Isolation and identification of *Pasteurella multocida* from healthy and clinical cases of sheep and study their antimicrobial sensitivity

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الخلاصة

اجريت هذه الدراسة في محافظة البصرة للفترة من كانون الاول 2017 لغاية نيسان 2018 حيث جمعت (75) عينة من الحيوانات الحقلية (اغنام) سليمة من مناطق مختلفة من محافظة البصرة واخرى مشكوك بها ومذبوحة في مجزرة القرنة حيث كانت العينات عبارة عن مسحات انفية ,انسجة رئوية واكباد مصابه .

اختبرت مقاومة عزلات *P. multocida* مع (5) مضادات حيوية مختلفة بعد ان تمت دراسة الصفات الشكلية للجرثومة المعزولة واشكال المستعمرات للعزلات الجرثومية على الوسط الانتقائي Clindamycin Gentamicin medium)) ودراسة الصفات الكيموحيوية واستخدام ايضا نظام API 20 E لتاكيد تشخيص الجرثومة. كانت نسبة العزل الاجمالية (33.3%) من المسحات الانفية ونحو(40%) من الرئات. لقد اظهرت النتائج ان نسبة الاصابة في الاناث كانت اكبر(38%) مما هي في الذكور(29.1%)..من ناحية اخرى بينت الدراسة ان جميع العزلات كانت مقاومة للامبسيلين والبستراسين والبنسلين .

Abstract

The study was entirely conducted in Basrah province which commenced from early of December 2017 until April 2018. Totally, seventy five samples were collected from the nasal passage of sheep's that randomly selected from the farms and from the lung and liver of slaughtered sheep at the Al-Qurnah abattoir. Five antimicrobial susceptibility testing were performed in order to identify *P. multocida*. Twenty one isolates (28%) of samples were identified as *P. multocida* through using selective media (Clindamycin Gentamicin medium). The morphological characterization identification of these bacteria was achieved using additional biochemical tests of Api20NE system. The total isolation was 33.3% from apparently healthy sheep and (40%) from the infected lungs. The frequency of bacterial isolates

was found to be slightly greater in female sheep (38%) than in male (29.1%). All isolates were observed to be resistant for three antibiotics comprised ampicillin, bacitracin, and penicillin.

Key Words: *Pasteurella multocida*, sheep, antimicrobial sensitivity, API 20E

Introduction

Pneumonic pasteurellosis, however, the most common example with a wide prevalence in ruminant animals. It is also known as respiratory mannheimiosis. The Respiratory tract infections disease, in its typical clinical form, is highly infectious, often fatal and with very serious economic impact in the animal industry, this disease common in various species of domestic and farm animals (1). It is well established that pneumonic pasteurellosis is responsible for the largest cause of mortality in feedlot animals in which the disease accounts for about 30% of total cattle death worldwide (2). The disease's global economic impact is well recognized and more than $1 billion are annually lost in beef cattle industry in north America alone. It has been shown that *Pasteurella multocida* is an important cause of the syndrome of animal disease known as pasteurellosis. In turkeys and chickens, *P. multocida* diseases include pneumonia in sheep, pigs, cattle, and acute septicemia **(**3). Pneumonic pasteurellosis, also known as respiratory mannheimiosis, is the most common example of ruminant animals with a wide prevalence. (2). *Pasteurella* is member of the family Pasteurallacea, which comprises three genera: *Pasteurella*, *Actinobacillus* and *Haemophilus* (4,5) *P. multocida* is non motile, non spore forming, short rod or coccobacillary (0.2 -0.4) by (0.5 – 2.5) mm in size . The Latin word multocidus consists of two sections or words, the first (multus) means many, and the next (cidus) means killer, so the whole multocida means the (killer for many animals) (6). Members of this species are responsible for a number of infections that are usually secondary to upper respiratory tract colonization, including avian cholera (in fowl, chickens and turkeys) (7,8). Before onset of the disease a selective effective treatment need to be administrated which can reduce probability of the disease occurrence. Various symptoms could be appeared on an illness animal for instance, temperature and loss appetence, increased salivation, and

respiratory distress. The treatment probably may be applied for other animals that may have considered a source of infection (carrier) with pasteuralla (9). Different studies were done in this area on other types of microorganisms (10,11,12), but few of them were deal with *P. multocida*, therefore this study was conducted to investigate the present of this bacterium and defending of their antibiotic susceptibility.

Materials and Methods:

Sample collection:

At the period from December 2017 until April 2018 samples were collected from different animals aged from (3-18) months of different sex. A total of 75 samples were collected, 45 from nasal cavity of sheep by sterilized swabs impregnated in nutrient broth after cleaning the nasal area by alcohol 70%. Thirteen samples also collected from infected lung and liver tissues from slaughtered animals showed clinical signs of disease. The surface of lung and liver was cutting by sterile dissecting instruments, a swab was taking from lung and liver according to (13).

Isolation and identification of bacteria: All samples were cultured on selective media (Clindamycin Gentamycin media) and MacConkey agar and incubated in37˚C for 24 hours.

Microscopic Examination: A slides were made using pure isolates selected from selective medium, then the slides stained with Gram stain. *Pasteurella multocida* give negative Gram stain. Another slide stained with methylene blue to indicate bipolarity shape of the bacteria.

Biochemical testes:

Catalase test :- The test was done by spreading single colony of bacteria from Nutrient agar on a clean slide, then (1-2) drops of Hydrogen peroxide 3% were added .The production of the bubbles considered as a positive reaction.

Oxidase test : The test was performed by adding many drops of Oxidase reagent (tetra methyl-P- phenyl di amine dehydrochlorid) on filter paper, then a single colony from nutrient agar was transported by sterile stick and was speeded on the moistened filter paper with reagent. The purple color appeared at (10) second was considered positive.

Motility test: Motility test was done on growing pure colony of nutrient broth which incubated at 37◦C for 24 hours

then one drop from them was taken and put on curved slide and covered with cover slide then tested under light microscope (4).

API 20NE system: Further confirmations were done by using API 20NE test kit (BioMérieux, Inc., France). The plastic strips holding twenty mini-test tubes were inoculated with the saline suspensions of the cultures according to manufacturer's directions.

Antibiotic susceptibility test: The antibiotic susceptibility test were done by agar disc diffusion method as described by (14). Discs used are listed in table 1.

Table 1:- Antimicrobial discs used in study.

Antibiotic discs	Assembly	Content(mcg)
Ampicillin	AM	25
Bacitracin	B	10
Neomycin	N	30
Chloramphenicol	C	10
Novobiocin	NV	30
Penicillines	P	25

Results

Out of 75 samples, Twenty one isolates (28%) of samples were identified as *P. multocida* through using selective CG T medium (table 2). The total isolation was 33.3% from apparently healthy sheep and (40%) from the infected lungs (Figure1). These isolates characterized on selective CG T medium (figure 2 A & B). Gram stained showed that these bacteria are Gram negative coccobacilli or short rods. Stained the isolates with methylene blue revealed the presence of bio polarity that revealed the stain of only the tip of the bacterium (Figure 3). It was catalase positive, oxidase positive, non-motile. These results were confirmed by using API 20 E kit; (Figure 4). All isolates were presumed to belong to *P. multocida* were produce indole, presence of ornithine decarboxylase that fermented mannitol, acid by fermentation of glucose and did not grow in MacConkey agar. The

percentage frequency of bacterial isolates was higher in female (38%) than in male (29.1%) (table 3). Infection was increased with decreasing of the age of the animal (Figure 5). Results of disc diffusion test (table 4) which illustrated by photograph (Figure 6 A-D) showed that these bacteria were resistant to ampicillin, bacitracin, penicillin and different degree of sensitivity to other antibiotics.

Table 2:- Percentage frequency of *Pasteurella multocida* in healthy cases and clinical specimen.

Sample type	Number of Sample	Number of Isolates	Percentage of Isolates
Nasal swabs	45	15	(33.3)%
Liver tissues	15	0	-
Lung tissues	15	6	(40)%
Total	75	21	(28)%

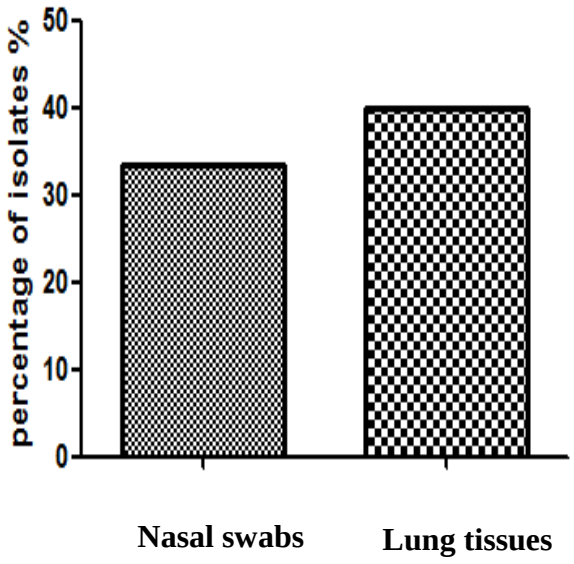


Figure 1:- Occurrence of *Pasteurella multocida* in nasal swabs and lung tissues

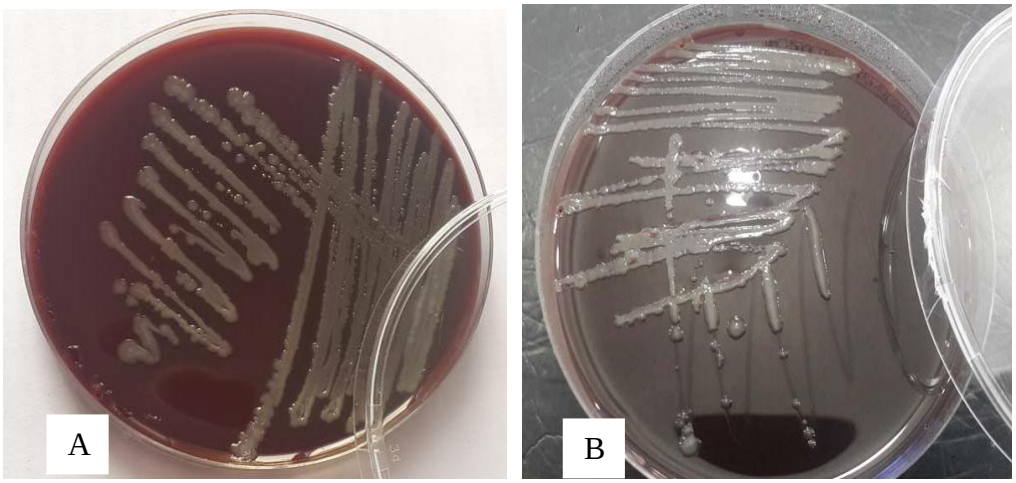
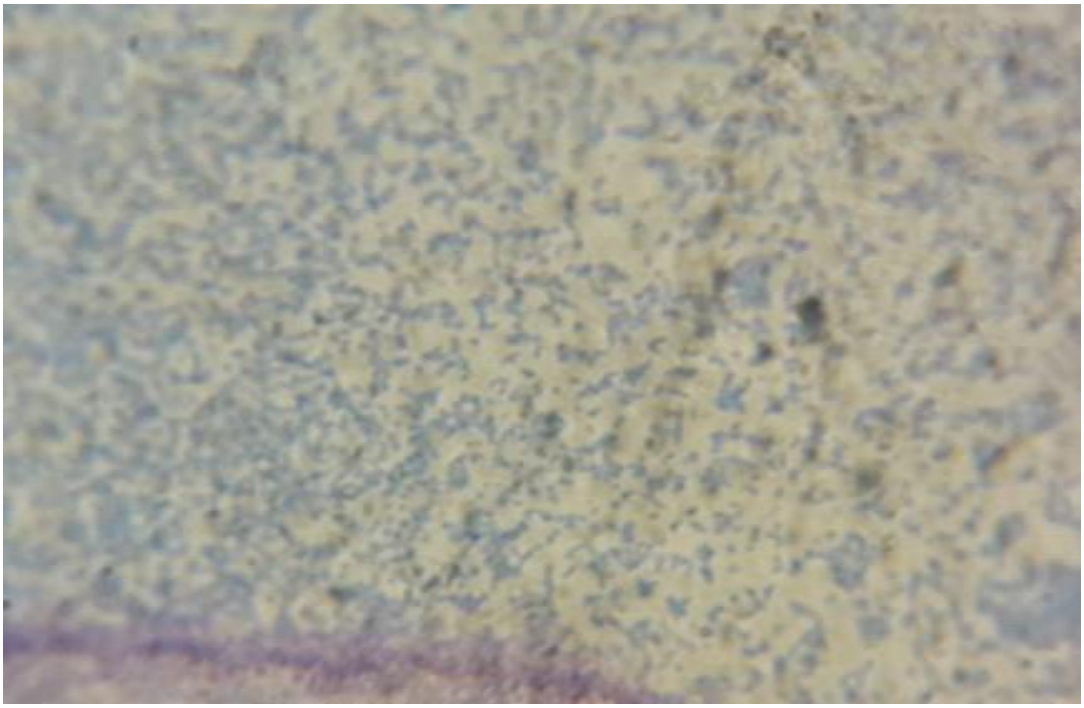


Figure 2:- (A&B):- ***Pasteurella multocida*** **on CGT agar Medium agar appeared as small, discrete, round, smooth, convex , opaque.**



Figure(3):- Bipolarity of ***Pasteurella multocida*** **isolated from stained with methylene blue .**

Table3. Percentage frequency of bacterial isolates in male and female in age groups.

Age (month)	No. of samples	Percentage Occurrence in male	Percentage occurrence in female	Percentage Occurrence
(3m-12m)	25	N=15 n=5(33.3)%	N=10 n=4(40)%	n=9(36)%
(13m-18m)	20	N=9 n=2(22.2)%	N=11 n=4(36.3)%	n=6(30)%
Total sample of bacterial isolates	45	N=24 n=7 (29.1)%	N=21 n= 8(38)%	n=15(33.3)%

N=Number of samples n= Number of isolates

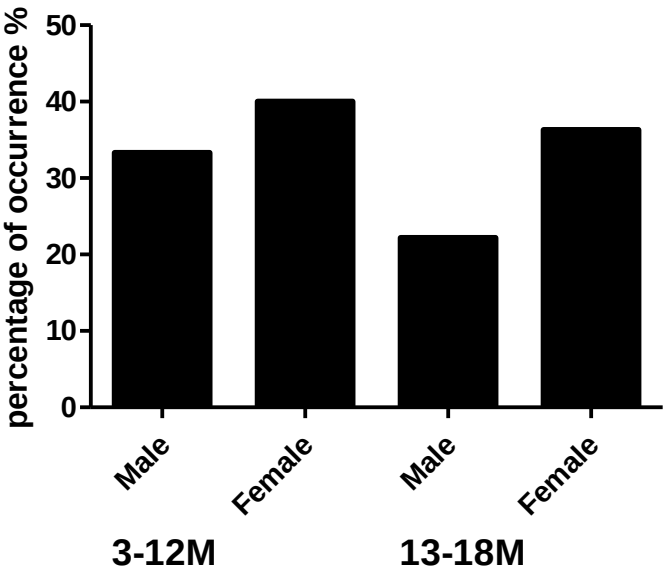


Figure 4:- The frequency of bacterial isolates in male and female. Infection were increased with decreasing of the age of the animal

Figure 5:- Results of API 20 NE system

Table 4:- Antibiotic sensitivity tests in millimeter against study isolates.

Bacterial	Amoxicilline	bacitracin	Neomycin	Chloramph.	Novobiocin	Penicilline
1-	0	0	2.3	4	2	0
2-	0	0	3	-	3	0
3-	0	0	3.5	3.5	2.5	0
4-	0	0	-	3	-	0
5-	0	0	2.5	4	3	0
6-	0	0	4	-	3	0
7-	0	0	-	-	2.5	0
8-	0	0	3	4	3	0
9-	0	0	2.5	3	4	0
10-	0	0	4	-	-	0
11-	0	0	-	3	2	0
12-	0	0	3	-	4	0

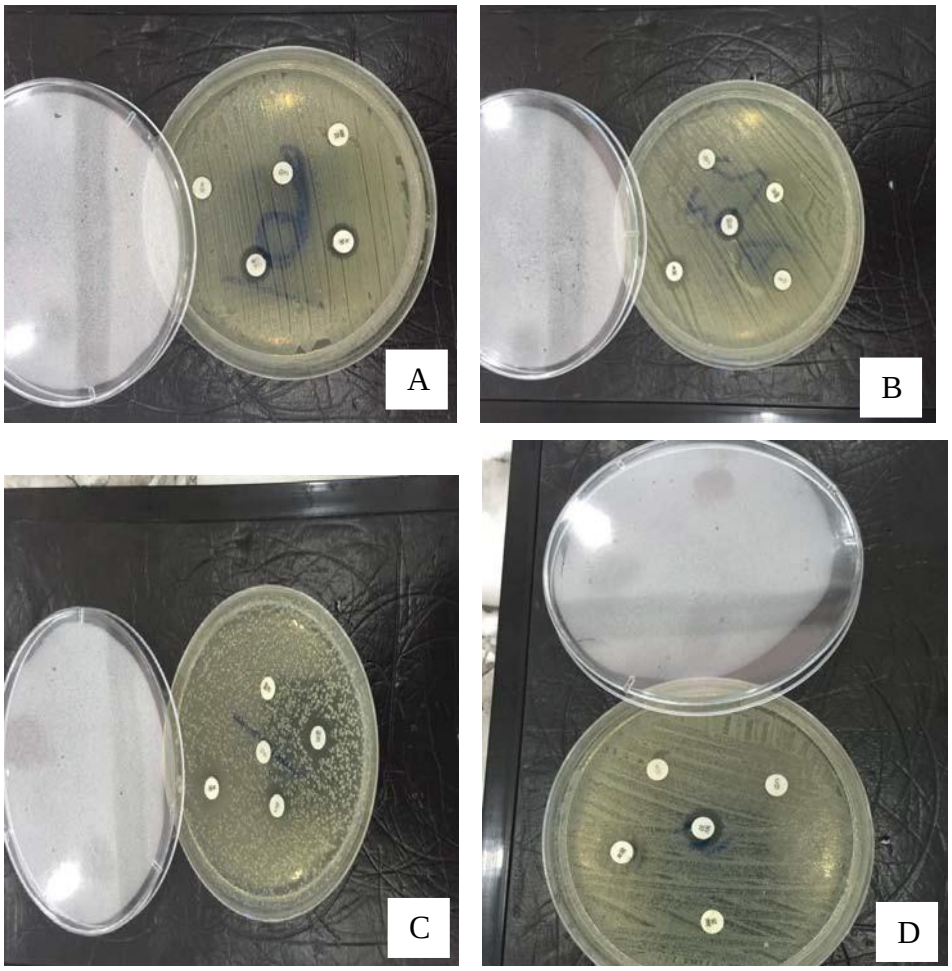


Figure 6:- (A, B, C & D) Antibacterial sensitivity test shows resistance and sensitive isolates.

Discussion:

In our investigation we showed that the percentage frequency of *Pasteurella multocida* in nasal swaps were (33.3%) and (40%) in lung tissues. That explained *P. multocida* considered commensal organism in upper respiratory tract and may be cause disease outbreak in sheep under extreme environ mental conditions that agree with (7) who consider *P. multocida* responsible for cause pasteurellosis the term that used to designate a number of infections in domestic animals and responsible for or associated with a wide range of systemic pulmonary and septicemia infections in various species of farm animals particularly ruminants. The cell capsule constitutes important virulence factors which play vital roles in the pathogenicity of pathogenic bacteria and establishment of infections. The

virulence mechanism of the cell capsule is mostly attributed to its ability to protect the invading organism against cellular and humoral defense mechanism of the host (15).The selective media used in study were more effective for isolation of these bacteria from mix culture than traditional blood agar ,these media allowed unimpaired growth of almost all strain of *P. multocida* ,while inhibition other bacteria. So, clindamycin, gentamicin inhibit growth of other gram negative bacteria whereas amphotericin by inhibit fungal growth (1). In present study, the test isolates ferment glucose and sorbitol but failed to ferment amylose, arabinose and inositol. This is in accordance with the finding of (16) and (17) that recorded most avian isolates ferment arabinose and inositol. The variability observed in fermentation reaction of carbohydrates might be due to geographic variation of isolates and use of chemotherapeutic agents as these factors influence the enzyme profiles of microbes (18). The majority of test isolates, also these isolates were predominant in female than in male(table 3) in smaller ages that may be due to decreased of immunity and resistance to antibiotics by bacteria, Also we found that these isolates found to be 100% resistant to amoxicillin. Penicillin table (4) that accepted with (19) where chloramphenicol, novobiocin, bacitracin neomycin found to be quite effective against *Pasteurella*. (18) Recorded that *Pasteurella* isolated from sheep were resistant to cephalexin and chloramphenicol, the sensitive to neomycin, chloramphenicol, and Novobiocin showed the necessity of invite antibiotic sensitivity prior treatment. (16, 20). In conclusion, the isolates were found to be slightly higher in female sheep than in male, and infection was increased with decreasing of the age of the animal. All isolates were resistant for ampicillin, bacitracin and penicillin, while showed different levels of sensitivity to other antibiotics.

References:

1-Knight,D.P;P Jane ;Speller,D.(2015). A selective media for pasteurella multocida and its used with animal and human specimens.*JClin Pathol* 1983;36:591-594 .

2-Boudreaux, C. M., (2004). A novel strategy of controlling bovine pneumonic pasteurellosis: Transfecting the upper respiratory tract of cattle with a gene coding for the antimicrobial peptide cecropin B. *M. Sc. Thesis*, Louisiana State University,USA.

3-Carter, G. R. (1967). Pasteurellosis: *Pasteurella multocida* and *Pasteurella haemolytica*. *Adv. Vet. Sci.* 11:321–379.

4- 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.

5- Diker, K.S., Akan, M. & Kaya, O. (2000). Evaluation of immunogenicity of Pasteurella haemolytica serotypes in experimental models.Turkish *JVAS* 24(2000)139-143.

6- Praveena,P. E., Periasamy, S., Kumar, A. A. and Singh,N. (2014) Pathology of Experimental Infection by *Pasteurella Multocida* Serotype A1 in Buffalo Calves. *Vet. Pathol.* 51(6):1109-12 doi:10.1177/0300985813516647

7- Mohamed, R. A. & Abdelsalam, E. B. (2008). A review on pneumonic Pasteurellosis (Respiratory Mannheimiosis) with emphasis on pathogenesis, virulence mechanism and predisposing factors. *BJVM* 11(3)139-160.

8- Ghosh, R.C., Hirpurkar, S.D. and Mondal, M. (2011). An outbreak of swine pasteurellosis in Chhattisgarh. *Indian J. Vet. Pathol*. 35 (1):87-88.

9- Prescott, J.F. and Baggot, J. D. 1988. Antimicrobial therapy in veterinary medicine. *Blackwell Scientific Publications*. 1-69.

10- Abbas, B. A., Khudor, M. H., & Touhali, I. S. (2011). Isolation, identification and immunological study of yeast from cattle and buffaloes in Basrah province, Iraq. *Iraqi Journal of Science*, *52*(1), 125-131.

11- Ibraheem, R. S., & Abbas, B. A. (2015). Study on *candida* species isolated from cows with molecular detection of some virulence gene. *Bas. J. Vet. Res*. 14(2):31-43.

12- Abbas, B. A., & Alghanim, A. M.

(2016). Detection of virulence genes in Escherichia coli isolated from frozen meat in Basrah market. *Bas. J. Vet. Res., 15*(3):134-145

13- Dunbar, M. R., M. J.Wolcott, R. B.Rimler, andB.M.Berlowsky. (2000). Systemic pasteurellosis in free- ranging neonatal pronghorn in Oregon. *J. Wildl. Dis* 36: 383–388.

14- 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.

15- Ashley, B. D., M. Noone, A. D. Dwarakanath & H. Manlick, 2004. Fatal Pasteurella dagmatis peritonitis and septicaemia in a patient with cirrhosis: A case report and review of the literature. *JCP*, 57, 210−212.

16- Prabhakar, T. G., Prabhakar, P. and Venkadabadi. (2010). Molecular characteriz*ation of* Pasteurella multocida isolated from an incidence of sheep pasterullosis in Karamadai Hill Tract of Tamil Nadu. Tamilnadu JVAS 6(2)81-87.

17- Mohamed-W, Mohamed A., Moemen A. Mohamed (2014) Molecular analysis of *Pasteurella multocida* strains isolated from fowl cholera infection in backyard chickens. *Asian Pac. J. Trop. Biomed.* 4(1): 8-12.

18- Mahmoud, S. Z. (2007). Some studies on *Pasteurella* spcies in sheep in Qena Governorate. *Beni-Suef Veterinary Medicine Journal* 17(1)105-107.

19- Shayegh, J., Parviz, M. and Hejazi, M.S. (2010). Species diversity of sheep and goat infecting Pasteurella multocida isolated based on SrRNA16 gene sequencing, *IJVR* No 33, Page 373.

20- Rangnekar, D.V. (2006) Livestock in the livelihoods of the underprivileged communities in India: A Review. *ILRI* Nairobi, Kenya.p32, 45