Incidence of *Yersina enterocolitica* in sheep in the south region of Iraq

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Summary

Yersiniosis is a zoonotic disease which infects many animal species. A preliminary study was done to detect the incidence of *Yersinia enterocolitica* in sheep. One thousand and two hundred fecal samples were collected randomly from sheep in four governorates in the southern region of Iraq: Thi-Qar, Al-Muthana, Messan and Basrah, in the period from July 2016 to June-2017. Enrichment *Yersinia* broth at 4°C for 48 hours was used for isolation of *Yersinia enterocolitica*. Selective Cefsulodin Irgasan Novobiocin and non selective media were used for bacterial culture. The identification of *Yersinia enterocolitica* was based on colony morphology and biochemical characters, API 20E and VITEK2 compact systems were used for the same purpose also. Data were analyzed by using SAS, Version 9-1. Chi- square test was used for comparison. The total isolation rate of *Y. enterocolitica* was 5.16% (62). High and low rates of isolation were recorded in Al-Muthana (6. 01%) and Basrah (3.86%). Similarly a significant high isolation rate was recorded in diarrheic sheep (17.4%), moreover the infection rate increased significantly (9.5%) in the young sheep (1day to 6 month's age). Furthermore, the recovery rate of *Y. enterocolitica* increased significantly during the cold months (12%) as compared with temperate and hot months (spring and summer), at the same instance, non significant variation among sex difference was detected. Fever, diarrhea and mild to moderate degree of dehydration were the most common clinical manifestations observed on the infected animals. Conclusively sheep were considered a source of infection to other species including human being, and the spread of microorganism increased markedly in the cold and wet environment.

Keywords: *Yersinia enterocolitica*, Zoonotic disease, Sheep, Iraq.

Introduction

Yersinia enterocolitica was discovered by Schleifstein and Coleman in 1939 in USA. However, most reports were published since the beginning of 1960s (1). *Y. enterocolitica* has a considerable clinical importance (2), it causes food borne illness, in addition, to its ability to infect different animal species (1). Yersiniosis was listed as a third disease after campylobacteriosis and salmonellosis in Europian Union (3). *Y enterocolitica* contains six heterogeneous strains and over 70 bio and sero types (4). The epidemiology of *Y. enterocolitica* is a complex one and remains poorly understood yet (5). The recovered infected animals may become carriers (6). The clinical symptoms mainly consist of sailorrhea, diarrhea, and loss of weight (7). Despite the clinical spectrum of *Y. enterocolitica*, the infections vary according to host ages and other factors (8). The virulence genes in both chromosomes and plasmids are necessary for their pathogenesis, beside that these genes had been widely used to identify the pathogenic strains (5). Interestingly, the human pathogenic strains had been isolated from animals, moreover a genotype relationship had been established between pathogenic strains isolated from human and sheep in Great Britain (9), and this indicated that sheep might be a potential reservoir for human pathogenic strains. *Y. enterocolitica* had been isolated from sheep carcasses' and butchers in Baghdad slaughter house in 1992 (10). Similar work in 1998 revealed high contamination of sheep and goats carcasses (11). *Y. enterocolitica* had been isolated from human patient also in the north of Iraq (12). The aim of the present work was to find the incidence of *Y. enterocolitica* infection in sheep in the south region of Iraq with referring to some epidemiological factors.

Materials and Methods

One thousand and two hundred sheep of different ages of both sexes, in the southern region of Iraq: Thi-Qar, Al-Muthana, Messan and Basrah governorates, during the period from July 2016 to June 2017, were used in this study. Histories of all animals were recorded in special cards prepared for this purpose. All animals were succumbed to clinical examination. Fecal swabs were taken from sheep and transported by a cool box to the laboratory of Veterinary Hospital in Thi-Qar governorate. Five milliliters of *Yersinia* enrichment broth (pH 7.4) were added to each sample and kept at 4°C for 48 hours (13). The samples were cultured by streaking on selective Cefsulodin Irgasan Novobiocin (CIN)* and non selective blood, nutrient and MacConkey agars, and were incubated at 25°C for 48 hours (14). Conventional biochemical tests including: Urease, Indole, Triple Sugar Iron and Catalase, were used for identification of suspected colonies (15). API 20E and VITEK2 systems** were used for more confirmative laboratory diagnosis of Yersinia organisms (16 and 17) respectively. Data were analyzed by using Statistical Analysis System (SAS) – version 9.1 (18). Chi-square test was used for comparison, $P<0.05$ was considered statistically significant.

*** Oxoid, England**

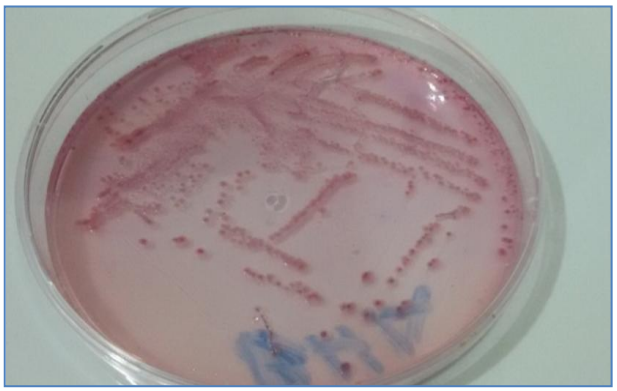
**** Reagents from bio Merieux, France**

Results and Discussion

Yersiniosis is an enteric infection affecting different species. In Iraq, no published data had been related to incidence of Yersiniosis in animals. However in northern of Iraq (Ninavah governorate), *Y. enterocolitica* was isolated from patients suffering from enteritis (12). Sheep were considered a major source of infection with this pathogen (4). Human infection by *Y. enterocolitica* in Ninavah governorate might reveal the role of farm animals in the transmission of Yersinia organisms. In fact the large animal population, in the north and south of Iraq initiates a risk of spreading yersiniosis.

The total rate of isolation in sheep was 5.16% (62), this was lower than the rate found in Great Britain (10.7%) and that reported (14.8%) in Australia (19 and 20). Moreover approximate low rates of infection (3%, 1%) in sheep were reported in Egypt and Nigeria (21 and 22). Furthermore in Iran (Neighboring country) the isolation- rate of *Yersinia* infection in sheep was higher (16%) than our finding (23). The variation in the incidence of *Y. enterocolitica* appeared approximately identical to other enteric enterobacteriacae infections, however many factors might influence the rate of infection: Geographical locations, management, ages, seasons, weather and isolation techniques (24).

The cold enrichment broth media highly facilitated the isolation of Yersinia species (25), although other suggested the opposite (26). The typical characteristic Yersinia colonies (Fig. 1) on selective agar (CIN) appeared with dark pink centers surrounded by translucent border, which were highly suggested to be belonged to *Y. enterocolitica* (27).



Figure, 1: *Yersinia enterocolitica* colonies on (CIN) agar.

Selective (CIN) media increased the recovery rate more than others (28). In fact this specific media is reducing the number of biochemical tests required for identification of Yersinia species (29). Moreover (CIN) agar is most suitable for clinical work (28). Additional confirmations for identification of *Y. enterocolitica* were required also. API20E and VITEK2 systems were used in this study; their results were highly coinciding with specific biochemical characters of *Y. enterocolitica* (Fig. 2). The high and low isolation rates were recorded in Al-Muthana (6.01%) and Basrah (3.86%) governorates, non significant variation between four governorates were observed, this might be owing to the similarity of their weather and environmental nature (Table, 1).

Figure, 2: Biochemical characters of *Y. enterocolitica* by API20E system.

Table, 1: Isolation of *Y. enterocolitica* from sheep in four governorates.

Governorates	No. of animals	Positive isolation	%
Thi- Qar	454	24	5.28
Al-Muthana	316	19	6.01
Messan	223	11	4.93
Basrah	207	8	3.86
Total	1200	62	5.16

Some authors reported no effects of different location on isolation rates (30). A significant high isolation rate was recorded in diarrheic sheep (17.4%) as compared with non diarrheic (1.7%) (Table, 2). The shedding of Yersinia pathogen increased obviously in diarrheic animals; this was noticed in other enteric enterobacteriacae infection (7 and 31). In the same instances, the isolation rate was increased significantly in sheep aged one day to 6 months (9.50%), and the rate was decreased to a minimum extent (0.76%) in sheep over 1 year old (Table, 3). This might be attributed to immaturity of the immune system and lack of previous exposure of young animals to multiple infections (32).

Table, 2: Isolation of *Y. enterocolitica* from diarrheic and non diarrheic sheep.

	Diarrheic	non diarrheic
Total No.	263	937
Positive isolates	46*	16
%	17.4	1.7

*** Significant difference at P≤0.05.**

Table, 3: Isolation of *Y. enterocolitica* from sheep according to age.

Age	No. animal	Positive isolates	%
1 day to 6 months	442	42**	9.5
> 6 month to 1 year	368	17*	4.6
Over 1 year	390	3	0.76
Total	1200	62	5.16

**** Significant difference at P≤0.05.**

The seasonal variation effectively influenced the rate of bacterial isolation. It increased significantly (12%) in cold (winter) months (November, December, January and February), whereas the rate decreased to 3.5% in the temperate months: October, September, March and April. Moreover *Yersinia* pathogen was not isolated in hot (Summer) months: May, June, July and August (Table, 4), this might owing to the high environmental temperature as it elevates to the maximum extent (over 40°C), similar finding was observed by others (33), in contrary no apparent seasonal effect on the isolation rate of *Y. enterocolitica* was reported also (34). The high prevalence rate of *Yersinia* infection was recorded in the wet season (7). High rain fall occurs mostly in winter season in Iraq; in turn this increases the grazing areas. Consequently this leads to an increase in the chances of animal infection. The large sheep populations and long grazing period will contribute to heavily contamination of pastures with sheep feces, and these were raising the infection rate (7). In addition, there are many factors that increase the isolation rate of *Y. enterocolitica*: particularly those causing stress or/and concurrent disease: Shearing, weaning, lambing, and the onset of cold, wet, windy weather and deficiency diseases. These factors reduce the host defense mechanism, particularly which initiated within the intestine (35).

Table, 4: Isolation of *Y. enterocolitica* according to months.

Season	Months	No. animals	No. positive	(%)
Cold months	November	100	10	10
	December	100	12	12
	January	100	16	16
	February	100	10	10
	Total	**400**	**48****	**12**
Temperate months	September	100	5	5
	October	100	3	3
	March	100	4	4
	April	100	2	2
	Total	**400**	**14***	**3.5**
Hot months	May	100	0	0
	June	100	0	0
	July	100	0	0
	August	100	0	0
	Total	**400**	**0**	**0**

*** Significant difference at p≤0.05**
**** High Significant difference at p≤0.05**

The year- round shedding of *Y. enterocolitica* was reported (33 and 35), also, consequently the zoonotic risk of such pathogen will increase (22). The cold and wet environment might increase the growth and multiplication of bacteria; this in turn increases the isolation rate also. No effect of sex difference on the isolation rate was noticed in this study. Sheep more than one year aged showed mild signs: soft feces without systemic reaction. These cases may represent the carrier state; however *Y. enterocolitica* was isolated from apparently healthy sheep, these animals increase the spreading of *Y. enterocolitica*. On the other hand sever signs were observed in younger sheep: fever, diarrhea and dehydration (mild to moderate), this was in agreement with others (31). Some considered enterotoxine produced by *Y. enterocolitica* (*yst*B) is analogue to that produced by *E. coli* (36). *Y. enterocolitica* enterotoxine might be the most important virulence factor of biotype A strain, which is responsible for long lasting diarrhea. Conclusively the *Y. enterocolitica* infection in sheep is a serious zoonosis to farmers, and sheep dairy products consumers in the southern region of Iraq, particularly in the winter and spring seasons.

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نسبة حدوث اليارسينيا المعوية القولونية في الأغنام في جنوب العراق

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الخلاصة

يعد داء اليارسينيوسيز من الأمراض الانتقالية والذي يصيب فصائل عديدة من الحيوانات وتعد هذه الدراسة الأولى من نوعها في الأغنام بالعراق وتهدف إلى تعيين نسب الإصابة بجراثيم اليارسينيا المعوية القولونية الممرضة. أجريت هذه الدراسة على 1200 عينة من براز الأغنام التي تربى في أربع محافظات في جنوب العراق (ذي قار والمثنى وميسان والبصرة). جمعت عينات البراز بشكل عشوائي في المدة من تموز 2016 إلى حزيران 2017. واستعمل المرق الغني لجراثيم اليرسينيا بدرجة 4 مئوي ولمدة 48 ساعة، ثم استخدمت الأوساط الانتقائية وغير الانتقائية الصلبة لزراعة الجراثيم. شخّصت جراثيم اليارسينيا المعوية القولونية استناداً إلى صفات مستعمراتها على الأطباق وخصائصها الكيموحيوية فضلا عن استعمال نظامي API20E و VITEK لتأكيد نوع جراثيم اليارسينيا المعوية القولونية. تم تحليل النتائج إحصائيا باستعمال نظام SAS, version 9-1 بجهاز الحاسوب وقورنت النتائج باستعمال اختبار Chi- square. بلغت نسبة عزل جراثيم اليارسينيا المعوية القولونية الكلية (62) 5.16% وسجلت أعلى نسبة عزل للجراثيم المذكورة في محافظة المثنى (6. 01%) وأوطئها في محافظة البصرة (3. 86%) وارتفعت نسبة عزل جراثيم اليارسينيا المعوية القولونية ارتفاعا معنويا في الأغنام التي تعاني من الإسهال (17.4%) كذلك ارتفعت نسبة الإصابة ارتفاعا معنويا في الأغنام الفتية (يوم- 6 شهور) إذ بلغت 9.5%. وسجل تأثير الفصول على نسبة عزل الجراثيم إذ بلغت أعلى نسبة في فصل الشتاء (الأشهر الباردة) 12% ولم تعزل هذه الجراثيم في فصل الصيف. ولم يلحظ تأثير الجنس على نسبة الإصابة في الأغنام. سجلت العلامات المرضية للحالات المصابة وكان أهمها الحمى والإسهال الذي تراوح مابين بسيط إلى متوسط الشدة. نستنتج من ذلك أن الأغنام تشكل مصدرا للإصابة بجراثيم اليارسينيا المعوية القولونية خاصة للعاملين بتربية الأغنام ومستهلكي منتجاتها ويزداد انتشار هذه الجراثيم في البيئة الباردة والرطبة.

الكلمات المفتاحية: اليارسينيا المعوية القولونية، الأمراض الأنتقالية، الأغنام ، العراق.