

ISOLATION AND IDENTIFICATION OF *SALMONELLA* SPP. FROM FECES AND RUMEN OF FARM ANIMALS AND STUDY THE ANTIMICROBIAL SENSITIVITY TEST

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ABSTRACT

About 50 samples were collected from November 2016 to March 2017 in city of Basra , from healthy farm animals the samples were taken from feces and rumen of animals at different age (male and female) and study the antibiotics susceptibility for five different antibiotics.

. (six) isolates (12%) of *salmonella spp* were identified by using selective media (xylose medium and macConky agar), The identification of this bacteria was achieved by using morphological and biochemical characterization (Api20 E system). The total isolation was four samples (20%) from healthy goats, (10%) from sheep and (10%) from cows.All isolates are resistant for ampicillines and Bacitracin ,penicillines but of sensitivity to other antibiotics.

INTRODUCTION

Enteric pathogens are a major source of morbidity and mortality throughout the world. It has been estimated that there are more than 3 million deaths associated with Gram-negative enteric pathogens worldwide due to diarrhea and enteric fever each year. Bacteria of genera such as *Escherichia*, *Campylobacter*, *Vibrio*, *Brucella*, *Shigella*, *Yersinia*, and *Salmonella* are responsible for causing enteric diseases (1)

Those *Salmonella* organisms which discovered by Daniel salmon (1850-1914) survive the low-pH environment proceed to the lumen of gastrointestinal tract (GIT) organs, including the small intestine, colon, and cecum. Epithelial and immune cells lining these GIT organs provide the initial protective barrier against *Salmonella spp* in the gut. *Salmonella* competes with the gut microflora to make the initial contact with enterocytes or M cells to colonize the GIT (2,3). *Salmonella* is a persistent pathogen in the environment, able to easily survival and proliferate (4). The most commonly isolated serovars worldwide from various animal sources continue to be *S. Enteritidis* carriers in a wide variety of animal species(5)

Salmonella is one of the major zoonotic foodborne pathogens worldwide. It can cause a variety of clinical manifestations from mild gastroenteritis to bacteremia and typhoid fever. The global burden of nontyphoidal *Salmonella* gastroenteritis has been estimated to be 93.8 million cases of gastroenteritis each year, with 155 000 deaths(6)

Sheep and goats are the most numerous domestic livestock that are especially important in the extreme climates of the world. Small ruminants in Africa are noted for their ability to convert low opportunity cost feed into high value products, namely; meat, milk, fiber, manure and hides(7)

Salmonellosis is caused by many species of salmonella where the genus salmonella is a typical member of the family *Enterobacteriaceae* characterized by Gram negative, straight sided, rod shaped bacteria, all of *salmonella spp* are motile by peritrichous flagella(8)

Salmonella have a wide variety of domestic abattoir, animal and wild animal hosts. The disease in the meat animals including sheep and goats arises from intensive rearing practices, use of contaminated feed and water, and cross contamination of carcass during slaughtering operations, stress associated with prolonged deprivation of feed and water, transport of animals from rearing farm to abattoir crowding and prolonged lairage in pens, parturition and administration of certain drug predispose animal to infection (9)

MATERIALS AND METHODS

Sample collection:-

During December 5th 2016 until March 15th 2017, a total 50 samples were collected from different animals (cows, sheeps, goats, calves). About 40 were collected from feces by sterilized syringe impregnated in center of feces and about 10 samples from ruminant of cows. The surface of rumen was cutting by sterile dissecting instruments, a syringe was taken from rumen fluid samples as described by (10).

Isolation and identification of bacteria:-

All samples were cultured on primary medium (pepton water) and incubated at 37°C for 24 h. The samples were then cultured on XLD and macConkey agar and incubated at 37°C for 24 h.

Microscopic Examination :-

A slide was made by using pure isolates selected from selective medium, and stained with Gram stain, *Salmonella* isolates give Gram negative stain

Biochemical testes :-

- A. **Catalase test** :- The test was done by spreading single colony of bacteria from nutrient agar on a clean slide, Drops (1-2) of hydrogen peroxide 3% were then added. The production of the bubbles give a positive reaction.
- B. **Oxidase test** :- The test was performed by adding many drops of oxidase reagent (tetra methyl-P- phenyl di amine dihydrochlorid) on filter paper, A single colony from nutrient agar was transported by sterile stick and spreaded on the moistened filter paper with reagent. The purple color give positive result.
- C. **Motility test**:- Motility test was done on growing pure colony of nutrient broth which incubated at 37°C for 24 hs. One drop from them was taken and put on curved slide and covered with cover slide and tested under light microscope (11).
- D. **Api 20 system**:- Further confirmations were done by using API 20E test kit (BioMérieux, Inc., France). The plastic strips holding twenty mini-test tubes were inoculated with the saline suspensions of the cultures as described by manufacturer's directions.

1. Preparation of the inoculums:

After checking the Api purity of tested bacteria, the isolate with 18-24 hrs. growth was transferred 20E to medium mix well to prepare a homogenous bacterial suspension with a turbidity equivalent to (0.5 McFarlands standard).

2. preparation of the strip

An incubation box prepared by distribution about 5 ml of tap water to create a humid

atmosphere.

3. Inoculation of the strip

By using micropipette, The microtubes filled with inoculated medium, the tip of the pipette was placed against the side of the capsule (upper part) to avoid bubbles formation at the base of the tube. The capsules of sugars filled with mineral oils. The incubation box was then closed by the lid and incubated at 37°C for 18-24h **Antibiotic susceptibility test :-**

The antibiotic susceptibility test were done by agar disc diffusion method as described by (12).

The isolated colonies of *Salmonella* were selected from the agar plate culture. The top of each colony was touched with a loop and the growth was transferred in to a tube containing 5ml normal saline and the turbidity of the actively growing broth culture was adjusted to 0.5 MacFarland standards.

Sterile cotton swabs were dipped into adjusted suspension, rotated several times and pressed firmly on the slide on the tube above the fluid level to remove excess inoculum from the swab.

The dried surface of the Muller –Hinton agar (MHA) plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, to ensure an even distribution of the inoculum. The predetermined antimicrobial discs were dispensed on to the surfaces of the inoculated agar plate. Each disc pressed down individually to ensure complete contact with agar surface.

The plates were then placed in an incubator for 18hrs. at 37°C. the resulting zones of inhibition were uniformly with confluent of growth. The diameters of the zones of complete inhibition were measured including diameter of the disk. The size of inhibition zones were estimated as described by (13).

Table(1)Antimicrobial discs used in study.

Antibiotic discs	Assembly	Content(mcg)
Ampicillines	AM	25
Bacitracines	B	10
Rifampines	R	30
Clindamycines	C	2
Gentamicin	GEN	10
Cefodoximes	C	10

RESULTS

From 50 samples we obtained 6(12)% isolates of *Salmonella* (Table 2).These isolates characterized by using selective media (XLD medium and MacConkey medium) (Figure 1) and then by gram stain *salmonella* bacteria showed gram negative long bacilli or short rods, it was catalase positive ,oxidase negative , motile. This finding was confirmed by using API 20 E kit;(Figure 5) all isolates were presumed to belong to *Salmonella* revealed production indole, by ornithine decarboxylase that fermentation mannitol ,acid by fermentation of glucose and pale colony(non lactose fermenter) in maCconkey agar . the percentage frequency of bacterial isolates were higher.

Results of disc diffusion test are shown in (table 4) (Figure 7) *Salmonella* bacteria revealed resistant to ampicillines and cefodoxime but sensitivity to other antibiotics

Table(2) percentage frequency of *Salmonella* in faeces samples and rumen specimen.

Sample t type	Number of Sample	Number of Isolates	Percentage of isolates
Faeces samples	40	2	(10)%
Rumen	10	4	(20)%
Total	50	6	(12)%

Table (3)percentage frequency of *Salmonella* Spp. goat,cows and sheep.

Sample t type	Number of Sample	Number of Isolates	Percentage of isolates
Goat	20	4	(20)%
Cows	10	1	(10)%
Sheep	10	1	(10)%

Table(4) percentage frequency of *salmonella* according sex and age groups.

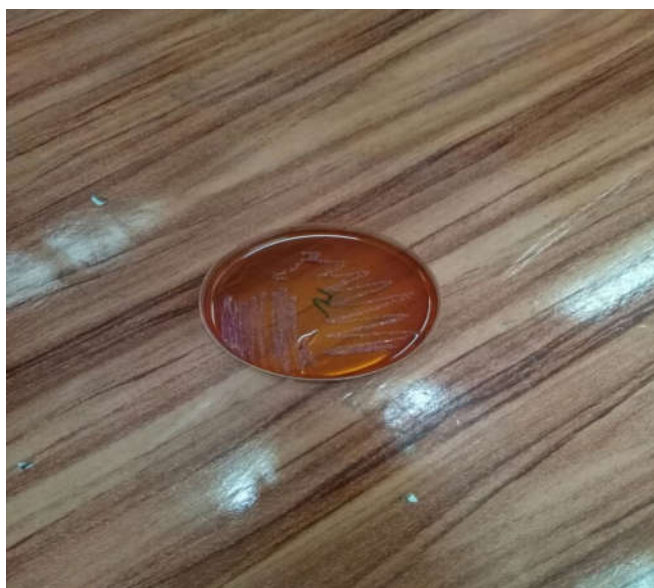
Age(month)	Number of samples	Percentage Occurance	Percentage occurance in male	Percentage occurance in female
(3m-12m)	25	n=7(28)%	N=15 n=4(26.6)%	N=10 n=3(30)%
(13m-18m)	15	n=5(33.3)	N=7 n=3(42.8)%	N=8 n =2(25)%
Total sample of bacterial isolates	40	n=12(30)%	N=22 n=7 (17.5)%	N=5(27.7)%

Table(5) Antibiotic sensitivity tests in millimeter against study isolates.

Bacterial	Ampicilline s	Bacitracin	Rifampin	Cefodoxim e	Clindamycine	Gentamicine
1-	0	0	0	0	2.7	1
2-	0	0	0	0	2.5	1
3-	0	0	5	0	2.5	2 7
4-	0	0	0	0	3	2
5-	0	0	1	0	3	2 5
6-	0	0	0.2	0	2	3 5



(A)



(B)

Figure(1) XLD medium, A,B for isolation *Salmonella spp* were appeared as small, red, round, smooth and convex .

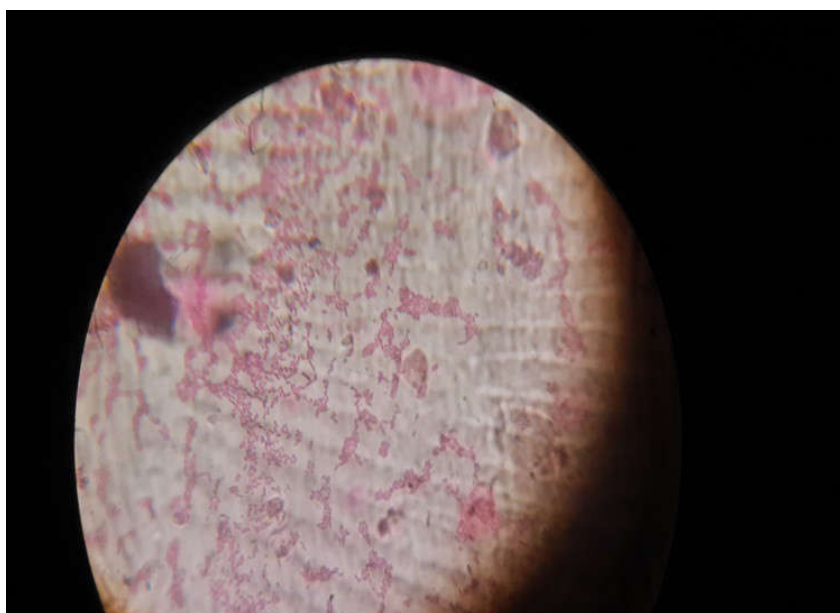


Figure (2):- Smear stained with grams stain showing bacilli cells like short rods ,singles, pairs.

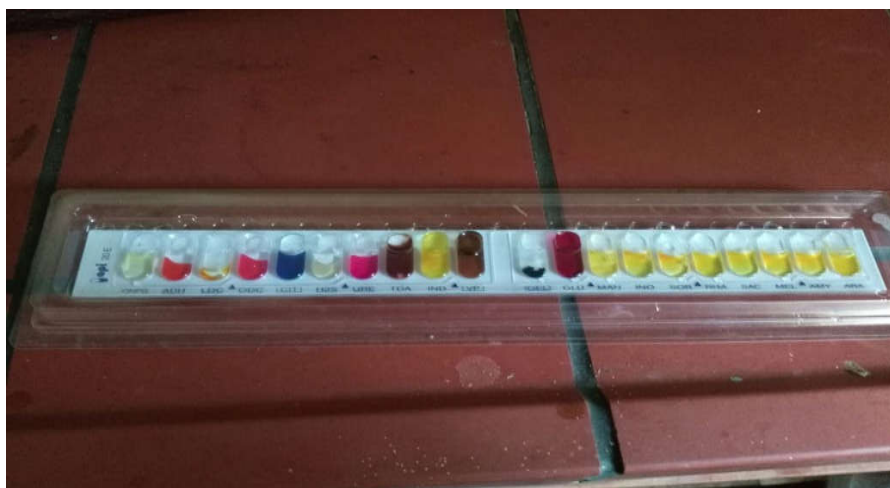


Figure (3):- Results of API 20 E system

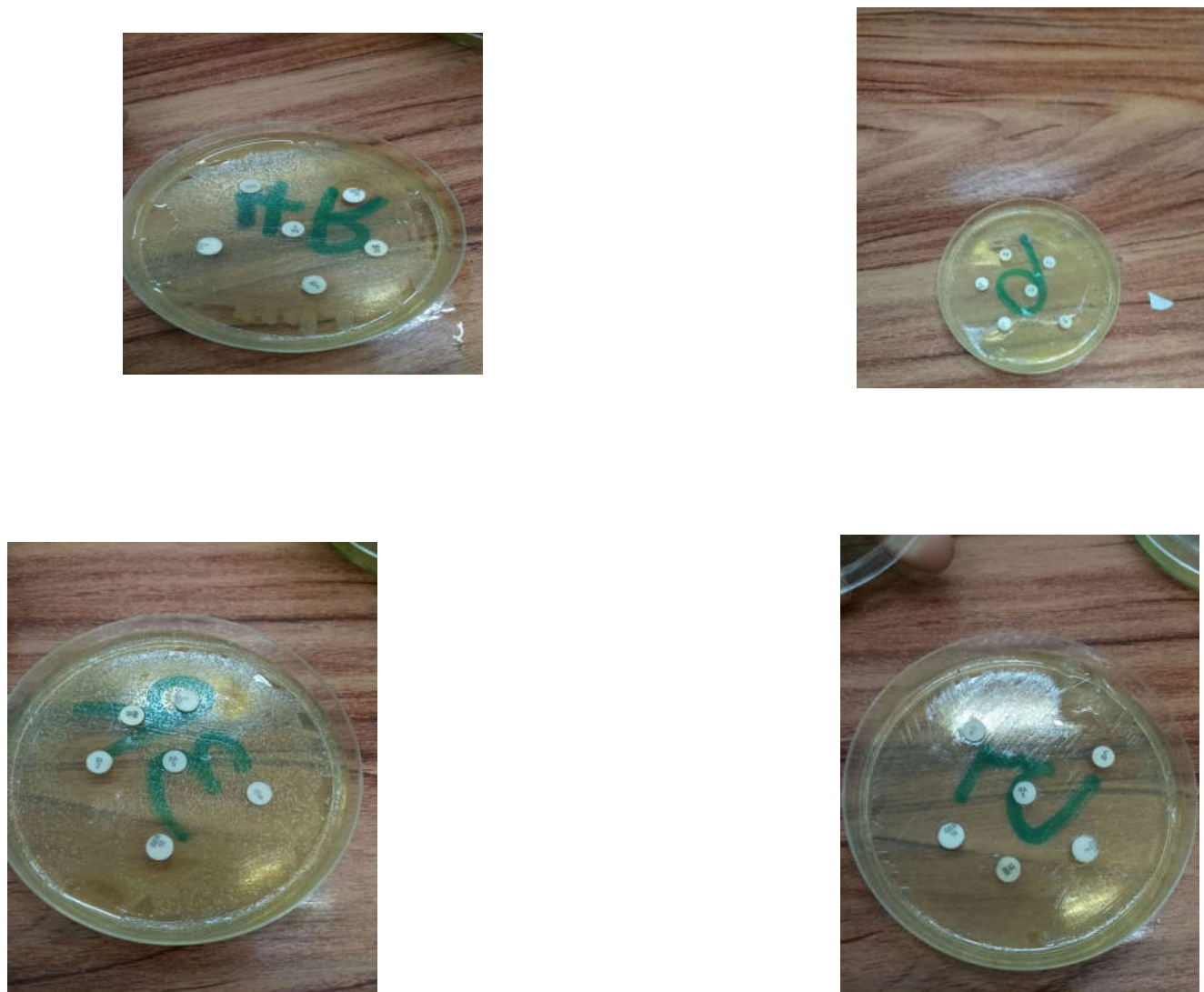


Figure (4) :- Antibacterial sensitivity test shows resistance and sensitive isolate

DISCUSSION

The current study revealed that the percentage frequency of *Salmonella* was(10%) in feces and (20%)in rumen(Table 2) and about(20%)from goat,(10%) cows,(10%)from sheeps (Table 3).This finding is in agreement with previous study(14) in which prevalence of *Salmonella* ranged 15.5% in sheep and 18.8% in goats.however,this finding disagreement with(15)in which higher prevalence of *Salmonella* in sheep (11.5%) as compared with goats (3%). The slight differences among the prevalence percentages might be the species differentiation,

hygienic, environmental and geographic variation and technical limitations of the laboratory of the study. The variety of *Salmonella* isolated from the samples of different animal species confirms the different source of contamination, making it even more interesting to study the different possible sources of contamination considering the variety of carrier individuals. In general, there is a high consistency between the literature reports and the findings of the present work. Thus, researchers of both human health and animal health should take appropriate precautions when working with *Salmonella* due to its different zoonotic potential and its role in public health, particularly when dealing with wildlife (16). responsible for cause Salmonellosis the term that used to designate a number of infections in domestic animals and responsible for or associated with a wide range of systemic and septicemia infections in various species of farm animals particularly ruminants. (17).

By using disk diffusion method, (six) isolates of salmonella were submitted for their anti microbial susceptibility toward 6 antimicrobials. Most isolates showed resistance to amoxicillines, bacitracines and cefodoximes but sensitive to clindamicines and gentamicines. These results were in agreement with previous study (12) who showed the necessity of invite such antibiotic sensitivity prior treatment.

RECOMMENDATION

The research recommends of the following:-

1. Studing serotype of *Salmonella* which determine the effective treatment and vaccination .
2. Molecular analysis for different clinical specimen collected from different animals to determine the distribution of *Salmonella* in different regions in Iraq.

عزل وتشخيص جراثيم السالمونيلا من كرش وبراز الحيوانات الحقلية ودراسة حساسية المضادات الحيوية

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الخلاصة

جمعت (٥٠) عينة من الحيوانات الحقلية (اغنام وابقار وعجول وماعز) سليمة للفترة من تشرين الثاني ٢٠١٦ لغاية اذار ٢٠١٧ ومن مناطق مختلفة من محافظة البصرة والعينات عبارة عن براز وكرش هذه الحيوانات. تمت دراسة الصفات الشكلية للجرثومة المعزولة واشكال المستعمرات للعزلات الجرثومية على الوسط الانتقائي (XLD

ووسط الماكونكي اكار) ودراسة الصفات الكيموحيوية واستخدام ايضا نظام API 20 E لتأكيد تشخيص الجرثومة وكذلك اختبرت مقاومة عزلات السالمونيلا مع (6) مضادات حيوية مختلفة وقد تمكنا من عزل الجرثومة من عينات الكرش وعينات البراز وتم الحصول على 6 عزلة (12%) . وكانت نسبة العزل الاجمالية (20%) من الماعز و(10%) من الابقار ونحو(10%) من الاغنام وقد اظهرت جميع عزلات السالمونيلا مقاومتها للامبيسلين والبيستراسين والبنسلين وجاءت بقية المضادات بعد ذلك على التوالي في تأثيرها على العزلات اذ كانت معظم العزلات حساسة لها وبنسب متفاوتة.

REFERENCES

- 1-Donnenberg, M.S.(2000). Pathogenic strategies of enteric bacteria. Nature 406:768 –774.
- 2-Ruby, T.;McLaughlin, L.; Gopinath, S.; Monack, D.(2012). Salmonella's long-term relationship with its host. FEMS Microbiol. Rev. 36:600 – 615.
- 3-Velge, P.; Wiedemann, A.; Rosselin, M.; Abed, N.; Boumart, Z.; Chausse ,A.M.; Grepinet ,O.; Namdari, F.; Roche, S.M.; Rossignol, A.; Virlogeux- Payant I. (2012). Multiplicity of Salmonella entry mechanisms, a new paradigm for Salmonella pathogenesis. Open Microbiol. 1:243–258.
- 4-Martelli, F. and R.H. Davies. (2012) Review Host Adapted Serotypes of *Salmonella Enterica*. *Salmonella Serovars* isolated from Table Eggs: An Overview. Food Res. Int. 45: 745-754.
- 5-Hohmann,E.L. (2001) Nontyphoidal Salmonellosis. Clin. Infect. Dis .32: 263-269.
- 6-Majowicz, S.E.; Musto, J.; Scallan, E.;Angulo, F.J.; Kirk, M.; O'Brien, S.J. ;Jones, T.F.; Fazil, A.; Hoekstra, R.M.(2010)The Global Burden of Nontyphoidal Salmonella Gastroenteritis.J. Clin. Infect. Dis. 50:882–889.
- 7-Wilsmore, T. (2006). Diseases of Small Ruminants in Ethiopia. The Veterinary Epidemiology and Economics Research Unit (VEERU) school of Agriculture policy and development, The University of read, UK pp: 6-7.
- 8-Varnam, A.H.; Evans, M.G. (1991) *Salmonella* In: Food Borne P. An Illustrated Text, Wolfe Publishing Ltd, Aylerbury, England pp: 51-85.
- 9-D'Aoust JY (1989) *Salmonellae* In: Doyle M (9th Ed) Food Borne Bacterial Pathogens. Marcel Dekker Inc New York, pp: 328-413.
- 10-López-Martín, J.I.;González-Acuña, D.;García, C.A.; Carrasco, L.O. (2016) Isolation and Antimicrobial Susceptibility of Salmonella Typhimurium and Salmonella Enteritidis in Fecal Samples from Animals. J Antimicro 2: 109. doi:10.4172/antimicro.1000109
- 11-Cruickshank, R., Duguid, J.P., Marimon, B.P. and Swain,R.A.H. (1975) Medical Microbiology. 2nd Edn. Vol. I, Church Hill, Livingstone, Edinburgh. p369-470.

- 12-Kirby, W.;M.M Bauer, A.W.;; Sherris, J.C. and Turck, M. (1966)** Antibiotic susceptibility testing using a standard single disc method. Am. J. Clin. Pathol. 45:493-496.
- 13-Imen,B.S.;Ridha,M.and Mahjoub,A.(2012).**Labratory typing methods of diagnostic of salmonella strains ,the old organisms that continued challenge salmonella-adangerous food borne pathogen,Dr.Barakat S,M(ed):ISBN:979-953-307-782-6,In Tech AAvailable from WWW.intechopen.com .
- 14-Woldemariarn, E. B.; Molla, D.; Alemayehu, and A. Muckle, (2005).** Prevalence and distribution of Salmonella in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia. Small Ruminant Res., 58: 19-24.
- 15-Wassie, M.A.,(2004).** A cross-sectional study on Salmonella in apparently healthy slaughtered sheep and goats at Addis Ababa and Modjo abattoirs, Ethiopia, Master thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia
- 16-López-Martín J, Junod T, Riquelme F, Contreras C, González-Acuña D (2011)** Detection of Salmonella and Mycobacterium species in Dominican gulls (*Larus dominicanus*) and gulls Franklin (Franklin's Gull) in the city of Talcahuano , Chile . Rev. Med Chile 139: 1496-1502.
- 17-Chandra, M.; Singh, B.R.; Shankar, H.; Agarwal, M., Agarwal, R.K.; Sharma, G. and N. Babu (2006).** Study on prevalence of Salmonella infection in goats. Small Ruminant Research, 65: 24-30.