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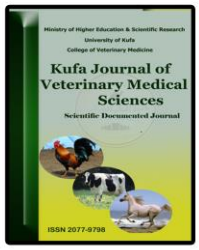

Detection of *Campylobacter* Spp. In Cheese of Awassi ewes milk and its effect in Public Health.

Ahmed M. S. Al-Shdidi Muna M.Asmael Rafid S.A.Al-Zubaidy

1&2 : College of Veterinary Medicine / Diyala University

3 : Faculty of Vet. Med. , Ferdowsi University of Mashhad, (Iran) .

E.mail : rafidsamir@yahoo.com

Abstract

The study conducted to indicate the experimentally comparative measurements of *Campylobacter* spp. Load with food poisoning and to investigate the effect of different types and concentrations of Emulsifying salts : (Sodium Carbonate Na_2CO_3 , triSodium Citrate $Na_3C_6H_5O_7$, Sodium tripolyphosphat (STPP) $Na_5P_3O_{10}$) , to choose the best mix of them that reduce the microbial load in locally produce cheese . 60 samples of Cheese locally produced from milk of Awassi ewes were been collected randomly from Local Awassi Flock of College of Agriculture at Baghdad University (Iraq) , (30 samples to each winter and spring season) to investigate their microbial load . Both of all winter and spring samples were in significant ($p<0.05$) microbial count of *Campylobacter* spp. . The Bacterio-(static&cidal) effect of Emulsifying salts on microbial activity was confirmed when The Total Bacterial Count (TBC) were highly significant ($p<0.01$) reduce in cooked cheese with 3% Emulsifying salts added . The *Campylobacter* spp. count in nutrient broth with 3% Emulsifying salts added were significant ($p<0.05$) reduce .

Key Words :- *Campylobacter* Spp. , Emulsifying salts , Cheese , Awassi ewes

دراسة لتلوث الجبن المصنع من لبن نعاج العواسي المحلي بجرثومة العطيفة وتأثيرها في الصحة العامة

1- أ.م.د. احمد محمد صالح الشديدي 2- أ.م.د. منى محمد إسماعيل

3- رافد سمير عبدالكريم

1- 2 : كلية الطب البيطري / جامعة ديالى

3 : كلية الطب البيطري / جامعة فردوسي مشهد (إيران)

الخلاصة :-

استهدفت التجربة دراسة درجة تلوث عينات من الجبن المصنع من اللبن المنتج محليا في بغداد (العراق) من نعاج العواسي بأعداد جراثيم العطيفة *Campylobacter* spp. المسببة للتسمم الغذائي بجراثيم العطيفة الملوثة للجبن المنتج من عينات هذا اللبن وتأثيرها على حمولته الجرثومية المؤثرة في الصحة العامة ودراسة تأثير استخدام أنواع وتراكيز مختلفة من أملاح الاستحلاب في هذة الحمولة الجرثومية . حيث جمعت بصورة عشوائية 60 عينة من الجبن المصنع محليا من ألبان نعاج قطيع أغنام العواسي التابع لكلية الزراعة / جامعة بغداد , العراق وبمعدل 30 عينة لكل من الموسم الشتوي من بداية كانون الأول إلى نهاية كانون الثاني و الموسم الربيعي من بداية آذار إلى نهاية نيسان , لدراسة حمولتها الجرثومية من جراثيم العطيفة التي تميزت بالارتفاع المعنوي ($p<0.05$) في كلا عينات لموسم الشتوي والربيعي . وأثبتت النتائج التأثير القاتل أو المثبط لنشاط الجراثيم بواسطة أملاح الاستحلاب المستخدمة في عملية الطبخ حيث انخفض معدل العد الجرثومي الكلي

في الجبن المطبوخ بفرق عالي المعنوية ($p<0.01$) بعد إضافة وخلط 3% من أملاح الاستحلاب مع الجبن مباشرة قبل عملية الطبخ ، وانخفض معدل أعداد الجراثيم بفرق معنوي ($p<0.05$) بعد إضافة 3% من أملاح الاستحلاب إلى المرق المغذي لنمو الجراثيم .

Introduction :

Zoonotic diseases are widely spreading among humans and animals in countries around the world and animal products is important in the transmission of these diseases to humans, The suggest local estimates of the national survey of livestock for the year 2008 by the Central Statistical Org**. (CSO)** and Ministry of Agriculture, Iraq [1] that the number of sheep in 2008 amounted to (7.722.375) constitute 63.86% of the number of total animals in diameter ratio, and *Awassi* breed constitute 60-65% of the total sheep local breeds [2], where there is widespread in some northern areas of the central region of the country and one of the most local breeds of sheep number in Iraq . The cheese factory in sheep breeders houses from the raw milk of ewes big role in the transfer of many of the pathogens to humans because of the high content of bacteria resulting from the non-use of thermal treatment or these transactions are insufficient to eliminate pathogenic bacteria in milk intended for the manufacture, or as a result of the pollution due to primitive methods used during production, transportation and trading. On this basis and because of the lack of such a cheese with health conditions, the incorrect thermal treatment and determine the quality of Emulsifying salts typically and appropriate proportions of emulsification cheese factory locally will lead to the elimination of this bacteria that resides in it and work on the reduction of germ payload that have a significant role in determining the quality and lengthen the period of validity for human consumption [3;4;5;6]. focusing on the role of the contamination of milk products in general and cheese produced locally in particular, in the process of epidemiological *Campylobacter* spp. And its relationship to Public Health and for the purpose of the consumer to know the true size of the problem locally, this study was carried out by following techniques to isolate and diagnose from the local milk products over the winter and spring season.

Campylobacterosis food poisoning is the most common cause of gastro-enteritis in the US, UK and around the world, especially in the developed and developing countries. It is a diverse transition to humans, but documented as the most commonly by eating poultry raw or non-cooked meat, in milk, on poultry meat, in un-Pasteurized milk, by domestic and wild animals infection, from the farm animals and water sources . *Campylobacter jejuni* have been identified as the main cause of human gastro-enteritis associated with food poisoning, but *C.coli* and other species and their sub-types can also be responsible gastro-enteritis [7]. Along with the risk to humans from gastro-enteritis can result in from *C.jejuni*, a (Guillain-Barré syndrome) [7] or acute idiopathic demyelinating polyneuropathy (AIDP), a rare condition, but seriously affects one person out of 100,000 people provoked occurrence prior *C.jejuni* infection among 30% of patients with this condition [8;9]. Nearly 845 thousand *Campylobacter* cases annually in the US happen or approximately 28% of the recorded outbreaks of food-borne illness which reported more than 6 thousands case of infection in 2009, only without a death [10;11]. recorded of several cases of outbreaks of *Campylobacter* milk-borne infection in the US since the beginning of the 70s of the last century, equivalent to 25% of all registered cases of morbidity of milk-borne infections [12;13] . Such cases as a result of raw milk contaminated

C.jejuni by infected people and lack of cooling the milk for several hours, and in 1981 scored outbreaks of *C.jejuni* due to consumption of insufficient pasteurized milk [13]. Recorded in the former SU outbreaks of *Campylobacter* associated with dairy products affected by the use of *C.jejuni* contaminated water in milk manufacturing processes [14]. Ensure *Campylobacter* infection in other dairy products such as yogurt and white cheese in England in 1981, signed for outbreaks caused by *C.jejuni* contaminated cheeses [15]. ***Campylobacterosis*** is a Zoonotic disease transmitted to humans by animals or animal products, causing Diarrhea more than *salmonella*, so that the number of cases of the disease reported in some countries outweigh the number of cases of *salmonella* disease, Its believed to be about 5-14% of all cases diarrhea in the world caused by the *Campylobacter*, and its frequent cause of travelers diarrhea [10;11], the longer, *Campylobacter* infection is the main problem for developing countries [16] because of the lack hygiene habits, contaminated water and food with infected fecal matter the main source of infection, the contaminated vegetables with infected feces and sewage, and can to convey houseflies and red cockroaches that are stockiest and transmission of the *Campylobacter* on their wings and legs after standing on a infected stool [17]. At least willing young adults under the age of 15-29 years of infection, followers of the personal hygiene habits of less in children, the incidence in males is higher than in females [18] *Campylobacter* cause of 49% of cases of food-borne illness and there is a high in the incidence rates and mortality rates among children under five years old, who make up 30% of the total infected . (Despite the incapability of children under the age of 6 months to be infected with dysentery) [19]. At the global level, talked about 220 million cases a year caused by the occurrence of 2.2 million deaths [18;20]. In endemic countries the *Campylobacter* infection is responsible for about 28% of all cases of diarrhea among children and more than 90% of deaths resulting from diarrhea [20]. The most important characteristic of the *Campylobacter* contaminated food is not to get any change in the taste and color and the smell of food, making it difficult to distinguish and caution him, although the increasing incidence of *Campylobacter* patterns of resistance to antibiotics of life made it difficult to control the treatment of infection [21;22;23].

- The general characteristics of *Campylobacter* bacteria :-

Theodor Escherich [24] a German-Austrian pediatrician in 1886 describe the transmission of *Campylobacter* in the form with parasitic infections in infants or summer of cholera disease which have been classified in a new genus for the first time in 1963 by Sebald&Veron [25] and were not isolated until 1972 [26]. In 1909 Notice researchers McFadyean&Stockman of unfamiliar bacterial shapes repeated presence in abortions disease endemic Epizootic in ewes have books in 1913 [27] the report made it clear that the existence of a relationship between the bacterium and cases of Infectious abortion in cattle and sheep, the first reference to the *Campylobacter* bacteria. And in the 50s of the last century [28] History of the disease such as hepatitis infectious infects poultry [30; 29]. Vinzent,*et.al.*1947 [31] scored the first case of *Campylobacter* human infection in pregnant women miscarried before the date of her birth after 5 weeks of illness. *Campylobacter* is a genus back to the ***Campylobacteraceae*** family, a Gram-negative bacteria, helical in shape, non spore-forming, mobile, uni- or bi-flagellated polar, Anaerobic or micro-philic, not grow in the presence of [O_2] more than 5%, thermo-philic grow at 42C (except *C.fetus* grow in 25C), Sensitive to cooling, freezing, killed by pasteurization, Sensitive to low pH below 4.5, as well as dry media and [salt] higher than 1% [33; 32] (except *C.fetus* Iraqi strain [34]). The

Campylobacter bacterium are sensitive to acidity and low pH not bear pH=2.5 for 2h. , but the chance of bringing the infection is greater when ingested with contaminated milk and unpasteurized milk products which making it easier to survive by crossing the stomach. Most *Campylobacter* strains infect the tissues of the jejunum, small intestine and colon, and produces a toxin cytolethal distending toxin which helps the bacteria to evade the immune system and stay in the cells for a limited period and that their disability cell division and the immune system, and it was believed in the past that its produce Cholera-Like Enterotoxin but it turned out later that it causes inflammation of the intestines respects (metastatic) bloody Odematus and exudative enteritis [36]. The large intestine (colon and rectum) is the main infection sites the fact that animated continue to stay in the small intestine and because of the movement of the ciliary cells of the intestine and rapid flow of liquids and intestinal peristaltic movement conversion intestinal growing. Establish colonies and invade mucosal epithelial cells and include pathogenicity of four phases: - invasion, intra-cellular multiplication, inter-&intracellular cells (cell-to-cell)-movement, and host cell-killing [35;36;37].

- *Campylobacter* pathogenesis :- *C.fetus* and *C.jejuni* Causing *Campylobacter* abortion and mastitis in goats and sheep, which is the treasurer naturally to *Campylobacteria* , where up insulation ratios have to 40-100% of the feces of the animals and most serotypes isolated similar to those isolated from human feces private *C.jejuni* and *C.coli* . *C.jejuni* is a common Zoonotic and one of the most major causes of gastro-enteritis in humans [35]. Symptoms of *Campylobacter* food poisoning occur in people usually after 2-5 (incubation) period of up to 10 days after eating contaminated food. The most common symptoms are diarrhea, typical symptoms include: diarrhea (which ranges between moderate and severe and often bloody), fever, nausea, vomiting, abdominal pain, headache, muscle pain. ***Campylobacteriosis*** [36;37;38;39] is the infection fecal-oral main route of *Campylobacter* infection in humans [19] where the transmission of the disease (especially in children under five years old) by contamination with infected fecal matter [18]. the germ with the infected stools in the recovery period for a period of 5 weeks and these germs highly infectious and may continue during the acute infection per gram of feces infected person may contain (10^9) bacteria , knowing that the minimum dose of infectious intake ranging between ($5x10^2$ - 10^5) bacteria [20;21]. Most outbreaks pathological reach its peak during the month of May and October and be individual cases in the summer [40]. In England the beginning of the month of May begins and reaches its peak in early June. This seasonal incidence may be linked to the movement of the object by flies [36]. It has been observed that there is epidemiological changes in the prevalence of serotypes quality and occurrence of dysentery, find that the serotype common in cold regions (America and Europe) is *C.fetus* and cause the incidence of dysentery during the winter months, while *C.jejuni* serotype is common in the tropics and cause the incidence of dysentery end of the summer, as it was noted that the epidemic dysentery be periodic cyclic lasts between each session (4-5 years), so this should be taken into account when applying vaccination programs to prevent infectious outbreaks [36;37].

- Emulsifying salts (E.salts) :-

By the experiences of researchers working in the cheese industry, a result of the search for a way to save the dry and semi-dry cheese observed the separation of cheese ingredients (water, fat and protein) from each other during cooking, which led to the search for chemical compounds have the ability to prevent separation in addition to its ability to spread protein in emulsion [3;4;41] . Habicht at 1934 used part of

E.salts known at the present time [42], which are organic compounds with mono-valent roots and positively charged and many other parity negatively charged, where its works as a dispersant of protein and thus help to dissolve as well as the emulsification of fat, but found that some of them not suitable for use in the cheese industry and for reasons that economic considerations . **Joha** E.salt found in 1936 and used in the cheese industry [43] there are about 20 species of these salts to fit all kinds of cheese industry, they vary salts in the capacity to bring about the spread of the protein and its effect on the pH of the mixture of cheese the user and its ability to resist pH changes ([1%] solutions) and has been divided into: acidic, alkaline and neutral E.salts , its works to stop calcium effectiveness, which affects the stability of the gel cheese , the first property of E.salt is the ability to dissolve the casein to produce a homogeneous fluid [44]. its has the ability to influence the properties of keeping the product of the bacteriological through its (Cidal or Static) antibacterial impact [45]. The table (1) shows the percentages of the components of four Mixes of E.salts used in the experiment and the results of the change in pH .

table (1) Emulsifying salts %	Mix(1)	Mix(2)	Mix(3)	Mix(4)
Sodium Carbonate (Na_2CO_3)	5	-	5	-
triSodium Citrate ($Na_3C_6H_5O_7$)	5	10	-	-
Sodium tripolyphosphat (STPP) ($Na_5P_3O_{10}$)	90	90	95	100
pH	10.23	10.23	10	9

- Materials and methods of work : -

- The Samples Preparation :

a-The process of the local soft cheese manufacture: -

It was, according to the [3;4]: by putting the milk drained in a vase and warms to the point of 38C and add the rennet at a rate of 2.5g/50kg milk and mix well for 5m. and cheese-forming process occur during 2-3h. and drain the clot formed with a piece of muslin cloth to get rid of whey and placed in molds refinery clot pressing weights.

b-Processed cheese manufacturing locally: -

It was, according to the [3;4]: Using a manufacture soft cheese where the local soft cheese cut into slices and mince electric with machine and put in the cooking pot and added 2% E.salts the mixture no.(2) in table (1) above then was cooked at 85C/30m. and then was packed in 100g. capacity containers .

- The Micro-biological tests:

a- Total Bacterial Count (TBC) :-

Followed the way of the Standard Plate Counting (SPC) in accordance with the [46;47] to calculate the counting of bacterial aggregate where they were taking 11g. of different parts of the cheese sample and placed in a blender added a 99mL of 2% Na citrate solution temperature of 45C and mix on high speed for 5m. to be obtained on the sample liquid was conducted on the sample string of decimal Dilutions and used two dishes to relieve the one and the transfer of (1&0.1)ml of the diluted to each dish and was attended by counting dishes plate count agar and poured in Dilution sample dishes and incubated at a temp. 42C/72 h. and calculated the number of colonies of bacteria formed.

b- isolate and diagnose the *Campylobacter* bacteria: -

Were in accordance with the [46;47] stages the following steps:

1. planting phase in nutrient broth (peptone water) in a container closed containers on Gas Pack provides 5-7% O_2 or containers Candle Jar provides 15-17% O_2 to provide the Microaerophilic condition=($N_2$85%, $CO_2$10%, $O_2$5%).
2. implant on the selective media -stage (Thiole media) prepared from horse blood in a container of anticoagulant freeze and then dissolved at room temperature frequently to ensure breaking of RBCs.

3. Microscopic examination by the Dark-field way.
4. conducting confirmatory biochemical tests, a definitive diagnosis :
(Oxidize+, Catalase+, Motility+).

c- The effect of Emulsifying salts (E.salts) on *Campylobacter* bacteria: -

After the diagnosis of bacteria depending on the [32;34;47] took the loop carrier one Standard Platinum 1Loopfil colonies of pure spores were grown in nutrient broth and incubated at 37C/48h. , then was taken 1 ml of nutrient broth-grown and conducted decimal Dilution required with buffer phosphate solution and by the number of spores in each 1 ml. of the broth, then added (2 , 2.5 , 3) g. of E.salt of the mixture no. (2) in table (1) above and used in the experiment per 100 ml. of the broth-grown and then by the number of germs after the addition of E.salts.

- Results and Discussion :-

Proven Micro-Biological results analysis that cheese samples locally produced from the milk collected at random from the herd sheep Awassi of the Faculty of Agriculture / University of Baghdad, Iraq, and at a rate of 30 samples for each of the winter season from the beginning of December to the end of January and the spring season from the beginning of March to the end April , all of these samples were of a low level of health and in terms of quality and not in conformity with local and international standards.

Table (2) Campylobacter/g isolation of cheese samples during the probationary period.

Month	no. of (+) samples/ total no.	Isolation %	Campylobacter TBC/g. CFU/g
December	**7/15**	**46.6**	**10^1× 7.5**
January	**6/15**	**40**	**10^1× 6.8**
December+January	**13/30**	**43.33**	**10^1× 7.15**
March	**9/15**	**60**	**10^3× 2.7**
April	**8/15**	**53.3**	**10^3× 3.2**
March+April	**17/30**	**56.66**	**10^3× 2.95**
Overall	**30/60**	**50**	**10^2× 5.1**

The results of Table (2) the number of positive samples to the total number of samples and the proportions of the isolation and the rates of the Campylobacter total bacterial count (TBC) in cheese in winter (month12 and 1) and spring (Month 3 and 4) seasons, we find that the overall average of the rates of presence of Campylobacter bacteria CFU/g in these products ratios were 50 %.

Table (3) isolation rates and the rate of Campylobacter TBC/g

	no. of (+) samples/ total no. **winter**	no. of (+) samples/ total no. **spring**	no. of (+) samples/ total no.	Isolation %	Campylobacter TBC/ g. CFU / g
Overall	**13/30**	**17/30**	**30/60**	**50**	**10^2× 5.05**

The results of the table (3) The number of positive samples to the total number of samples and the proportions of the isolation and the rates of the Campylobacter total bacteria count (TBC) in cheese samples that have been collected, including the winter seasons and spring, and we find that the overall rate ratios of the rates of presence of Campylobacter bacteria CFU/g in these products was 50%.

Table (4) compared to the TBC of milk samples for each of the winter season and spring rates

CFU	winter season TBC±SE	spring season TBC±SE	Significance level
TBC	**$\pm10^2\times9.5$ -$10^2\times1.20$ $10^2\times0.087$**	**$\pm10^2\times12.083$ -$10^2\times1.80$ $10^2\times0.086$**	**
Campylobacter / g Counting	**$\pm10^2\times27.5$-$10^2\times$ 5.1 $10^2\times0.377$**	**$\pm10^2\times11.5$-$10^2\times$ 5.1 $10^2\times16.18$**	**

- SE = Standard error
-** Highly significant difference (P<0.01).

The results table (4) The seasons of the year effect on the total bacterial counting (TBC) and the Campylobacter bacteria, where the statistical test results showed a highly significant difference (P<0.01) in the total bacterial counting (TBC) rates CFU / g of the season spring for the winter season and attributed this high count the reasons many of them inefficient thermal treatment and pasteurization of raw milk. And an appropriate degree air to the growth and reproduction of these germs heat and an increase subtracting the germs with the media during the spring season and misapplication of health law at the production, marketing and supply in addition to the rapid multiplication of bacteria in milk products when they become a temperature close to the optimum for their growth during the spring season, where it is under conditions of cooling and thawing repeated because of a power outage during storage in addition to survival for long periods in the retail and not consumed shortly before the citizen exposing them to these conditions and for longer periods. It also notes from the results table (4) there is a highly significant difference (P<0.01) in the Campylobacter total bacterial count rates of the CFU/g of the season spring for the winter season and attributed this rise of advanced reasons as there is a relationship between growth and reproduction of Campylobacter bacteria in the milk and temperatures various observation during the seasons of the year, fecal-oral infection considered the main route of Campylobacter infection in sheep, and be wild bird carriers of the infection [36;37], and the proliferation of domestic flies and the red cockroaches, which is the Campylobacter treasurer carrier can transmit the Campylobacter on the legs and wings yet stand on a infected stool [17].

Table (5) count rates of Campylobacter/g in samples nutrient broth affected by adding Emulsifying salts%

Emulsifying Salt %	0%	2%	2.5%	3%	Significance level
pH	**7.2**	**9.8**	**9.85**	**9.9**	**-**
Campylobacter /g Counting	**b $10^2\times$ 5.1**	**b $10^1\times1.8$**	**a $10^1\times1.3$**	**a $10^1\times1.0$**	*

- Small different English letters within the same column indicate significant difference (P<0.05).
* Significant difference (P<0.05)

The results of the table (5) the existence of a significant difference (P<0.05) in the Campylobacter bacteria count rates before and after the addition of (2, 2.5 and 3%) of Emulsifying salts into nutrient broth and attributed this difference is due to changes in the pH of the broth nutrient to become the baseline by Emulsifying salts additives and is not valid for the growth of these bacteria. Where he was pH 7.2 and became after the addition of Emulsifying salts (9.8 and 9.85 and 9.9), respectively, resulting in a reduction of Campylobacter bacteria grow in nutrient broth samples.

Table (6) the effect of adding Mixture No. (2) of 3% on the Campylobacter TBC/g.

Emulsifying Salt %	0%	3%	Significance level
TBC	**10^2×1.2**	**10^1×6.7**	**
Campylobacter/g Counting	**10^2× 5.1**	**10^1×8.4**	*

** Highly significant difference (P<0.01).
* Significant difference (P<0.05).

The results table (6) that for some Emulsifying salts (Cidal) or (Static) antibacterial effect [45] When adding the concentration of 3% of the Emulsifying salts of the mixture (2) used in the experiment there was a decrease highly significant (P<0.01) in the Total Bacterial Count (TBC) from 1.2×10^2 to 6.7×10^1 CFU/g and a significant decrease (P<0.05) in Campylobacter bacterial count from 5.1×10^2 to 8.4×10^1 and attributed this difference as a result of bacterio-static effect by Emulsifying salts on the Bacteria .

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