائر فى حالة الاصابة بمرض الكوكسيديا 495.23 جنيه على التوالى ، اما فى الحالات الغير مصابة كان صافى ربح/100 طائر 658.97 جنيه على التوالى.

Radiological Investigation of the African Brush-tailed Porcupine (*Atherurus africanus*) Appendicular Skeleton

El-Shafey, A.A[1]. Akraiem A.[2] and Abdel-Galil, A.S.A[3].

1. Dept. Anat. & Embry. Fac. Vet. Med., Benha Univ. Egypt. elshafey74@yahoo.com
2. Dept. Surgery, Fac. Vet. Med., Omar El-Mokhtar Univ., Libya .
3. Dept. Surgery, Anesthesiology and Radiology, Fac. Vet. Med. Benha Univ., Egypt.

ABSTRACT

The present study aimed to describe the normal radiographic anatomy of the healthy African Brush-tailed Porcupine appendicular skeleton to fill a gap in the field of comparative anatomy and to establish an anatomical basis for diagnosis of skeletal diseases and surgical interference of the limbs of this important zoo-animal. Four (two male and two females) free-living porcupines (*Atherurus africanus*) from the El-Jabal Alakhdar region of Libya were selected for this study. Lateral, craniocaudal and caudocranial radiographs from the thoracic and pelvic limbs were obtained. The important anatomic structures of the appendicular skeleton were revealed, seen clearly and labeled in two corresponding photographs of radiograph and bones of porcupine limbs. The various boney structures of the limbs were recorded. The radiograph pictures from these porcupines were compared with the normal canine and feline skeletal radiographic anatomy.

INTRODUCTION

African Brush-tailed Porcupine (*Atherurus africanus*) is a hystricomorph rodent, which lives in the forests of west and central Africa, it is a favorite source of meat for rural population, and its price is often high in comparison with that of other domestic animals[1]. Radiographic examination is a method that can play an important role in the diagnosis of a wide variety of skeletal diseases.

The macroanatomical study of domestic animals skeletons[2], porcupine[3] and hedgehog[4] had been reported. Radiographic anatomy of the appendicular skeleton is performed increasingly in many animals[5, 6] such as dogs, cats[7,8] and hedgehog[9], but to our knowledge there is no any earlier study has been reported on the normal radiographic anatomy of the appendicular skeleton of African Brush-tailed Porcupine (*Atherurus africanus*).

The aim of this study was to provide an atlas of the normal radiographic anatomy of the bones of the porcupine limbs which might be necessary to describe any abnormalities that may be present.

MATERIAL AND METHODS

The present study was carried on four healthy adult free-living porcupines (Atherurus africanus, Fig.1) (two males, two females) from the El-jabal Alakhdar region of Libya. The porcupines were anesthetized by injecting (35 mg/kg ketamine + 5 mg/kg xylazine IM). Lateral, craniocaudal and caudocranial x-ray radiographs from the thoracic and pelvic limbs were obtained. The radiographic films were stored digitally. After radiographic images were obtained, the porcupines were slaughtered and subjected to boiling method of skeleton preparation techniques to correspond with the radiographic images. The radiographic images were labeled by comparison with the boney skeleton. The nomenclature was adopted according to the Nomina Anatomica Veterinaria[11]. Some structures present in the bones could not see on the corresponding radiographic images. Some Radiographic images artifacts were noted.

Fig.1**:** A photograph of African Brush-tailed Porcupine (Atherurus africanus).

RESULTS

The results of the present study consisted of eleven radiographic images and eleven boney images. The important anatomic structures of the appendiclar skeleton were revealed, seen clearly and labeled in two corresponding photographs of radiograph and bones of porcupine limbs. The various boney structures of the limbs were recorded.

The thoracic limb radiographs revealed that, the scapula had a long acromion process reaching the level of the glenoid cavity (Fig. 2,3 A&B). The spine of the scapula divided the lateral surface into supraspinous and infraspinous fossae by ratio 2:1 (Fig. 2, 3. 4, 5 A&B).

The clavicle was observed as a complete bone connected to the scapula and manubrium of the sternum (Fig. 4 A&B).

The radial and olecranon fossae of the humerus communicated with each other through an oval supratrochlear foramen (Fig. 6 A&B).

The ulna was longer and larger than the radius and separated from it by interossous space (Fig. 6 A&B).

There were seven carpal bones, three in the proximal row and four in the distal rows, the radial and intermediate bones fused in on bone. There were five metacarpal bones and five digits in the thoracic limb. Each digit had three phalanges except the first one which had two phalanges only (Fig.7 A&B).

The pelvic limb radiographs revealed that, the wing of the ilium was long and its gluteal surface was less concave. In the lateral view, the pubis and ischium are relatively small (Fig. 8, 9 A&B).

The highest point of the greater trochanter of the femur lied at the same level of the head (Fig. 10 A&B).

The fibula was separated from the tibia by long interossous space (Fig. 11 A&B).

There were seven tarsal bones arranged in three rows, Calcaneus and Talus in the proximal row, Central tarsal bone in the middle row and First tarsal bone, Second, Third and Fourth tarsal bones in the distal row. There were five metacarpal bones and five digits in the pelvic limb each digit had three phalanges except the first digit which had two phalanges only (Fig. 12 A&B).

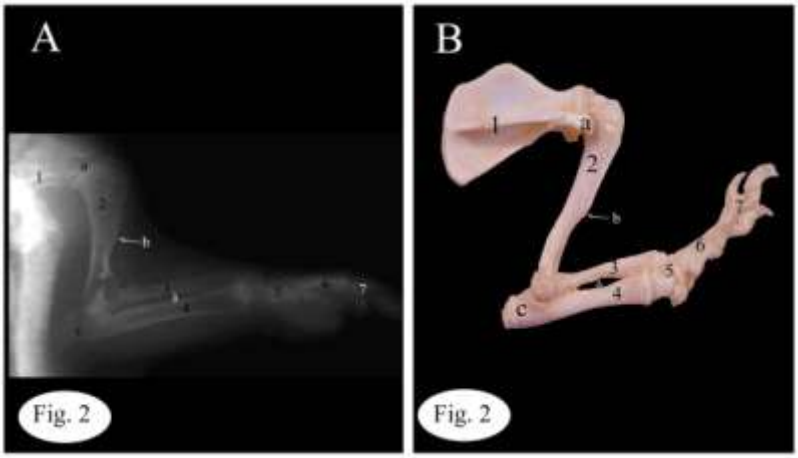

Fig.2: Lateromedial radiographic image (A) and boney representation (B) of the right forelimb. 1. Scapula. 2. Humerus. 3. Radius. 4. Ulna. 5. Carpal bones. 6. Metacarpal bones. 7. Digits. a. Acromion process. b. Deltoid tuberosity of the humerus. c. Olecranon tuberosity. d. Interosseus spaces.

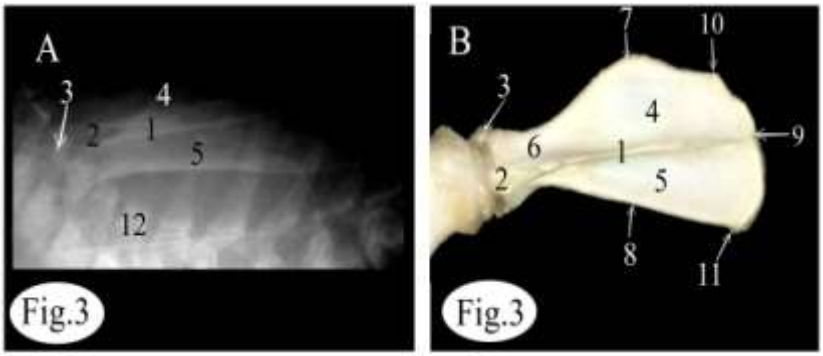

Fig.3: Dorsolateral radiographic image (A) and boney representation (B) of the left scapula. 1. Spine of scapula. 2. Acromion process. 3. Supraglenoid tuberosity. 4. Supraspinous fossa. 5. Infraspinous fossa. 6. Neck. 7. Cranial border. 8. Caudal border. 9. Dorsal border. 10. Cranial angle. 11. Caudal angle. 12. 4th thoracic vertebra.

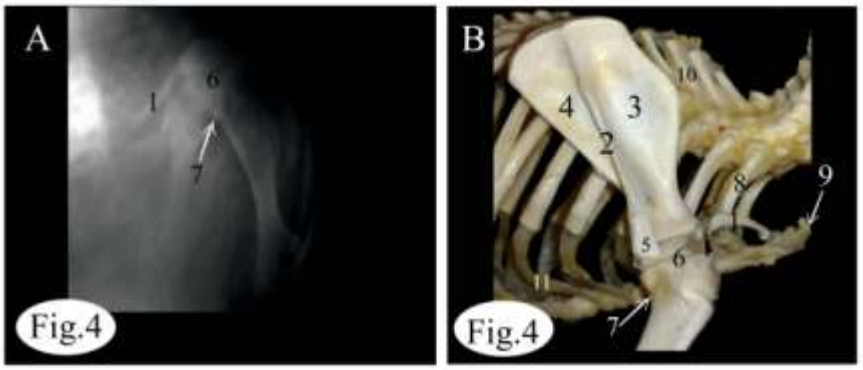

Fig.4: Dorsolateral radiographic image (A) and boney representation (B) of the right shoulder joint.

1. Clavicale. 2. Spine of scapula. 3. Supraspinous fossa. 4. Infraspinous fossa. 5. Acromion process. 6. Head of the humerus. 7. Neck of the humerus. 8. Frist rib. 9. Manubrium of the sternum. 10. 4th thoracic vertebra. 11. Costal cartilage of 5th rib.

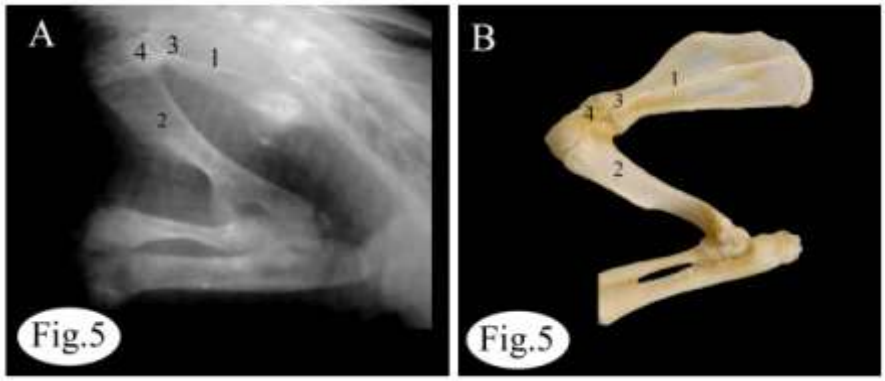

Fig.5: Caudolateral radiographic image (A) and boney representation (B) of the left shoulder joint (complete flexion).

1. Scapula. 2. Humerus. 3. Neck of the scapula. 4. Head of the humerus.

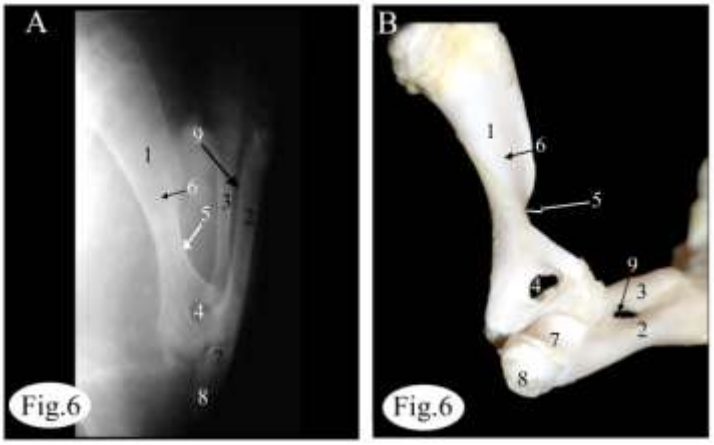

Fig6: Lateromedial radiographic image (A) and boney representation (B) of the right elbow joint. 1. Humerus. 2. Ulna. 3. Radius. 4. Supratrochlear foramen 5. Crest of the humerus. 6. Sulcus m.brachialis.7. Olecranon.8.Olecranon tuberosity.9. Interosseus space.

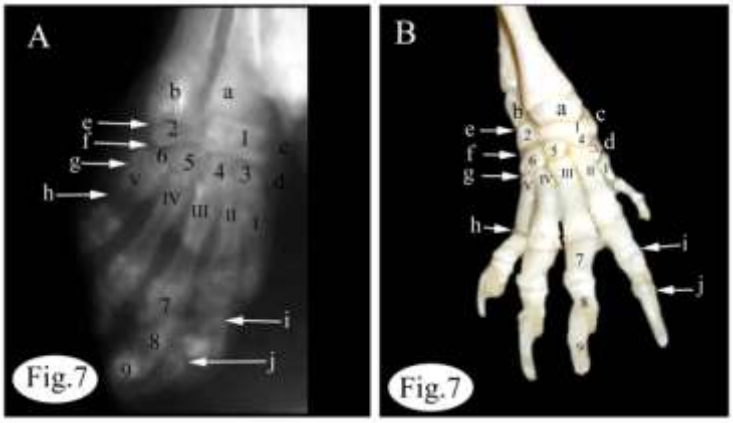

Fig.7: Dorsopalmar radiographic image (A) and boney representation (B) of the right manus. a. Distal epiphysis of radius. b. Distal epiphysis of ulna. c. Proximal row of carpal bones. d. Distal row of carpal bones. e. Radiocarpal joint. f. intercarpal joint. g. Carpometacarpal joint. h. Metacarpophalangeal joint (Fetlock joint). i. Proximal interphalangeal joint (Pastern joint). j. Distal interphalangeal joint (Coffin joint) 1. Intermedioradial carpal bone. 2. Ulnar carpal bone. 3. First carpal bone. 4. Second carpal bone. 5. Third carpal bone. 6. Fourth carpal bone. I-V Metacarpal bones. 7. Proximal phalanx of digit III. 8. Middle phalanx of digit III. 9. Distal phalanx of digit III.

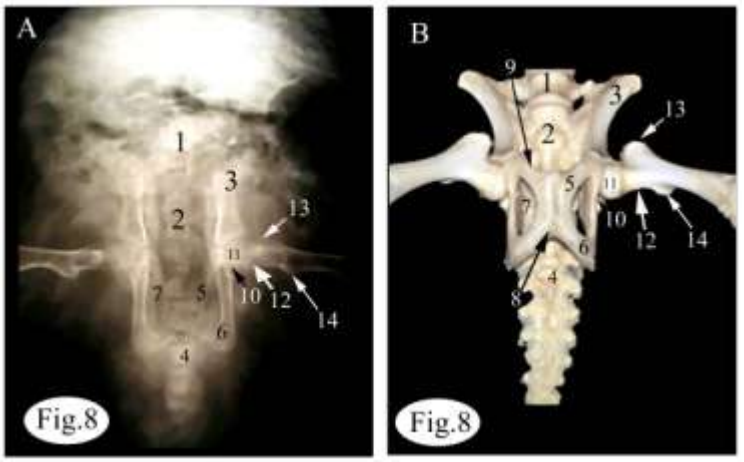

Fig.8: Ventrodorsal radiographic image (A) and boney representation (B) of the pelvis. 1. Last (6^{th}) lumbar vertebra. 2. Sacrum. 3. Ilium. 4. First caudal vertebra. 5. Pubis. 6. Ischium. 7. Obturator foramen. 8. Ischial arch. 9. Pectin of pubis. 10. Acetabulum. 11. Head of femur. 12. Neck of femur. 13. Greater trochanter. 14. Lesser trochanter of femur.

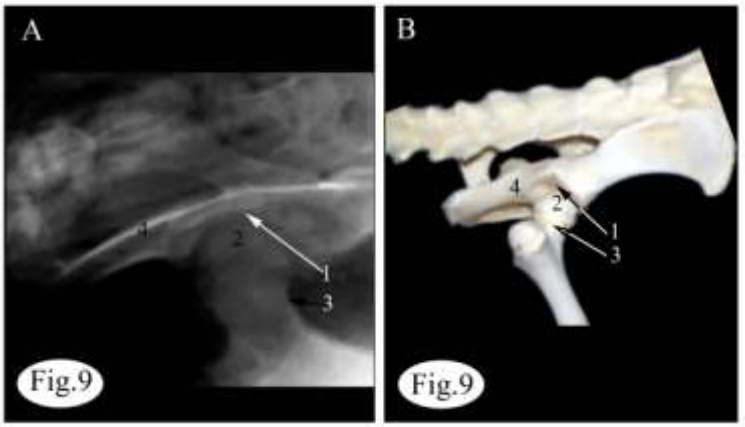

Fig.9: Lateromedial radiographic image (A) and boney representation (B) of the right hip joint.
1. Acetebulum. 2. Head of the femur. 3. Neck of the femur. 4. Ischium.

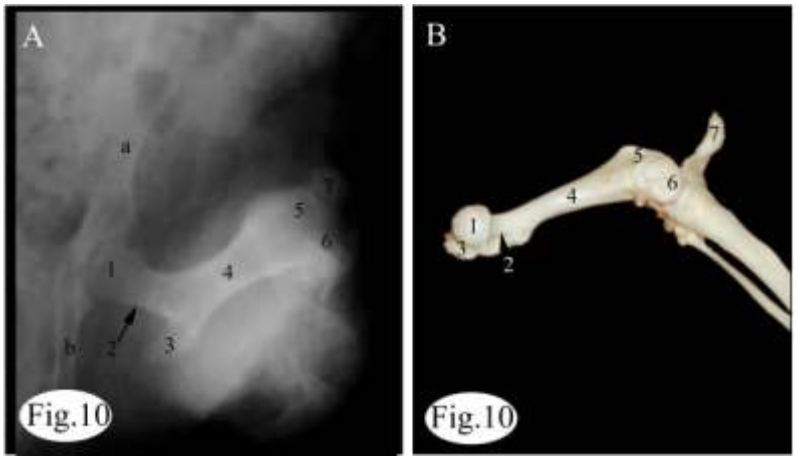

Fig.10: Mediolateral radiographic image (A) and boney representation (B) of the left femur.

1. Head of the femur. 2. Neck of the femur. 3. Greater trochanter of femur. 4. Body of the femur. 5. Trochlea of femur. 6. Medial condyle of femur. 7. Patella. a. Ilium. b. Ischium.

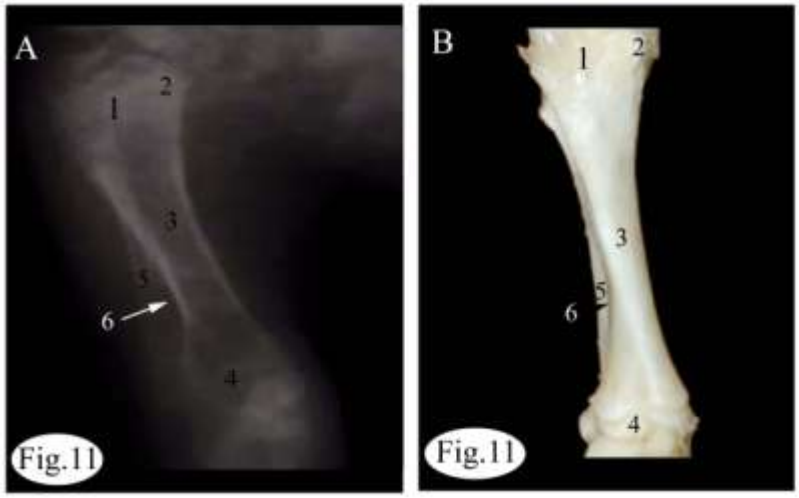

Fig.11: Mediolateral radiographic image (A) and boney representation (B) of the left tibia.

1. Proximal epiphysis of tibia. 2. Medial condyle of the tibia. 3. Body of the tabia. 4. Distal epiphysis of tibia. 5. Fibula. 6. Spatium between tibia and fibula.

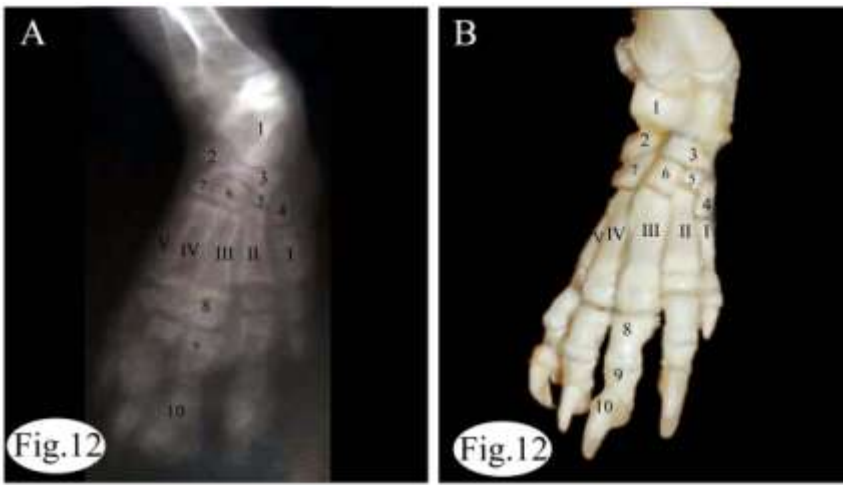

Fig.12: Plantarodorsal radiographic image (A) and boney representation (B) of the left pes.

1. Calcaneus. 2. Talus. 3. Central tarsal bone. 4. First tarsal bone. 5. Second tarsal bone. 6. Third tarsal bone. 7. Fourth tarsal bone. I-V. Metatarsal bones. 8. Proximal phalanx of digit III. 9. Middle phalanx of digit III. 10. Distal phalanx of digit III.

DISCUSSION

This article presents the first series of labeled radiographical images of African Bruch-tailed porcupine and allow for visualization of the normal structure of the porcupine apendicular skeleton from macroscopic and radiological images.

The present investigation had revealed both similarities and differences between the African Bruch-tailed porcupine apendicular skeleton and dog and cat [7, 8] and hedgehog [9].

Radiological images of the African Bruch-tailed porcupine provide complete details of the anatomical structure of the appendicular skeleton and correlates well with corresponding boney specimens.

The radiological images could be useful in studies of the abnormalities and lesions of the appendicular skeleton of the African Bruch-tailed porcupine[5].

The tarsal bones, carpal bones and digits are similar in the number to that of the dog [11].

In conclusions as demonstrated in the this study, labeled radiological images of the normal African Bruch-tailed porcupine appendicular skeleton provides excellent visualization of many anatomic structures of the appendicular skeleton and establish an anatomical basis for diagnosis of skeletal diseases and surgical interference of the limbs of the African Bruch-tailed porcupine which is an important zoo-animal and valuable favorite source of meat for rural population.

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الملخص العربي

فحص شعاعي للهيكل الطرفي للشيهم الأفريقي

أنور عبد الفتاح الشافعي[1]، المهدي محمد إكريم[2]، عاطف سيد احمد عبد الجليل[3]

1. قسم التشريح والأجنة بكلية الطب البيطري جامعة بنها- مصر.
2. قسم الجراحة بكلية الطب البيطري جامعة عمر المختار – ليبيا.
3. قسم الجراحة والتخدير والاشعة بكلية الطب البيطرى جامعة بنها – مصر.

تهدف هذه الدراسة إلي وصف التشريح بالأشعة السينية للهيكل الطرفي للشيهم الأفريقي لسد فجوة في مجال التشريح المقارن ولبناء أساس تشريحي لتشخيص أمراض الأطراف والتدخل الجراحي الأمثل فيها في هذا الحيوان الهام. استعملت لهذه الدراسة أربعة شياهم برية (ذكرين وأنثيين) طبيعية سريرياً ، جمعت من منطقة الجبل الأخضر بليبيا .

تم الفحص بالأشعة وحشياً أنسياً وأمامياً خلفياً وخلفياً أمامياً لكل من الطرف الصدري والطرف الحوضي . تم توضيح التراكيب التشريحية الهامة وتم وضع البيانات عليها في صورتين متطابقتين لكل من صور الأشعة وصور العظام. تم مقارنة صور الأشعة التشريحية للشيهم مع مثيلاتها في الكلاب والقطط.