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Atherosclerosis Induced By *Enterococcus Faecalis* In Japanese Quails *(Coturnix Coturnix)*

Mohammed. G. Saeed[1], Entisar. R. Al-Kennany
Department of Pathology and Poultry Diseases,
College of Veterinary Medicine,
University of Mosul,
Mosul, Iraq

ABSTRACT

This study was conducted to determine the ability of *Enterococcus faecalis* bacteria to cause atherosclerotic lesions in quails which are susceptible to atherosclerosis. Identification of *E. faecalis* in small intestine and cecum of quails was done by culturing on differential and selective media and API 20 Strep. The experimental infection was conducted by dividing 252 quail birds aged 21 days randomly into 6 groups, 42 birds in each group. The 1st group (G1) was a control group, the 2nd group (G2) was injected with the antibiotic Amoxicillin- Clavulanic acid (Amoxiclav) 1000 mg. The 3rd group (G3) was injected with manganese as an antioxidant. The 4th group (G4) was inoculated with *E. faecalis* suspension 0.5 ml. intraperitoneally i.p. with a concentration of 10^6 CFU/ml. The 5th group (G5) was inoculated with bacterial suspension plus antibiotic and the 6th group (G6) was inoculated with bacterial suspension plus antioxidant manganese. Results of the biochemical tests of lipids profile in the serum showed a significant increase in total cholesterol, triglycerides, LDL-c, VLDL-c and atherogenic index, and a significant decrease in the level of high-density lipoprotein cholesterol HDL-c in the group (G4) compared with (G1) and other groups over periods of 3, 7 and 30 days post infection PI. There were general increase rates of lipids observed in all groups of quails after 60 and 90 days PI, and this increase was in direct correlation with their age. The microscopic changes of the aorta appeared in (G4) after 1 day PI and were severe at 7 days PI. They consisted of the presence of bacterial colonies in endothelium, hypertrophy and hyperplasia of endothelial cells, intensive localization of fatty droplets and foam cells in the intimal and medial layers which extended then to all layers of aorta, proliferation of vascular smooth muscle cells VSMCs, proliferation and irregularity of collagen fibers, fragmentation of elastic fibers and infiltration of heterophils. This study concludes that *E. faecalis* led to the development of primary lesions of atherosclerosis in the aorta of quails.

Keywords: *Atherosclerosis, Enterococcus faecalis Japanese quails, Coturnix coturnix)*

INTRODUCTION

Atherosclerosis is a disease of the large and medium arteries. It is the leading cause of heart attacks and strokes, and the reason for 50% of all deaths in industrialized countries (1). It is a vascular disease of great importance in humans, but rarely leads to clinical disease in animals. The presence of lesions of this disease that occurs naturally in pigs, large birds, and in dogs that suffer from hypothyroidism and is associated with high cholesterol (2). This disease is characterized by the accumulation of lipids in the large arteries in the form of plaques filled with a lipid which is called atheroma (2,3).

[1] Corresponding author: mgsaeed@uomosul.edu.iq

Recent studies have shown that biological pathogens may lead to the induction of primary lesions of atherosclerosis (4,5). Among the pathogens that are involved in the pathogenesis of atherosclerosis are bacteria such as *Chlamydia pneumonia*, *Helicobacter Pylori*, (5) and *Enterococcus faecalis* (6,7). One study found that the tissues of atherosclerosis taken from patients with heart and coronary artery diseases contain more than 50 species of bacteria, including *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae* and species of Streptococcus. The researchers concluded that the bacterial colonies which are found in plaques of atherosclerosis may be more important than the individual factors causing the disease. The studies also showed that bacteria are not causal factors in pathogenesis of coronary and heart disease, but can colonize atherosclerotic lesions secondarily and lead to the development of lesions in the vessel wall and can serve as an additional factor to accelerate the progression of the disease (8).

Studies are limited on the subject of *Enterococcus faecalis* that causes atherosclerosis, which is why we conducted this study to observe the effects of this bacteria in quails. The bacterium *E. faecalis* has several virulence factors and may cause oxidative stress and induce atherosclerosis. The latter is due to the ability of the bacteria to secrete extracellular superoxide and hydrogen peroxide (9). This feature is a unique or unusual phenotype for this species of *E. faecalis* and through a survey conducted among the strains of *E. faecalis*, almost all strains or about 95% of them produce extracellular superoxide which is one of the of prevalent virulence factors found in pathogenicity (10, 11). A study conducted by (6) noticed the role of oxidative stress associated with *E. faecalis* through experimental infection in rats, by which the bacteria release extracellular superoxide which, in turn, induces lesions of endocarditis and the development of atherosclerosis. The reactive oxygen radical species ROS are also produced during phagocytosis by the inflammatory mechanism of host cells against bacteria, and also cause damage to cell membranes and then release lipids outside the cells and increase lipids levels in the blood. ROS are produced by smooth vascular muscle cells, endothelium, fibroblasts and infiltrate leukocytes (12). ROS affect gene transcription, lead to damaged DNA, and increase production of inflammatory mediators, as well as the oxidization of low density lipoprotein LDL in atherosclerotic lesions (13).

MATERIALS AND METHODS

Experimental animals: In this experiment we used 21-day-old Japanese quails *(Coturnix Coturnix)*, each weighing between 80-100 grams. The quail eggs were obtained from the Agricultural Research Corporation / Research Department of Nineveh. The quail eggs were incubated in an incubator of the Animal House of the College of Veterinary Medicine - University of Mosul, and after hatching, the quail chicks were placed in standard laboratory conditions in terms of temperature and lighting and were supplied with a standard diet for quails throughout the duration of the experiment.

Bacterial isolation: *E. faecalis* were isolated from the intestine and cecum of quails through a sterilized method. The isolations were cultured in a brain heart infusion broth BHIB and were incubated at 37 °C for 24 hours and then cultured on MacConkey agar and incubated at 37 °C for 24 hours. A swab was then taken and cultured on the differential media, Azide blood agar and Edward blood agar then incubated at 37 °C for 24 hours. Swabs from these media were cultured on the selective media Enterococcus agar. The biochemical tests were done by the API 20 Strep (company bioMérieux French) for an accurate diagnosis (14), and were incubated for 24 hours before the results were taken.

Experimental infection: We obtained different concentrations of bacterial suspension of *E. faecalis* by using a viable bacterial count by method of Miles & Misra (15). Three concentrations of bacterial suspension were chosen at 10^5 and 10^6 and 10^7 CFU/ml based on previous studies. Depending on the preliminary experiment, the concentration 10^6 CFU/ml was selected to be inoculated in main experiment. For the main experiment, 252 randomly selected quail birds aged 21 days were divided into 6 groups, 42 birds for each group. The first group (G1) was injected with normal saline as a control group, the second group (G2) was injected with the antibiotic Amoxicillin- Clavulanic acid (Amoxiclav) 1000 mg. (The antibiotic was chosen by way of an antibiotic sensitivity test), and was injected intraperitoneally (IP), daily for four days. The third group (G3) was injected with antioxidant manganese (in the form of MnCl2.4H2O) where the dose determined was based on other studies at 3 mg / kg of body weight i.p, and was

calculated according to the molecular weight of each of MnCl2.4H2O 197.9 g / mol , chlorine 35.5 g / mol and manganese 54.9 g / mol, where it was dissolved with distilled water and injected daily for five days. The fourth group (G4) was inoculated with *E. faecalis* suspension 0.5 ml. i.p with a concentration of 10^6 CFU/ml. The fifth group (G5) was inoculated with bacterial suspension 10^6 CFU/ml plus antibiotic and the sixth group (G6) was inoculated with bacterial suspension plus antioxidant manganese.

The 6 quails in each group were sacrificed at different intervals: 12 hours, 1, 3, 7, 30, 60 and 90 days post infection, starting with the last dose in each group, and the blood. was collected from the jugular vein for all sacrificed birds, and was later centrifuged to obtain the serum to be used later in biochemical measurements. Postmortem was performed to notice macroscopic changes of thoracic and abdominal aorta; then necropsy samples were taken from the aorta and fixed in a 10% neutral buffer formalin and then embedded in paraffin, sectioned at 5 uM, and stained with hematoxylin and eosin (H&E). Selected sections were stained with Gram's tissue modified stain for bacteria (16).

Biochemical tests:

Lipid profile:

1- Measurement of the level of total cholesterol:

2- Measurement of the level of triglycerides (TG):

3- Measurement of the level of high density lipoprotein cholesterol (HDL-c):

We used kits manufactured by the French company Biolabo to measure the concentration of total cholesterol, triglycerides, and high density lipoprotein cholesterol.

4 - Measurement of the level of low density lipoprotein Cholesterol (LDL-c):

The LDL-c was calculated by application of Friedewald equation (Friedewald et al., 1972) :

(Total LDL-c (mg / dl) = (Cholesterol) - (HDL-c) - (Triglycerides / 5)

And the final value per unit of mg / liter Dessie.

5- Measurement of very low density lipoprotein cholesterol (VLDL-c):

It was calculated by dividing the value of triglycerides TG by 5 (Friedewald et al., 1972) as follows:

VLDL-c = TG / 5

Measurement of Atherogenic Index: It was based on the following equation : Atherogenic index=TC/HDL-c.

Statistical analysis: Data were analyzed for the various animals groups statistically using one way analysis of variance and the results subjected to Least Significant Difference (LSD) test and Dunnet test for comparison with the control group. This occurred by using the software of statistical analysis SPSS and the level of significance for all tests at less than 0.05 ($P < 0.05$).

RESULTS

Bacterial isolation: Enterococci bacteria grew on the Azide blood agar as white to gray color colonies, white pinhead size colonies on the Edward blood agar and single pinhead size red to brown color colonies on the selective Enterococcus agar. The biochemical test of bacteria API 20 Strep showed that the type of bacterial isolation is *E. faecalis* 99% according to the bacteria guide.

Total cholesterol: Results of statistical analysis showed a significant increase in the level of total cholesterol in serum of quails in the group (G4) at 3 and 7 days p.i compared with the control group (G1) and other groups, but at 30 days p.i compared only with (G2) and (G3). The level of total cholesterol was low in (G2) and (G3). The manganese injected group recorded a significant lower level of total cholesterol compared to other groups of quails at 3 and 7 days p.i and with (G4) only after 30 days p.i. At 60 and 90 days p.i there appeared a high level of total cholesterol in all the quail groups compared to the early periods of the experiment and this increase was in positive correlation with age (Table 1).

Table 1. Values of total cholesterol (TC) mg/dl. in serum of quails.

DURATIONS / GROUPS	**12 hrs. p.i.**	**24 hrs. p.i.**	**3 days p.i.**	**7 days p.i**	**30 days p.i.**	**60 days p.i.**	**90 days p.i.**
CONTROL (G1)	90.07±37 b A	108.98±38 b A	105.85±22 b BCD	126.97±28 b BC	200.29±46 b B	321.36±56 a A	341.30±68 a A
ANTIBIOTIC (G2)	92.73±37 b A	125.44±38 b A	94.23±22 b BCD	108.6 ±28 b BC	195.93±46 b B	258.01±56 a A	358.21±68 a A
MANGANESE (G3)	77.54±37 b A	89.68±38 b A	69.66±22 b D	87.65±28 b C	185.57±46 a B	245.59±56 a A	327.31±68 a A
BACTERIA (G4)	117.43±37 c A	147.69±38 c A	251.42±22 b A	266.19±28 b A	275.35±46 b A	334.62±56 a A	381.89 ±68 a A
BACTERIA+ ANTIBIOTIC (G5)	104.92±37 b A	128.95±38 b A	177.69±22 b B	176.85±28 b B	235.35±46 ab AB	242.52±56 ab A	377.48± 68 a A
BACTERIA + MANGANESE (G6)	102.05±37 b A	131.84±38 b A	168.37±22 b C	168.04±28 b B	237.09±46 a AB	337.92±56 ac A	367.49±68 ac A

The value represent the mean ± standard error (n=6/group)
The capital letters represent the differences among groups in significant differences ($P < 0.05$).
The small letters represent the differences among durations in significant differences ($P < 0.05$).

Triglycerides (TG):The statistical analysis showed a significant increase in the level of triglycerides in the serum of quails infected with bacteria (G4) compared with the control group (G1) and (G5) at 3 days p.i, and with (G1) and all other quail groups at 7 days p.i, and with the (G1), (G2) and (G3) at 30 days p.i, and with (G5) and (G6) at 60 days p.i, and finally with (G1) and all other quail groups at 90 days p.i. There were no differences between the groups. The other feature was a general increase in the level of triglycerides at 30, 60 and 90 days p.i in all groups, this increase was in positive correlation with the age of quails (Table 2).

Table 2. Values of Triglyceride (TG) mg/dl. in serum of quails.

DURATIONS / GROUPS	12 hrs. p.i.	24 hrs. p.i.	3 days p.i.	7 days p.i	30 days p.i.	60 days p.i.	90 days p.i.
CONTROL (G1)	63.85±13 b A	67.21±15 b A	62.72±17 b B	62.14±15 b B	74.961±26 b B	80±35 b AB	110.55±53 a B
ANTIBIOTIC (G2)	61.85±13	57.57±15	70.69±17 ab AB	52.28±15 b B	73.386±26	53.62±35 ab B	89.92±53 a b

	ab A	ab A			a B		
MANGANESE (G3)	69.78±13 b A	56.43±15 b A	63.79±17 b AB	53.40±15 b B	65.05±26 ab B	72.83±35 ab B	127.17±53 a B
BACTERIA (G4)	57.13±13 b A	58.43±15 b A	91.29±17 a A	105.22±15 a A	142.36±26 a A	152.36±35 a A	285.59±53 a A
BACTERIA + ANTIBIOTIC (G5)	44.67±13 b A	64.65±15 b A	54.15±17 b B	68.13±15 b B	100.39±26 a AB	107.56±35 a AB	152.36±53 a B
BACTERIA + MANGANESE (G6)	46.9±13 b A	51.01±15 b A	74.63±17 Bb AB	50.84±15 b B	94.01±26 a AB	105.2±35 a AB	149.84±53 a B
The value represent the mean ± standard error (n=6/group) *Significantly different from the control value (P < 0.05) Letters in the column indicate significant differences among groups (P < 0.05) : (a)Antibiotic, (b)Manganese, (c) Bacteria , (d) Bacteria + antibiotic, (e) Bacteria + Manganese							

High density lipoprotein Cholesterol (HDL-c): The results showed a significant decrease in the level of HDL-c in the serum of quails infected with bacteria (G4) compared with (G5) and (G6) at12, 24 hours and 3 days p.i, and compared (G1) and all other groups at 7 and 30 days p.i. There were a significant increases in the level of HDL-c in the (G6) compared with (G4) and (G5) at 3 days p.i. In general there were low levels of HDL-c in quail in all groups at 90 days p.i, and this decline was a positive correlation with age (Table 3).

Table 3. Values of High density lipoprotein Cholesterol (HDL-C) mg/dl. in serum of quails.

DURATIONS / GROUPS	12 hrs. p.i.	24 hrs. p.i.	3 days p.i.	7 days p.i	30 days p.i.	60 days p.i.	90 days p.i.
CONTROL (G1)	66.67±13 b ABC	60.99±13 b AB	75.85±16 b ABC	72.11±15 b A	69.08±10 b AB	69.23±12 b B	43.15±8 a A
ANTIBIOTIC (G2)	59.29±13 c ABC	76.05±13 c A	70.02±16 c BC	68.64±15 c A	72.65±10 c AB	96.39±12 a A	48.4±8 b A
MANGANESE (G3)	67.85±13 b ABC	76.05±13 b A	74.95±16 b BC	75.06±15 b A	68.91±10 b A	89.08±12 b AB	31.45±8 a A
BACTERIA (G4)	49.36±13 a C	44.94±13 a A	48.02±16 a C	36.39±15 a B	40.36±10 a C	68.87±12 b B	33.39±8 a A
BACTERIA + ANTIBIOTIC (G5)	84.80±13 b AB	72.38±13 b AB	94.58±16 b AB	86.14±15 b A	91.50±10 b A	95.90±12 b B	38.32±8 a A
BACTERIA + MANGANESE (G6)	78.45±13 b AB	49.91±13 a AB	109.29±16 b A	71.55±15 b A	54.5±10 a BC	63.95±12 b B	47.59±8 a A
The value represent the mean ± standard error (n=6/group) *Significantly different from the control value (P < 0.05) Letters in the column indicate significant differences among groups (P < 0.05) : (a)Antibiotic, (b)Manganese, (c) Bacteria , (d) Bacteria + antibiotic, (e) Bacteria + Manganese							

Low density lipoprotein Cholesterol (LDL-c): The statistical analysis showed a significant increase in the level of LDL-c in the serum of quails infected with bacteria (G4) compared with the control group and all others quail groups at 12 hours, 1, 3, 7, and 30 days p.i. Also showed a significant decrease in the level of LDL-c in the manganese treated group (G3) compared with the other groups at 24 hours and 7 days p.i. It was noticed a general increase in the level of l LDL-c in the serum of quails in all groups at 30, 60 and 90 days p.i, and this increase was a direct correlation with age (Table 4).

Table 4. Values of Low density lipoprotein Cholesterol (HDL-C) mg/dl. in serum of quails.

DURATIONS / GROUPS	12 hrs. p.i.	24 hrs. p.i.	3 days p.i.	7 days p.i	30 days p.i.	60 days p.i.	90 days p.i.
CONTROL (G1)	10.63±28 c B	34.54±75 c B	17.45±63 c B	42.43±76 c B	116.21±86 b B	236.13±94 a A	276.04±55 a A
ANTIBIOTIC (G2)	21.07±28 c B	37.87±75 c B	10.072±63 c B	29.5±76 c B	108.6±86 b B	150.89±94 b A	291.82±55 a A
MANGANESE (G3)	12.93±28 c B	12.34±75 c B	11.95±63 c B	21.91±76 c C	103.65±86 b B	141.94±94 b A	270.42±55 a A
BACTERIA (G4)	42.64±28 b A	91.06±75 b A	185.14±63 b A	208.75±76 a A	206.51±86 a A	235.27±94 a A	291.38±55 a A
BACTERIA + ANTIBIOTIC (G5)	11.18±28 c B	43.64±75 c B	72.28 ±63 bc B	77.08±76 bc B	123.77±86 b B	125.1±94 b B	308.68±55 a A
BACTERIA + MANGANESE (G6)	14.22±28 c B	71.72±75 bc AB	44.15±63 c B	86.32±76 bc B	163.78±86 b B	252.93±94 a A	289.93±55 a A

The value represent the mean ± standard error (n=6/group)
*Significantly different from the control value ($P < 0.05$)
Letters in the column indicate significant differences among groups ($P < 0.05$) : (a)Antibiotic, (b)Manganese, (c) Bacteria , (d) Bacteria + antibiotic, (e) Bacteria + Manganese

Very Low density lipoprotein Cholesterol (VLDL-c): The statistical analysis showed a significant increase in the level of VLDL-c in the serum of quails infected with bacteria (G4) compared with the control group and other groups at 3 and 7 days p.i, and with (G1), (G2) and (G3) at 30 and 60 days p.i, and with all other quail groups at 90 days p.i. There were a general increase in the level of VLDL-c at 60 and 90 days p.i in all quail groups, and this increase was a direct correlation with age (Table 5).

Table 5. Values of very low density lipoprotein (VLDL-c) mg/dl. in serum of quails

DURATIONS / GROUPS	12 hrs. p.i.	24 hrs. p.i.	3 days p.i.	7 days p.i	30 days p.i.	60 days p.i.	90 days p.i.
CONTROL (G1)	12.77±13 b A	13.44±15 b A	12.54±17 b B	12.42±15 b B	14.9±26 b B	16±35 ab AB	22.11±53 b B
ANTIBIOTIC (G2)	12.37±13 b A	11.51±15 b A	14.13±17 b AB	10.45±15 b B	14.67±26 b AB	10.72±35 b B	17.98±53 a B
MANGANESE (G3)	13.95±13 b A	11.28±15 b A	12.75±17 b AB	10.68±15 b B	13.01±26 b B	14.56±35 b B	25.43±53 a B

BACTERIA (G4)	11.42±13 c A	11.68 ±15 c A	18.25±17 c A	21.04±15 bc A	28.47±26 b A	30.47±35 b A	61.11±53 a A
BACTERIA + ANTIBIOTIC (G5)	8.93±13 c A	12.93±15 c A	10.83±17 c B	13.62±15 bc B	20.07±26 ab AB	21.51±35 ab AB	30.47±53 a B
BACTERIA + MANGANESE (G6)	9.38±13 b A	13.2±15 b A	14.92±17 b AB	10.16±15 b B	18.8±26 ab AB	21.04±35 a AB	29.96±53 a B
*Significantly different from control value (P < 0.05) Letters in the column indicate significant differences among groups (P < 0.05) : (a)Antibiotic, (b)Manganese, (c) Bacteria , (d) Bacteria + antibiotic, (e) Bacteria + Manganese The value represent the mean ± standard error (n=6/group)							

Atherogenic Index: There was a significant increase in the atherogenic index in a quail (G4) compared with the control group and the other groups at 12 and 24 hours, 3, 7 and 30 days p.i, and with (G2) and (G3) at 60 days p.i. There was a significant increase in atherogenic index in (G6) compared with (G1) at 24 hours, 30 and 60 days p.i. A general increase was observed in atherogenic index at 90 days p.i, and this increase was positive in correlation with age (Table 6).

Table 6. Values of atherogenic index

DURATIONS / GROUPS	12 hrs. p.i.	24 hrs. p.i.	3 days p.i.	7 days p.i	30 days p.i.	60 days p.i.	90 days p.i.
CONTROL (G1)	1.35±1.09 b B	1.78±1.5 b B	1.39±1.77 b B	1.76±2.41 b B	2.89±2.81 b B	4.64±2.85 a B	7.90±6.06 a A
ANTIBIOTIC (G2)	1.56±1.09 b B	1.64±1.5 b B	1.34±1.77 b B	1.58±2.41 b B	2.69±2.81 b B	2.67±2.85 a B	7.81±6.06 a A
MANGANESE (G3)	1.14±1.09 b B	1.17±1.5 b B	0.92±1.77 b B	1.16±2.41 b B	2.69±2.81 b B	3.31±2.85 b B	6.34±6.06 a A
BACTERIA (G4)	2.37±1.09 b A	3.28±1.5 b A	5.23±1.77 a A	7.31±2.41 a A	6.82±2.81 a A	4.85±2.85 a A	8.43±6.06 a A
BACTERIA + ANTIBIOTIC (G5)	1.23±1.09 b B	1.78±1.5 b B	1.87±1.77 b B	2.05±2.41 b B	2.57±2.81 b B	4.52±2.85 a A	7.31±6.06 a A
BACTERIA + MANGANESE (G6)	1.3±1.09 b B	2.64±1.5 b B	1.54±1.77 b B	2.36±2.41 b B	4.3±2.81 a A	5.28±2.85 a A	6.79±6.06 a A
The value represent the mean ± standard error (n=6/group) *Significantly different from the control value (P < 0.05) Letters in the column indicate significant differences among groups (P < 0.05) : (a)Antibiotic, (b)Manganese, (c) Bacteria , (d) Bacteria + antibiotic, (e) Bacteria + Manganese							

Macroscopic changes of aorta: When a vertical open of aorta there were appearance of fatty streaks on the lining of the aorta at periods of 30, 60 and 90 days p.i in quail groups (G4), (G5) and (G6) compared with (G1), (G2) and (G3).

Microscopic changes of aorta (figures 1, 2 and 3): Aortic sections showed slight changes for the period of 12 hours p.i represented by hypertrophy and proliferation of endothelial cells and hypertrophy of vascular smooth muscle cells VSMCs in the (G4), in addition to the presence of bacterial colonies of *E.faecalis* as dark blue cocci on the endothelium in groups (G4), (G5) and (G6). The histopathological lesions at 24-hour and 3 days p.i were more severe and represented by hypertrophy and hyperplasia of endothelial cells and localization of fatty vacuoles in intimal layer

and hypertrophy and the proliferation of VSMCs toward intima in the group (G4). These lesions also appeared in (G5) and (G6) in addition to the localization of the fatty vacuoles and foam cells in intimal and medial layers.

The lesions were very severe at 7 days p.i which were represented by hypertrophy and hyperplasia of endothelial cells, intensive localization of fatty vacuoles and foam cells in all layers of the aorta, accumulation of platelets, red blood cells and fibrin composed of recent thrombus adherent to endothelium, proliferation of VSMCs, fragmentation of elastic fibers and infiltration of inflammatory cells in group (G4). These lesions also appeared but were less severe in the groups (G5) and (G6) compared to the group (G3), (G1) and (G2).
The lesions retreated at 30 days p.i which were represented by the localization of foam cells in all layers of the aorta and proliferation of VSMCs. The histopathological changes were more severe at the period of 60 and 90 days p.i, comparing to the period of 30 days p.i in group (G4) which were represented by hypertrophy and hyperplasia of endothelial cells, intensive localization of fatty vacuoles and foam cells in all layers of the aorta and proliferation of VSMCs. These lesions were also observed but were less severe in the groups (G5) and (G6), localization of fatty vacuoles and foam cells were present in the groups(G1) and (G2), while there were no histopathological changes in the group (G3) which showed an improvement compared with the control group.

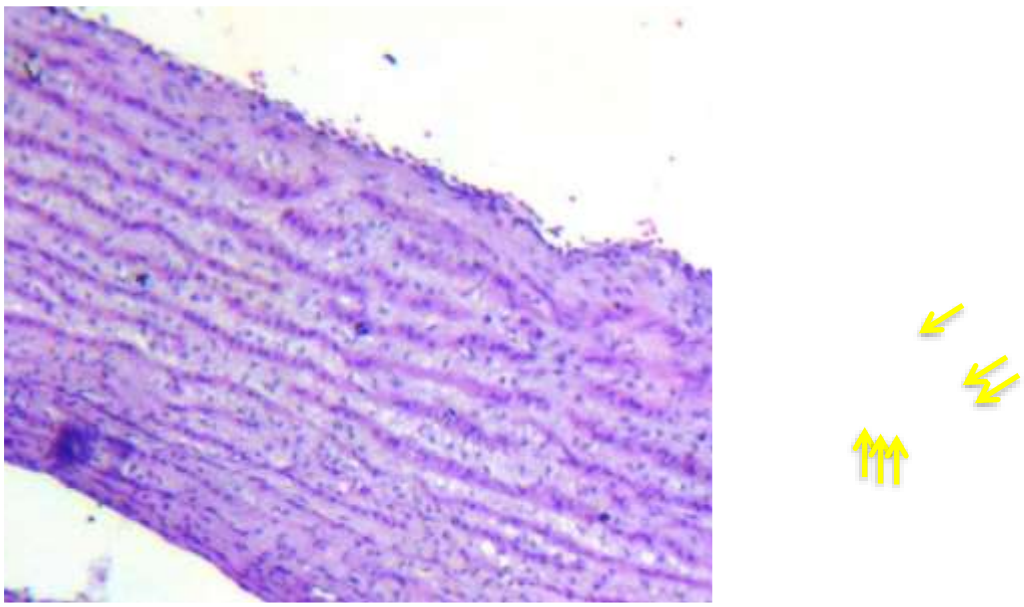

Figure 1. Aorta section of quail from (G5) at 3 days PI. shows hypertrophy and hyperplasia of endothelial cells ↑, localization of fatty droplets (foam cells) in intimal and medial layers ↑↑ and proliferation of VSMCs toward intima ↑↑↑. H&E stain. 210X

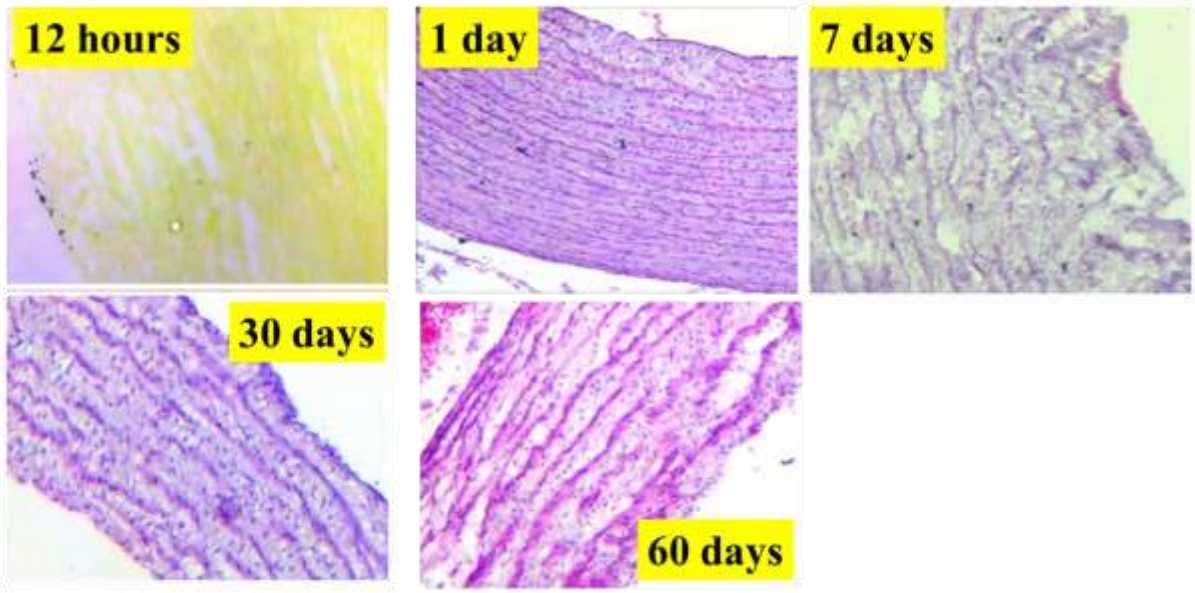

Figure 2. Aorta section of quail from (G4) at different time points. (12 hours) shows the presence of bacteria colonies of E. faecalis in blue - black color cocci in endothelium ↑, Gram's tissue modified stain. (1day) shows the hypertrophy of the endothelial cells ↑, localization of fatty droplets inintima ↑↑ and hypertrophy and proliferation of VSMCs toward intima ↑↑↑. (7 days) shows intensive localization of foam cells in all layers ↑, adhesion of recent thrombus on endothelium ↑↑, proliferation of VSMCs ↑↑↑, fragmentation of elastic fibers and infiltration of inflammatory cells. (30 days and 60 days) shows hypertrophy and severe hyperplasia of the endothelial cells ↑, localization of foam cells in all layers ↑↑, and proliferation of VSMCs ↑↑↑. H&E stain.

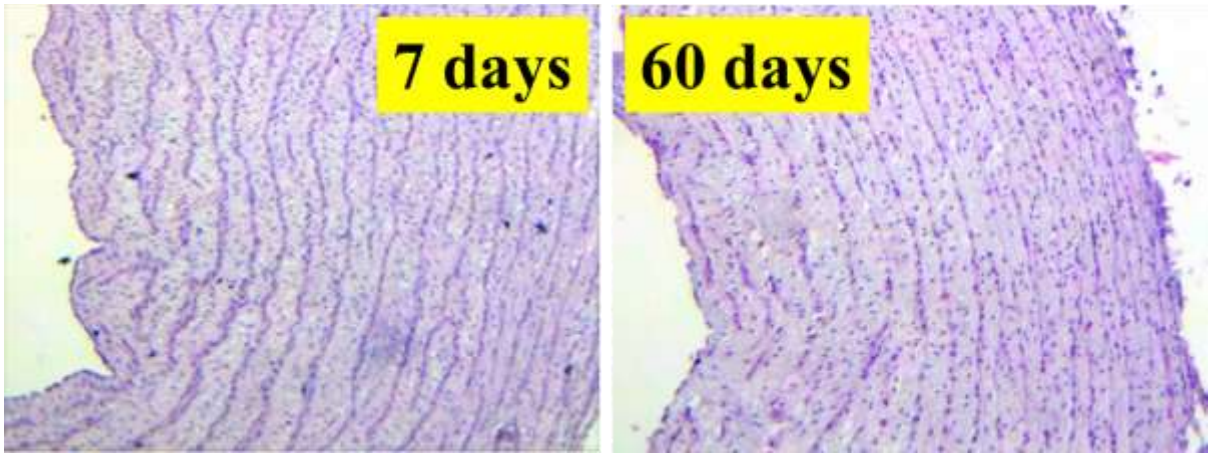

Figure 3. Aorta section of quail from (G3). (7 days) shows the absence of pathological changes compared with (G1) and the other groups. (90 days) shows the absence of pathological changes compared with (G1) and the other groups.. H&E stain. 210X

DISCUSSION

Natural intestinal flora include many types of positive and negative gram bacteria in mammals and birds, which include enterococci bacteria. Enterococci is located in a common and widespread environment of poultry and is considered an important pathogen in conditions of stress; immunosuppression, therefore, may interfere with many diseases (17,18). The results of the bacterial isolation appeared after bacterial biochemical tests through analytic profile index API 20 Strep system, and the confirmed diagnosis by identifying the species of enterococci which was *E.faecalis*, this species being considered the predominant in most animals and poultry. This result agrees with many research studies (7,19, 20, 21).

We selected three concentrations of bacterial suspensions of *E.faecalis*, at 10^5 and 10^6 and 10^7 CFU/ml based on previous studies of experimental infection of *E.faecalis*, in which the researchers reported that the lethal dose LD_{50} of this bacteria in mice is 3.2 x 10^8 CFU - 2.2 x 10^8 CFU by i.p injection inducing mortality after 24-36 hours (22). In another model of neutropenic Swiss Webster mice the lethal dose LD_{80} of *E.faecalis* was 10^7 CFU (23). In another study, rats were injected with *E. faecalis* strain HH22 concentration of 5 x 10^5 CFU inside the heart to induce endocarditis (24). Moreover, injection of bacterial suspensions of *E.faecalis* at 10^8 CFU intra ear vein in rabbits led to the occurrence of endocarditis (25).

In other studies, rats were injected with *E.faecalis* at a concentration of 3 x 10^7 CFU/ml and of 7.8 x 10^7 CFU/ml of i.p, and this induced lesions of atherosclerosis and endocarditis (6,7). Through these studies, it was the adoption of different concentrations of *E.faecalis* bacterial suspension which caused infection in quails, and we conducted these three concentrations in our study, which was designed to determine the appropriate dose of bacterial suspension to be used in the main experiment.

Results revealed the appearance of clinical signs and symptoms in the (G4), which are similar to the clinical signs and symptoms recorded in *E. faecalis* infection in chicken in acute phase (21, 26, 27) and in rats (6). Lethargy, lack of activity, and ruffled feathers are general symptoms that occur with most bacterial infections or in oxidative stress. The lack of egg production may be attributed to the stress caused by *E.faecalis* through producing superoxide or by reactive oxygen species ROS produced by immune cells in the event of mechanism of phagocytosis of bacteria, as studies indicate that quails exposed to heat stress experience disturbances in ovulation as a result of the lack of releasing LH Luteinizing hormone from the pituitary gland (28).

The inability to move in individual cases of quails may be due to the transmission of *E.faecalis* through the blood to the joints and the incidence of amyloidosis arthropathy is the causative agent in chickens (29,30), while for torticollis in one of the birds may be due to transmission of the bacteria to the brain and induction of encephalomalacia as it happens in small chicks of chicken infection (26,27). The appearance of clinical signs after 1 and 3 days PI and then its disappearance after this period may be attributed to the effectiveness of the bacteria after injection i.p and transmitted to the bloodstream and then disappearing as a result of the activation of the immune system and defense mechanism of quails in (G4) against these bacteria. Furthermore, the quails have the ability to resist many of the infections that infect other birds which could refer to the possession of a defensive and resistant immune system (31, 32).

The results showed an improvement and an increase in the production of eggs in the (G3) compared with the control group and the other groups, and this is consistent with numerous studies indicating the role of manganese with other metals to improve the production and quality of eggs in quails and chickens when those metals are added to the feed (33).

Results of lipids profile in the serum of quails showed a significant increase in the levels of total cholesterol, triglycerides, LDL-c, VLDL-c and atherogenic and a significant decrease in the level of HDL-c in the group (G4) compared with the (G1) and other groups, especially in the periods 3, 7 and 30 days PI. Since the quails are susceptible to the induction of lesions of atherosclerosis, they are also susceptible to the high lipid levels in blood (34, 35), which can be attributed to virulence factors produced by *E.faecalis*, such as hemolycin (36) and superoxide. Hemolycin is an enzyme leading to damaged cells through degradation of membranes rich in lipids as well as the decomposition of red blood cells, leading to further release of lipids and increase in plasma levels (20, 37). The superoxide produced by *E.faecalis* is a reactive oxygen species ROS, which leads to modifications in proteins, nucleic acids and lipids, and consequently produces tissue damage. The production of superoxide by *E.faecalis* is more common in *E.faecalis* strains which is considered unique to this species of bacteria and one of the important virulence factors in its pathogenicity (10, 11). The significant increase of lipid levels were at their peak during the interval periods of 3, 7 and 30 days. PI may be attributed to the production of virulence factors by *E.faecalis* especially superoxide at the stage of bacteremia in these periods, which leads to endothelial cell injury and the destruction of cell membrane, and then releases phospholipids. This contributes to the increased production of ROS of quail and oxidation of lipoproteins that promote oxidative stress (7,38). The ROS either produced by bacteria or by host phagocytic cells and endothelial

cells are responsible for lipids peroxidation of lipoprotein of cellular membranes which are exposed heavily to lipids peroxidation due to the fact they contain high concentrations of unsaturated fatty acids (39). This oxidation leads to produce lipid hydroperoxide LOOH then it hydrolyze to aldehyde such as Malonaldehyde MDA and the consequences are the production of linkage membrane proteins and changes in fluidity of membranes and the formation of more lipoproteins, which appear in high levels in serum (40, 41).

The accumulation of LDL-c beneath endothelial cells of the aorta induced by superoxide actions leads to the oxidation of LDL-c molecules to oxidative ox-LDL and this in turn produces many Oxysterol that have the capability to inhibit the activation of the LDL-c receptors and stimulate acyl-co A cholesterol acyltransferase enzymes leading to increased cholesterol esters and thus increase the levels of lipid in the blood (38, 42, 43, 44, 45).

The results showed a low level of HDL-c in the blood serum of the group (G4) with a significant difference in periods 12 hours, 1, 3, 7, 30 and 60 days PI especially in the two periods 7 and 30 days PI comparing with the all groups. Research has proven that excess cholesterol in blood leads to a low level of HDL-c (46). Moreover, the migration of monocytes induced by ox-LDL was inhibited by antioxidants and HDL-c, which prevent oxidative modifications of LDL-c through two paths: the first can catch prooxidant compounds, and the second can replace the ox-LDL with HDL-c. Furthermore, HDL-c has an important role in the metabolism of cholesterol through the reverse cholesterol transport and hence HDL-c acts to inhibit lipid oxidation (47). This relationship is inverse when levels of LDL-c and VLDL-c are high; the level of HDL-c is low, especially during the following two periods: 7 and 30 days after infection. As it was observed in the two interval periods of 7 and 30 days PI, there was a significant increase of lipids (total cholesterol, triglycerides LDL-c and VLDL-c) in group (G4) at its peak in these two periods compared with all groups.

Levels of lipids in serum of quail treatment with manganese (G3) approached the values of the control group at all intervals, and a significant decrease was recorded in total cholesterol levels at intervals 3 and 7 days compared with (G1) and a significant decrease in LDL-c in the period of 7 days compared with (G1). The reason for this result may be due to the role of manganese as an antioxidant in the prevention and reduction of the increased lipids in plasma by inhibiting the lipids peroxidation (48, 49). This corroborates the results of another study (50), where adding manganese 120 mg / kg to the diet alone or with chromium in quails exposed to heat stress led to a reduction in total cholesterol levels in blood plasma. Our result as well agrees with studies (51, 52) about the role of manganese in reducing lipid levels in the serum of chickens exposed to heat stress when this metal was added to the diet in amount of 240 mg / kg, suggesting the role of manganese involved in the metabolism of lipids in chicken. Other studies also confirmed that adding manganese to the other metals vitamin E, and selenium in chickens improved the action of the enzyme superoxide dismutase SOD in the liver, heart, kidney and brain, reducing the level of lipids in the plasma (53). However, in our study, perhaps a dose of manganese which was given in the group (G6) was minimal, which is why we suggest subsequent studies on preventive doses of manganese in quails.

Results showed a general increase in the levels of total cholesterol, triglycerides, LDL-c, VLDL-c and atherogenic index and low level of HDL-c in all groups including control group (G1) after periods of 60 and 90 days PI, and these were in direct correlation with age. The increase in the levels of lipids was also proportional to age. This confirms the sensitivity of the quails to the high level of lipids and atherosclerosis, and this result matches studies which showed that the lipid levels increased in aging in quails (54, 55, 56). A study by Shih et al. (1983) proved that in quails susceptible to atherosclerosis, the lesions develop at the age of 8 weeks, whereas in other breeds of quails, atherosclerosis developed at the age of 12 weeks, and this is consistent with what we achieved by increasing the level of lipids after two periods in all groups including (G1). The study of (57) revealed that injury of the arteries and changes in the level of cholesterol become severe over time in quails. The correlation between levels of blood lipids and atherosclerosis in quails with age progression needs to be further studied.

The appearance of fatty streaks on the lining of the aorta at periods of 30, 60 and 90 days PI in in quail groups (G4), (G5) and (G6) compared with (G1), (G2) and (G3) confirms the induction of atherosclerosis in the aorta, which is concomitant to the biochemical results of significant elevations of lipids in serum, and a significant decrease in the level of HDL-c in those periods, indicating the occurrence of atherosclerotic lesions in the aorta.

The microscopic changes of aorta showed the induction of atherogenic lesions in (G4) after 24 hours PI, and they were severe after 7 days PI. The appearance of aortic lesions in quails confirms the sensitivity of these birds to the occurrence of atherosclerosis (20,21) which are consistent with studies that proved the occurrence of primary lesions of atherosclerosis *E.faecalis* infection through the events of oxidative damage in chickens (21) and rats (6,7). *E.faecalis* possesses many virulance factors like superoxide, lipotechoic acid LTA, aggregation substances AS, cytolycin, hyelorenidase and lipase (11), which facilitate the spread of bacteria in the tissues of the host, especially hyelorenidase factor (spread) and enable the induction of primary lesions of atherosclerosis, as well as bacteria that stimulate the release of cytokines and chemokines, leading to more injury, characterized by an inflammatory response. The lesions attributed to injury of the endothelium of the aorta resulting from oxidative stress by producing superoxide of bacteria which causes change in the permeability of endothelial cells associated with the inflammatory response against bacterial invasion, and which contributes to the activation of polymorph nuclear cells PMNCs and mono nuclear clls MNCs which accumulate at the site of injury and produce cytokines and chemokines (interleukins and tumor necrosis factor TNF-a), and which lead to attract more inflammatory cells which contribute to additional injury to the vessel wall where it produce ROS which exacerbate oxidative stress. There is a consensus that atherosclerosis represents a state of increased oxidative stress, which is characterized by oxidation of lipids and proteins in the vascular wall. According to the hypothesis of the oxidative modification, the LDL oxidized early to oxidized ox-LDL, which contributes to the progression of atherosclerotic lesions (38, 58).

The potential pathogenesis occurs through the transmission of bacteria in the blood vessels through the bloodstream after injected i.p in groups (G4), (G5) and (G6), which were confirmed by the presence of bacteria in an intimal layer of aorta in 1 and 3 days PI and in the medial layer in day 7 PI observed by gram's tissue modified stain (Brown and Bren). As mentioned previously, the virulence factors of *E. faecalis*, change the anticoagulant phenotype of endothelial cells to procoagulant (11, 59) such as aggregation substances AS, which adhere bacteria to endothelial cells (60).

Another dysfunction which is the expansion of the endothelial cells, as well as the cytokines, chemokines, and adhesion molecules, contributes to the injury of the endothelial cells and cellular events of inflammatory responses. The virulence factor surface adhesions enable bacteria to activate and accumulate the platelets and then adhere to each other as well as IgG and immune response that stimulates cytokines and proinflammatory substances, which activate platelets (61), as well as the response inflammatory active and platelet activating factor PAF which facilitates accumulation of platelets and formation of micro thrombi in location of injury (62).

Another principal effect of *E.faecalis* is to stimulate the proliferation of VSMCs by stimulating the production of growth factors GF or increasing the expression of growth factor receptors that include fibroblast growth factors FGF, epidermal growth factors EGF, and platelet derived growth factors PDGF, which stimulate the synthesis of DNA and proliferation of endothelial cells and VSMCs (63).

The foam cells characterize lesions of atherosclerosis which is a formation and accumulation of fatty droplets within the cytoplasm and between VSMCs and mononuclear cells as a result of oxidation and localization lipoprotein of LDL-c by ROS, and which leads to the occurrence of the injury in the lining of the blood vessel resulting in an imbalance in the permeability of the endothelium which increases the permeability of the plasma proteins such as LDL-c, albumin and fibrinogen, making it easier to combine LDL-c in the vessel wall after its association with extracellular proteins such as collagen and glycoseamin. It may also be the result of a low hydrolyzing of esters of cholesterol in the cytoplasm and lysosomes. Oxidation of LDL-c by mononuclear cells occurs due to the presence of attractive receptors on their surface and thus consists of foam cells and stimulates VSMCs in releasing extracellular proteins which include proteoglycanes, collagen, and elastic fibers that are involved in the formation of lipid droplets, increasing the action of scavenger receptors on the surface of mononuclear cells,which is an additional mechanism for the pathogen in the lipids accumulation (64, 65).

There were increased and irregular collagen fibers appearing in the intimal and medial layers of aorta, and also fragmentation of elastic fibers in (G4) after 7 and 30 days PI, with the progress of lesions of atherosclerosis, which is due to deposition of extracellular matrix ECM in intima, which is rich in fibers of collagen and elastin, and which

were deposited as a network of thin collagen fibers around VSMCs or basal lamina in the initial phase of atherosclerosis, leading to accumulation of more fibers; while in the stage of fiberatherma the collagen fibers are thick in the intima between the foam cells (66). Collagen fiber growth continues through the development of atherogenic plaques and it contributes to 60% of the total protein content. Collagen type I is the highest amount of about 70 % and is associated with collagen type III while the collagen type V increases with the lesion (67). The elastin lamellea were fragmented in more advanced lesions of atherosclerosis which is due to disruptions in elastic fibers in the media (68), where there are less amounts of elastic fibers in the progress of plaques which is due to deposition of elastin with calcium ions which are affected by enzymes hydrolyzed elastin (66).

VSMCs considered prevalent cells within the cells of atherosclerosis lesions which include macrophages laden lipids, lymphocytes and endothelial cells. The functional role of VSMCs in atherosclerosis can be summed by phenotyping transformation from (quiescent) contracting cells to the active (synthetic) cell formation of ECM, and proliferation, migration and releasing of inflammatory mediators that include interferon, interleukins, TNF-a, and different kinds of growth factors and production of ROS (69, 70, 71, 72).

The results of our study showed that the microscopic changes of the aorta were more severe after 60 and 90 days PI, compared with the early periods in experiment in the (G4) and less severe in the (G5) and (G6) as well as localization of lipid droplets in the (G1) and (G2), while this did not appear in (G3), as compared to the (G1) after 90 days PI. This was attributed to the development of lesions of atherosclerosis with age progression in conjunction with significant in increase lipids in plasma. Localization of fatty droplets in the aorta of groups (G1) and (G2) indicate the sensitivity of quails in regard to the induction of primary lesions of self-atherosclerosis and confirmed a high significant level of lipids in all quail groups, including the control group (G1) after 90 days of the experiment (111 days old), but that a group treated with manganese alone (G3) showed an improvement compared with (G1). These findings are evidence of manganese as an essential metal and an antioxidant in the protection of primary lesions for atherosclerosis.

REFERENCES:

1. Fruchart JC, Duriez P. Fundamental data on atherosclerosis. InAnnales d'endocrinologie 2001 Feb 1 (Vol. 62, No. 1 Pt 2, pp. 93-100).
2. Zachary JF, McGavin MD, editors. Pathologic Basis of Veterinary Disease5: Pathologic Basis of Veterinary Disease. Elsevier Health Sciences; 2012.
3. Cheville NF. Cell pathology. Iowa State University Press; 1983.
4. Ruef J, Peter K, Nordt TK, Runge MS, Kübler W, Bode C. Oxidative stress and atherosclerosis: its relationship to growth factors, thrombus formation and therapeutic approaches. Thrombosis and haemostasis. 1999;82(S 01):32-7.
5. Morré SA, Stooker W, Lagrand WK, Van Den Brule AJ, Niessen HW. Microorganisms in the aetiology of atherosclerosis. Journal of clinical pathology. 2000 Sep 1;53(9):647-54.
6. Al-Kennany ER. Role of Oxidative Stress Associated with Experimental *Enterococcus faecalis* Infection for Initiation and Development of Atherosclerosis in Rat. Iraq. J. Vet. Sc.; 2004; 18 : 135-144.
7. Hassan H.H.O. (2006). The pathology of vegetative endocarditis and atherosclerosis induced experimentally in rats. Master thesis, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.
8. Ott SJ, El Mokhtari NE, Musfeldt M, Hellmig S, Freitag S, Rehman A, Kühbacher T, Nikolaus S, Namsolleck P, Blaut M, Hampe J. Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary heart disease. Circulation. 2006 Feb 21;113(7):929-37.
9. Huycke MM, Joyce W, Wack MF. Augmented production of extracellular superoxide by blood isolates of Enterococcus faecalis. Journal of Infectious Diseases. 1996 Mar 1;173(3):743-5.
10. Gilmore MS, Clewell DB, Courvalin P, Dunny GM, Murray BE, Rice LB, editors. The enterococci: pathogenesis, molecular biology, and antibiotic resistance. Washington, DC: ASM press; 2002.
11. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. Clinical microbiology reviews. 1994 Oct;7(4):462-78.

12. Tsimikas S, Palinski W, Witztum JL. Circulating autoantibodies to oxidized LDL correlate with arterial accumulation and depletion of oxidized LDL in LDL receptor–deficient mice. Arteriosclerosis, thrombosis, and vascular biology. 2001 Jan;21(1):95-100.
13. Kita T, Kume N, Minami M, Hayashida K, Murayama T, Sano H, Moriwaki H, Kataoka H, Nishi E, Horiuchi H, Arai H. Role of oxidized LDL in atherosclerosis. Annals of the New York Academy of Sciences. 2001 Dec;947(1):199-206.
14. Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC. Veterinary microbiology and microbial disease. Blackwell science; 2002.
15. Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. Epidemiology & Infection. 1938 Nov;38(6):732-49.
16. Luna LG. Manual of histological staining methods of the armed forces institute of pathology. 3rd ed. New York : Mac Graw Hill Book Company. 1968; 38-76.
17. Diveriese L, Van de kerchove A, and Scheifer H. Characterization and identification of Enterococcus spp. Isolated from animals. Int J System Bacteriology. 1987 ; 37,251-259.
18. Kahn C. and Line S. The Merck Veterinary Manual. 10th Edition ; Merck Sharp & Dohme Corp., asubsidiary of Merck & Co.,Inc.Whitehouse Station, NJ USA. 2011; 978-0-911910-93-3.
19. Facklam, R.R. and Teixeria, L.M. (1997). Enterococcus. In : Collier, A., Balow, S. and Sussman, M., (Eds.), Microbiology and Microbial infections. Topiey and Wilson, l9th ed., Edward Arnold, London, PP. 669-682.
20. Hanno GN. (2002). A Study on Diagnosis and Pathogenicity of Enterococcus Isolated from Urinary Tract Infection and Its Ability to Cause Experimental Endocarditis., Ph. D. Thesis, College of Science, University of Mosul, Mosul, Iraq.
21. R Al-Kenany E. Effect of taurine and vitamine E in treating atherosclerosis induced experimentally by hydrogen peroxide in rabbits. Iraqi Journal of Veterinary Sciences. 2005 Jun 28;19(1):45-56.
22. Schlievert PM, Chuang-Smith ON, Peterson ML, Cook LC, Dunny GM. Enterococcus faecalis endocarditis severity in rabbits is reduced by IgG Fabs interfering with aggregation substance. PloS one. 2010 Oct 4;5(10):e13194.
23. Onyeji CO, Nicolau DP, Nightingale CH, Bow L. Modulation of efficacies and pharmacokinetics of antibiotics by granulocyte colony-stimulating factor in neutropenic mice with multidrug-resistant Enterococcus faecalis infection. Journal of Antimicrobial Chemotherapy. 2000 Sep 1;46(3):429-36.
24. Hindes RG, Willey SH, Eliopoulos GM, Rice LB, Eliopoulos CT, Murray BE, Moellering Jr RC. Treatment of experimental endocarditis caused by a beta-lactamase-producing strain of Enterococcus faecalis with high-level resistance to gentamicin. Antimicrobial agents and chemotherapy. 1989 Jul;33(7):1019-22.
25. Dubé L, Caillon J, Jacqueline C, Bugnon D, Potel G, Asseray N. The optimal aminoglycoside and its dosage for the treatment of severe Enterococcus faecalis infection. An experimental study in the rabbit endocarditis model. European journal of clinical microbiology & infectious diseases. 2012 Oct;31:2545-7.
26. Saif YM, Fadley AM, Glisson JR, McDougald LR, Nolan LK, and Swayne DE. (2008). Diseases of Poultry, 12th ed. Blackwell Publishing Professional, 2121 State Avenue, Ames, USA.
27. Aiello SE, Moses MA, Lane KAG, Schindler ST, Short SC, Steigerwald MA. (2012). Overview of Enterococcosis in Poultry. The Merck Veterinary Manual ; Merck Sharp & Dohme Corp., asubsidiary of Merck & Co.,Inc.White house Station, NJ USA. http://www.merckmanuals.com.
28. Donoghue DJ, Krueger BF, Hargis BM, Miller AM, Halawani ME. Thermal stress reduces serum luteinizing hormone and bioassayable hypothalamic content of luteinizing hormone-releasing hormone in hens. Biology of reproduction. 1989 Sep 1;41(3):419-24.
29. Landman WJ, vd Bogaard AE, Doornenbal P, Tooten PC, Elbers AR, Gruys E. The role of various agents in chicken amyloid arthropathy. Amyloid. 1998 Jan 1;5(4):266-78.
30. Landman WJ, Veldman KT, Mevius DJ, Van Eck JH. Aerosol transmission of arthropathic and amyloidogenic Enterococcus faecalis. Avian diseases. 2001 Oct 1:1014-23.
31. Shiina T, Shimizu S, Hosomichi K, Kohara S, Watanabe S, Hanzawa K, Beck S, Kulski JK, Inoko H. Comparative genomic analysis of two avian (quail and chicken) MHC regions. The Journal of Immunology. 2004 Jun 1;172(11):6751-63.

32. Shomali T, Mosleh N, Alaeddini A. Levamisole enhances cell-mediated immune responses and reduces shedding of H9N2 avian influenza virus in Japanese quails (Coturnix coturnix japonica). American Journal of Animal and Veterinary Sciences. 2012;7(1):16-20.
33. Mabe I, Rapp C, Bain MM, Nys Y. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poultry science. 2003 Dec 1;82(12):1903-13.
34. Iwasaki H, Oku H, Toda T, NASU T, MIYAGI T, CHINEN I. Increased cholesterol absorption by hyperlipidemia atherosclerosis prone (LAP) Japanese quail. Journal of nutritional science and vitaminology. 2000;46(5):235-9.
35. Li X, Schulte P, Godin DV, Cheng KM. Differential mRNA expression of seven genes involved in cholesterol metabolism and transport in the liver of atherosclerosis-susceptible and-resistant Japanese quail strains. Genetics Selection Evolution. 2012 Dec;44(1):1-1.
36. Furumura MT, Figueiredo P, Carbonell GV, Darini AL, Yano T. Virulence-associated characteristics of Enterococcus faecalis strains isolated from clinical sources. Brazilian Journal of Microbiology. 2006;37:230-6.
37. Van Tyne D, Martin MJ, Gilmore MS. Structure, function, and biology of the Enterococcus faecalis cytolysin. Toxins. 2013 Apr 29;5(5):895-911.
38. Al-Kennany ER. *A study on the capability of oxidative stress by hydrogen peroxide to induce atheromatous lesions experimentally in chickens* (Doctoral dissertation, Ph. D. College of Veterinary Medicine, University of Mosul, Mosul, Iraq).
39. Sorensen M, Sanz A, Gomez J, Pamplona R, Portero-Otin M, Gredilla R, Barja G. Effects of fasting on oxidative stress in rat liver mitochondria. Free Radical Research. 2006 Jan 1;40(4):339-47.
40. Beckman KB, Ames BN. (1997). Oxidative decay of DNA. J Biol Chem., 272:19633–19636.
41. Kate IE, Lucky OO. Effects of Vernonia amygdalina Del. extract on cholesterol level and lipid peroxidation status in rats given red dye adulterated palm oil diets. British Journal of Pharmaceutical Research. 2012 May 15;2(2):98-107.
42. Morin RJ, Peng SK. Effects of cholesterol oxidation derivatives on cholesterol esterifying and cholesteryl ester hydrolytic enzyme activity of cultured rabbit aortic smooth muscle cells. Lipids. 1989 Mar;24(3):217-20.
43. Jialal I, Freeman DA, Grundy SM. (1991). Varying susceptibility of different low density lipoprotein to oxidative modification. Arteriosel Thrombi. 11:482–488.
44. Khudiar KK. (2000). The role of aqueous extracts of olive (Olea europaca) leaves and garlic (Allium sativum) in ameliorating the effects of experimentally induced atherosclerosis in rats. PhD theses. College of Veterinary Medicine, University of Baghdad.
45. Al Attar HY, Jihad TW. (2012). Effect of Pimpinella anisum and Foeniculum vulgare Seeds Extracts in Protect From Oxidative Stress Induced By Hydrogen Peroxide in Swiss Albino Mice. Tikrit Journal of Pure Science, 17 (3):60-66.
46. Nenseter MS, Gudmundsen O, Malterud KE, Berg T, Drevon CA. Effect of cholesterol feeding on the susceptibility of lipoproteins to oxidative modification. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism. 1994 Jul 14;1213(2):207-14.
47. Jialal I, Devaraj S. Low-density lipoprotein oxidation, antioxidants, and atherosclerosis: a clinical biochemistry perspective. Clinical Chemistry. 1996 Apr 1;42(4):498-506.
48. Sziráki I, Rauhala P, Chiueh CC. Novel protective effect of manganese against ferrous citrate-induced lipid peroxidation and nigrostriatal neurodegeneration in vivo. Brain research. 1995 Nov 6;698(1-2):285-7.
49. Valachova K, Kogan G, Gemeiner P, Šoltes L. (2010). Protective eff ects of manganese(II) chloride on hyaluronan degradation by oxidative system ascorbate plus cupric chloride. *Interdisc Toxicol* 3, 26–34.
50. Gültekin M, Uyanik F, Güçlü BK, Eren M. EFFECTS OF CHROMIUM AND MANGANESE ON PERFORMANCE, EGG QUALITY AND SERUM LIPID LEVELS OF JAPANESE QUAIL EXPOSED TO HEAT STRESS. World Poultry Science Association (WPSA), 2nd Mediterranean Summit of WPSA, Antalya, Turkey, 4-7 October 2009. 2009:117-22.
51. Sands JS, Smith MO. Effects of dietary manganese proteinate or chromium picolinate supplementation on plasma insulin, glucagon, glucose and serum lipids in broiler chickens reared under thermoneutral or heat stress conditions. International Journal of Poultry Science. 2002;1(5):145-9.
52. Uyanik F, Atasever A, Eren M, Arik T. Effects of dietary manganese supplementation on performance, serum enzymes, glucose and lipids in broilers. Indian Veterinary Journal. 2004 May 1;81(5):537-40.

53. Öztürk-Ürek R, Bozkaya LA, Tarhan L. The effects of some antioxidant vitamin-and trace element-supplemented diets on activities of SOD, CAT, GSH-Px and LPO levels in chicken tissues. Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease. 2001 Jun;19(2):125-32.
54. Dalton MN. (2000). Effects of Dietary Fats on Reproductive Performance, Egg Quality, Fatty Acid Composition of Tissue and Yolk and Prostaglandin Levels of Embryonic Tissues in Japanese Quail (*Coturnix coturnix japonica*). Thesis of Master of Science in Animal and Poultry Sciences, Faculty of the Virginia Polytechnic Institute and State University, Virginia, USA.
55. Bavelaar FJ, Beynen AC. (2004). The Relation Between Diet, Plasma Cholesterol and Atherosclerosis in Pigeons, Quails and Chickens. International Journal of Poultry Scienc ; 3 (11): 671-684.
56. Krupakaran RP. Serum biochemical profile of Japanese quails (Coturnix coturnix japonica). Indian Journal of Fundamental and Applied Life Sciences. 2013;3(1):182-3.
57. FANN JI, ANGELL SK, CAHILL PD, KOSEK JC, MILLER DC. Effects of fish oil on arteriosclerosis in the Japanese quail. Cardiovascular research. 1989 Jul 1;23(7):631-8.
58. Stocker R, Keaney Jr JF. Role of oxidative modifications in atherosclerosis. Physiological reviews. 2004 Oct;84(4):1381-478.
59. Chuang ON, Schlievert PM, Wells CL, Manias DA, Tripp TJ, Dunny GM. Multiple functional domains of Enterococcus faecalis aggregation substance Asc10 contribute to endocarditis virulence. Infection and immunity. 2009 Jan;77(1):539-48.
60. McCormick JK, Hirt H, Waters CM, Tripp TJ, Dunny GM, Schlievert PM. (2001). Antibodies to a surface-exposed, N-terminal domain of aggregation substance are not protective in the rabbit model of Enterococcus faecalis infective endocarditis. Infect Immun. ;69(5):3305-14.
61. Rasmussen M, Johansson D, Söbirk SK, Mörgelin M, Shannon O. Clinical isolates of Enterococcus faecalis aggregate human platelets. Microbes and infection. 2010 Apr 1;12(4):295-301.
62. Okada H, Woodcock-Mitchell J, Mitchell J, Sakamoto T, Marutsuka K, Sobel BE, Fujii S. Induction of plasminogen activator inhibitor type 1 and type 1 collagen expression in rat cardiac microvascular endothelial cells by interleukin-1 and its dependence on oxygen-centered free radicals. Circulation. 1998 Jun 2;97(21):2175-82.
63. Boonanantanasarn K, Gill AL, Yap Y, Jayaprakash V, Sullivan MA, Gill SR. (2012). Enterococcus faecalis enhances cell proliferation through hydrogen peroxide-mediated epidermal growth factor receptor activation. Infect Immun. ;80(10):3545-58.
64. Salisbury BG, Falcone DJ, and Minick CR. (1999). Insoluble low-density lipoprotein- proteoglycan complex enhance cholesterol extra accumulation in macrophage. Am J Pathol. ; 20:6-11.
65. Ross R. Atherosclerosis—an inflammatory disease. New England journal of medicine. 1999 Jan 14;340(2):115-26.
66. Tanasković I, Lačković V, Gluvić Z, Lačković M, Nešić V, Stanković V, Labudović-Borović M, Radović S, Ašanin B. The influence of extracellular matrix composition on the pathogenesis of coronary atherosclerosis. Archives of Biological Sciences. 2011;63(2):333-43.
67. Katsuda S, Kaji T. Atherosclerosis and extracellular matrix. Journal of atherosclerosis and thrombosis. 2003;10(5):267-74.
68. Ayabe N, Suganuma E, Babaev R, Fogo A, Swift L, Linton MF, Fazio S, Ichikawa I, Kon V. (2004). Angiotensin II amplifies macrophage-driven atherosclerosis. Arterioscler Thromb Vasc Biol.; 24: 2143–2148.
69. Maytin M, Leopold J, Loscalzo J. Oxidant stress in the vasculature. Current atherosclerosis reports. 1999 Sep;1(2):156-64.
70. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The New England journal of medicine. 2000 Jan 1;342(3):154-60.
71. Ait-Oufella H, Taleb S, Mallat Z, Tedgui A. Recent advances on the role of cytokines in atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2011 May;31(5):969-79.
72. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. Nature Reviews Immunology. 2013 Oct;13(10):709-21.