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Virulence Studies For Listeria spp. Recovered From Raw Ruminant Milk

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ABSTRACT

In this study, 512 milk samples (103 raw cow's milk, 100 raw buffaloes milk, 107 raw goats milk, 102 raw ewes milk and 100 raw she-camels milk) .The incidence of Listeria among the different ruminant milk species was 6.1% . The isolated Listeria spp. were distributed as 9 isolates from cows, 8 isolates from buffaloes, 6 isolates from goats, 8 isolates from ewes and 4 isolates from she –camels raw milk. The Listeria spp. were distributed as 7 isolates of L. monocytogenes, 4 isolates L. innocua, 8 isolates L. welshimeri, 7 strains L. seelegeri and 5 isolates L. grayi in a percentage of 1.4%, 0.8%, 1.6%, 1.4% and 0.98% respectively. The results of virulence study revealed that all of the tested Listeria isolates were strong positive for Congo red after 24 hrs. Virulence of the Listeria isolates was also investigated by detection of hemolysis on blood agar, i/p injection to mice and intraocular distillation in rabbits. Hemolysis on blood agar, death of mice and eye inflammation in rabbits within 24h after injection in an incidence of 100% in case of L. monocytogenes isolates (virulent). On the other hand the suspected isolates of L. innocua, L. seelegeri, L. welshimeri and L. grayi showed negative results (a virulent).

Key words: (Listeria isolation, virulence, Hemolysis).

INTRODUCTION

Listeria spp. is gram positive, non spore forming, rod shaped bacteria 10.5 µm and 1-1.5 µm in length microaerophilic, belonging to the Firmicutes phylum. The genus Listeria belongs to the Clostridium sub-branch, together with Staphylococcus, Streptococcus, Lactobacillus, and Bronchothrix. This phylogenetic position of Listeria is consistent with its low G+C DNA content (36-42%) (Rocourt et al., 1982) . The genus Listeria harbors two pathogenic species, Listeria monocytogenes and L. ivanovii, and eight nonpathogenic species that include L. innocua, L. seeligeri, L. welshimeri, L. grayi, L. marthii, L. rocourtiae, L. fleischmannii, and L. weihenstephanensis (Abu Mraheil et al., 2013).

Raw milk is one of the most common paths for transmission of L. monocytogenes, mainly due to sick animals in the farm. It is important to point out that healthy animals are often carriers L. monocytogenes and as such can be source of contamination of the environment, or food including raw and processed food (milk and dairy product) (Kasalica et al., 2011). There is an opinion that the main source of contamination to animals by L. monocytogenes is poor quality of silage (Fenlon, 1986). As already stated, facilities of dairy plants are excellent environment for development and growth of L. monocytogenes considering high moisture in milk and dairy products and their residue that remain on equipment used in production. L. monocytogenes has the ability to form phybrils through which it attaches/adheres to solid surfaces, creating biofilm, which is reason why it is very difficult to remove the organism from the equipment and production facilities, where it multiplies on surfaces resulting in re-contamination of dairy products (Sleator et al., 2003).

Listeriosis with milk consumption association supports the hypothesis that L. monocytogenes is a pathogen transmitted to humans from infected animals or their products and that the consumption of the microorganism is an infection mechanism (Lunden et al., 2004). The results suggest that milk should be considered as a possible infection vehicle on sporadic listeriosis cases and that even when pasteurization is a highly effective method to eliminate pathogens from milk, it might not always be 100% effective concerning Listeria spp (Fleming et al.,1985). So the aims of the

the incidence of virulent Listeria spp. recovered from ruminant raw milk.

MATERIALS AND METHODS

A total of 512 samples of raw milk (103 cows , 100 buffaloes , 107 goats, 102 ewes and 100 she- camels) collected from different Governorates (El Giza, El Kaluobia, EL Fayoum, El Sharkia, El Behaira)the samples transported to microbiology department , faculty of veterinary medicine , cairo university under refrigeration in ice box .

- **Isolation and identification of Listeria spp.**

For the isolation and identification of Listeriasppfrom milk samples, the techniques recommended bythe International Organization for Standards (ISO 11290-1, 1996) were implemented. Isolation and identification of Listeria was performed using the double enrichment procedure, the first (Half Fraser) and the second (Fraser) enrichment broths. A

- **VirulencestudiesforListeriaisolates(Berkhoff and Vinal, 1986)Congo red binding test**

Listeria was tested for their growth status on Congo red medium. Congo red binding ability is one of the indicators of virulence among Listeria isolates. Congo red positive (CR+) serovars was indicated by the development of bright or orange red colonies. Different intensities in the dye uptake were expressed as (+) and (++) whereas Congo red negative (CR-) organisms showed white colonies due to Listeria did not bind to the dye.

Blood hemolysis

A thick 5 % sheep blood agar was used for stabbing. Grid was drawn to 20-25 spaces on plate bottom. One culture was stapped per grid space. A positive control (L. monocytogenes) and negative control (L. innocua) were used, then incubated at 35°C for 48 hrs

Mouse virulence assay

Three mice were used for each isolate. Each mouse was injected intraperitoneally with 0.1 ml of the bacterial suspension. Three mice were kept as control. The death rate and post-mortem changes as well as the reisolation of the organism from the internal organs and heart blood were recorded.

Rabbit (Anton's eye test)

Each rabbit was infected with 0.1 ml of the bacterial suspension by instillation into the conjunctiva. Conjunctivitis within 24 h was recorded.

- **Sample**

loopfulof growth from Fraser II was subcultured onto PALCAM selective agar supplemented with SR 0150E (polymyxin B 5 mg, acriflavineHCl 2.5 mg) (Oxoid) and incubated micro-aerobically (5% O_2, 10% CO_2, and 85% N_2). Colonies suspected to be Listeria were transferred onto tryptic soya yeast extract agar (TSYEA) (Difco, Bacton, PA, USA) and incubated at 30°C for 18–24 hours. Those putative Listeria colonies were tested for purity, in addition to morphological and biochemical characteristics using API Listeria system (bioMe'rieux, Marcy l'Etoile, France). Presumptive Listeria colonies were maintained at 4°C on Trypticase soy agar with 0.6% yeast extract slants, incubated at 37°C for 24 hours, and stored at 4°C.

RESULTS

The incidence of Listeria among the different ruminant species was clearly indicated that all of the tested 512 ruminant raw milk revealed that the percentage of Listeria isolation reached 6.1%. The isolated Listeria spp. Were distributed as 9 isolates from cow's milk, 8 isolates from buffalo's milk, 6 isolates from goat's milk, 8 isolates from ewe's milk and 4 isolates from she–camel's raw milk. The Listeria spp. were distributed as 7 strains of L. monocytogenes, 4 strains L. innocua, 8 strains L. welshimeri, 7 strains L. seelegeri and 5 strains L. grayi in a percentage of 1.4%, 0.8%, 1.6%, 1.4% and 0.98% respectively.Virulence of theListeria isolates was also investigated by detection of hemolysis on blood agar, i/p injection to mice and intraocular distillation in rabbits. Hemolysis on blood agar, death to mice and conjunctivitis in rabbits within 24h after injection in an incidence of 100% in case of L .monocytogenes isolates (virulent). On the other hand the suspected isolates of L. innocua, L. seelegeri, L. welshimeri and L. grayi showed negative results (a virulent).

DISCUSSION

Listeriosis outbreaks have mostly been linked to consumption of raw milk or dairy product made of unpasteurized milk. Previous outbreaks of listeriosis have been linked to a variety of foods especially processed meats (such as hot dogs, deli meats, and pate). Today, most are linked to consumption of raw milk or cheese made from unpasteurized milk. The public health importance of listeriosis is not always recognized, particularly since Listeria is a relatively rare disease compared with other common foodborne illnesses such as salmonellosis. However, because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to foodborne illness: second after salmonellosis. Changes in the manner food is produced, distributed and stored have created the potential for widespread outbreaks involving many countries (Johansson et al., 2002) .Food infection is transmitted predominantly in an ape-zootica. Because, although the soil is the source, transmission to man is mainly produced from the environment through animals and food surfaces. In this way, L. monocytogenes should be considered as environmental bacteria whose transmission to humans occurs mainly through consumption of foods that have been contaminated during its manufacture and production (WHO, 1988).

Even though most of the disease cases are due to the intake of non-pasteurized milk or sub products made with it, outbreaks that occurred in spite of pasteurization show that this process does not eliminate the risk of later contamination. Therefore, pasteurized foods have the same risk consequence (Bulaet al., 1995). Incorrect milk pasteurization and its subsequent contamination are the most possible explanations for the presence of pathogens in pasteurized milk. Some of the milk bacteria produced by cows with bovine mastitis may survive after pasteurization and replicate themselves at refrigeration temperatures. Even when contamination with pasteurized milk bacteria is demonstrated, it is hard to determine the way and the source of such contamination. Raw milk is an essentially dangerous product and, as such, it should never be added to the pasteurized product (Flemingetal.,1985).

Among ruminant's Vazquez –Boland et al., (1992) recorded that Listeria infection is transmitted by consumption of spoiled silage, in which these bacteria multiply readily, resulting in herd outbreaks.L. monocytogenes detected in 2% of cow 's milk samples while buffalo's milk samples were free from the pathogen .On the other hand L. innocua could be detected in 2% of cow 's milk samples, while L. welshimeri was isolated from 2% of buffalo's milk samples (El-kholy and El-Leboudy, 1995). Survey of two Northern Ireland milk processing plants for L. monocytogenes, samples included the milk processing environment, Processing equipment, raw and pasteurized milk. In raw milk, the incidence Listeriaspp was 44.4% (22.2% L. monocytogenes). On one occasion L. welshimeri was isolated from pasteurized milk probably demonstrating post-pasteurization contamination of product (Kells and Gilmour, 2004).In the present study the prevalence of Listeria spp. in ruminant raw milk clearly indicate that all of the tested 512 ruminant's raw milk revealed a percentage of Listeria isolation reached 6.1%. The isolated Listeria spp. were distributed as 9 isolates from cow's milk, 8 isolates from buffalo's milk, 6 isolates from goat's milk, 8 isolatesfrom ewe's milk and 4 isolates from she –camel's milk. The Listeria spp. were distributed as 7 strains of L. monocytogenes, 4 strains L. innocua, 8 strains L. welshimeri, 7 strains L. seelegeri and 5 strains L. grayi in a percentage of 1.4%, 0.8%, 1.6%, 1.4% and 0.98%, respectively.

Listeria can attach to and enter mammalian cells. The bacterium is thought to attach to epithelial cells of the GI tract by means of D-galactose residues on the bacterial surface which adhere to D-galactose receptors on the host cells. This is the opposite to the way that most other bacterial pathogens are known to adhere, i.e., the bacterium displays the protein or carbohydrate ligand on its surface and the host displays the amino acid or sugar residue to which the ligand binds. Macrophages are well known to have "mannose binding receptors" on their surface whose function presumably is to ligand to bacterial surface polysaccharides that terminate in mannose, as a prelude to phagocytic uptake.

The bacteria are then taken up by induced phagocytosis, analogous to the situation in Shigella. After engulfment, the bacterium may escape from the phagosome before phagolysosome fusion occurs mediated by a toxin, which also acts as a hemolysin, listeriolysin O (LLO). This toxin is one of the so-called SH-activated hemolysins, which are produced by a number of other Gram-positive bacteria, such as group A streptococci (streptolysin O), pneumococci (pneumolysin), and C.perfringens. Survival of the bacterium within the phagolysosome also occurs, aided by the bacterium's ability to produce catalase and superoxide dismutase which neutralize the effects of the phagocytic oxidative burst. So the present study give attention for detection and characterization of virulent Listeria spp. through congo red , hemolysis , lab animal inoculation which revealed that all recovered isolates were congored positive while hemolysis on blood agar, death to mice and eye inflammation in rabbits within 24h after injection in an incidence of 100% in case of L monocytogenes isolates (virulent). On the other hand the suspected isolates of L. innocua, L. seelegeri, L. welshimeri and L. grayi showed negative results (a virulent) (Todar, 2009).

CONCLUSION

virulentL.monocytogeneswas recovered from raw milk and thisneed good hygienic practice to avoid its transmission to human through milk.

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الملخص العربى

دراسات علي الضراوة لانواع الليستريا المعزولة من ألبان المجترات الخام

في هذه الدراسة تم فحص 512 عينة لبن من المجترات المختلفة لتحديد نسبة تواجد ميكروب الليستريا فيه مقسمة الي (103 لبن ابقار و 100 لبن جاموس و 107لبن ماعز و 102 لبن أغنام 100 لبن نوق) و كانت نسبة عزل ميكروب الليستريا من هذه العينات هي 1.6 % مقسمة الي (9 معزولات من لبن الابقار و 8 معزولات من لبن الجاموس و 6 معزولات من لبن الماعز و 4 معزولات من لبن الجمال) و تم تصنيف هذه المعزولات الي (7 عترات ليستريا مونوسيتوجينز و 4 عترات ليستريا انوكا و 8 عترات ليستريا وليشيمري و 7 عترات ليستريا سيليجيري و 5 عترات ليستريا جراي) بنسبة 98%., 1.4 %, 1.6 %, 8%., 1.4 % بالترتيب و بدراسة ضراوة هذه الميكروبات كانت كل الانواع المعزولة ايجابية لاختبار الكونجو ريد بينما تكسير الدم علي اطباق الاجار و موت الفئران المحقونة والتهاب عيون الارانب كانت مميزة فقط لانواع الليستريا مونوسيتوجينز عن باقي الانواع المعزولة