İstanbul Üniv. Vet. Fak. Derg.
35 (I), ??-??, 2009

J. Fac. Vet. Med. Istanbul Univ.
35 (I), 49-53, 2009

ISOLATION OF *Mannheimia Haemolytica* FROM THE UHT MILK

Aysun YILMAZ[1], Gözde DALKILIÇ KAYA[1]

Geliş Tarihi : 27.11.2008
Kabul Tarihi : 04.02.2009

UHT Sütten *Mannheimia Haemolytica* İzolasyonu

Özet

Bu çalışma ile UHT sütten *Mannheimia haemolytica* izolasyonu ve identifikasyonu tanımlandı. Etken insanlarda da infeksiyona neden olduğu için önemlidir. Bu çalışmada, 2008 yılında ticari bir firmadan temin edilen yarı-yağlı, 3 paket UHT sütün bakteriyel analizi yapıldı. Sütte organoleptik açıdan anormal görünüm ve pıhtı saptandı. Süt homojenize edildikten sonra PCA ve koyun kanlı agara ekimler yapıldı. Gram boyama sonucunda Gram negatif kokobasiller görüldü ve etken API-20E identifikasyon sistemi kullanılarak *Pasteurella pneumotropica/ Mannheimia haemolytica* olarak identifiye edildi.

Anahtar Kelimeler: İzolasyon, *Mannheimia haemolytica*, UHT süt

Abstract

This study describes the isolation and identification of *Mannheimia haemolytica* from the UHT milk. This is important for public health since human might get infected with this bacteria. Three package of semi-skimmed UHT drinking milk with cacao was obtained from a commercial company and analysed in a private laboratory (Istanbul, Turkey) bacteriologically. The gross apparence of the milk was not normal. It was thickened and clotted. Milk homogenates were analysed by using PCA and sheep blood agar. After Gram's staining Gram negative coccobasils were observed. *Pasteurella pneumotropica/ Mannheimia haemolytica* was identified according to the API 20E.

Key Words: Isolation, *Mannheimia haemolytica*, UHT milk

1 Cevre Industrial Analysis Laboratory, Merkez Mahallesi, Ceylan Sokak, No 24, Mart Plaza, Kat 2, Kağıthane, Istanbul, Turkey

Corresponding Author: Aysun Yilmaz, Cevre Industrial Analysis Laboratory, Merkez Mahallesi, Ceylan Sokak, No 24, Mart Plaza, Kat 2, Kağıthane , Istanbul, Turkey, Tel: +90 212 3210900, Fax: +90 212 3210975, Email: aysunyilmaz@cevreanaliz.com

Introduction

During milking, the milk may be contaminated with some microorgainsms including *Mannheimia haemolytica* (**5**). The major contamination is emerged from milking equipment, teat and mammary glands. In addition, the cooling capacity of bulk tank, storage temparature and storage time of the milk affects the growing of contaminant microorganisms in milk (**2,8**).

Mannheimia haemolytica is a Gram negative, weakly haemolytic coccobacillus. It was first described as *Pasteurella haemolytica* in 1932 and separated as A and T (**11**). In 1999, the agent was classified as *Mannheimia haemolytica* (**3**). To make the identification and serotyping of *Pasteurella haemolytica* in those days, the bacteria has been considered as 3 different species as *Mannheimia haemolytica, Mannheimia glucosida and Pasteurella trehalosi* (**12**). However, theoretically it has to be considered as a single species as *Mannheimia haemolytica* (**2**).

Mannheimia haemolytica is a primary respiratory pathogen of sheep and cattle and also isolated from mastitic sheep and cows (**2, 15**). *Mannheimia and Pasteurella* are commonly found in upper respiratory flora of domesticated animals but human do not harbour these bacteria (**2**). However, human gets infection with this bacteria mainly by close contact to domesticated animals via wounds. *Mannheimia haemolytica* was found in cases of endocarditis, wound infections, croup cases and in urine of people (**10, 14, 16, 17**). This study describes the isolation and identification of *Mannheimia haemolytica* from the UHT milk.

Materials and methods

Description of milk samples

Three package of 200 ml of semi-skimmed UHT drinking milk with cacao was obtained from a commercial company in 2008 and analysed in a private Laboratory (Istanbul, Turkey) bacteriologically. The milk was produced according to the communique of raw and heat processed milk. The gross apparence of the milk was not normal. It was thickened and clotted.

Isolation and identification

The package was opened aseptically in sterile conditions for bacteriological analysis. 600 ml of milk was aseptically transferred into a sterile container and homogenized for sampling. Twenty-five mililiters of sample was homogenized for 2

min at medium speed in a Seward Stomachers 400 Laboratory Blender (Seward) in 225 ml buffered peptone-water (BPW) (1.07228, Merck). An aliquot (1 ml) of sample and further decimal dilutions of the milk homogenate in BPW were transferred into petri dishes and 12-15 ml of Plate Count Agar (105.463, Merck) (1g skimmed milk powder (LP 0031, Oxoid) was added per 1 liter of PCA) was poured on inoculated petri dishes. The plates were incubated at 30°C for 72 hours according to EN ISO 4833-2003 (**6**). 0,2, 0,3 and 0,4 ml of sample and decimal dilutions from homogenate was inoculated by spread plate method on ready to use Columbia agar plates containing 5% sheep blood (43041, Biomerieux). The plates were incubated at both 30°C for 72 hours and 37°C for 48 hours.

Colonies grown on sheep blood agar were recultured by streak plate method using a sterile loop on PCA and sheep blood agar for isolation and incubated at 35°C for 48 hours. For identification Gram's staining was done and examined on microscope. API 20 E (Biomerieux) identification system were used according to manufacturers instructions. All analysis were performed in duplicate.

Results and discussion

This study describes the isolation of *Mannheimia haemolytica* from the UHT milk. This is important for public health since human might get infected with this bacteria. Several investigators have pointed out that milk may serve as a vehicle for human pathogens like *Helicobacter, Campylobacter, Salmonella, Listeria, Pasteurella* and others (**5, 9, 13, 15**). It has been reported that UHT milk can be a source of human pathogens (**5, 9**). In this stsudy, clotted UHT milk was analysed for the presence of pathogen microrganisms. After Gram's staining Gram negative coccobasils were observed. *Pasteurella pneumotropica/ Mannheimia haemolytica* was identified according to the API 20E.

The major contamination of milk occurs during milking and is emerged from milking equipment, teat and mammary glands. The bedding used in cattle husbandry, feces, mud, and feed may cause contamination of teat. The general contaminating agents are *Micrococcus* spp., coagulase-negative *Staphylococcus* spp., *Enterococcus* spp., Coryneforms, Coliforms and *Bacillus* spp. (**4, 7**). It should be emphasised that many agents like *Staphylococcus* spp., *Streptococcus*, spp, *Lactococcus* spp., *Enterococcus* spp., haemolytic *Streptococcus* spp., *Pasteurella* spp., *Mannheimia haemolytica* and *Arcanobacterium pyogenes* contaminates the milk originated from cows with mastitis (**1,15**). The results of above studies reflects that it is more likely *M. haemolytica* isolated in this study may have been originated from the milk of mastitic cow but might be an environmental contamination. However,milk analysed in this study was thickened

and clotted. This indicates that the agent found in UHT milk in this study might have been emerged from the milk of mastitic cow since milk from cows with clinical mastitis is thickened and clotted (**4, 15**) .Therefore, care should be taken to avoid the clotted milk going into food chain. However, this type of milk may go into food chain accidentally by milk from cows with subclinical mastitis. When you consider the zoonotic agents caming from milk, the species of bacteria is more important than the number of agents. In other words, it is important whether it causes disease in people or not (**4**).

In conclusion, poor hygiene was found to be associated with the elevated level of microorganisms in milk (**2, 8**) However, spesific agents like *Mannheimia* usually comes form the milk of cows with mastitis. Therefore, preventive measuraments should be taken for poor hygiene and for milk obtained from mastitic cows not to enter food chain.

References

1. **Aagaard, K.:** Annual Report from the Veterinary and Milk Quality Department. The Danish Dairy Board, 2001, Arhus C, Denmark.

2. **Adamu, J.Y.:** *Mannheimia haemolytica*: phylogeny and genetic analysis of its major virulence factors. Israel. J Vet Med, 2007; 62 (1): 6-13.

3. **Angen, Ø., Mutters, R., Caugant, D.A., Oslen, J.E., Bisgaard, M.:** Taxonomic relationships of the [*Pasteurella*] *haemolytica* complex as evaluated by DNA-DNA hybridization and 16rRNA sequencing with proposal of *Mannheimia haemolytica* gen. nov., comb. nov., *Mannheimia granulomatis* comb. nov., *Mannheimia glucosida* sp. nov., *Mannheimia ruminalis* sp. nov. and *Mannheimia varigena* sp. nov. Int J Syst Bacteriol, 1999; 49: 67-86.

4. **Bramley, A. J., McKinnon, C.H.:** The microbiology of raw milk. Pages 163–208 in Dairy Microbiology, 1999, vol. 1. R. K. Robinson, ed. Elsevier, London, England.

5. **De Buyser, M.L., Dufour, B., Maire, M., Lafarge, V.:** Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. Int J Food Microbiol, 2001; 67(1-2): 1-17.

6. **EN ISO 4833:2003:** Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony-count technique at 30 degrees C.

7. **Hogan, J. S., Smith, K.L., Hoblet, K.H., Todhunter, D.A., Schoenberger, P.S., Hueston, W.D., Pritchard, D.E., Bowman, G.L., Heider, L.E., Brockett, B.L., Conrad, H.R.:**

Bacterial counts in bedding materials used on nine commercial dairies. J Dairy Sci, 1989; 72: 250–258.

8. **Holm, C., Jepsen, L., Larsen, M., Jespersen, L.:** Predominant Microflora of Downgraded Danish Bulk Tank Milk. J Dairy Sci, 2004; 87: 1151-1157.
9. **Jimenez, M., Soler, P., Venanzi, J.D., Cante, P., Varela, C., Martinez Navarro, F.:** An outbreak of *Campylobacter jejuni* enteritis in a school of Madrid, Spain. Euro Surveill, 2005;10(4): 118-121.
10. **Lejbkowicz, F., Davidkin, V., Gorenshtein, S.:** *Pasteurella haemolytica* in human urine. Scand J Infect Dis, 2003; 35(8): 512-514.
11. **Newsome, I.E., Cross, F.:** Some bipolar organisms found in pneumonia of sheep. J Am Vet Med Assoc, 1932; 80: 711-719.
12. **Odugbo, M.O., Odama, L.E., Umoh, J.U., Makinde, A.A.:** Serotypes of *Pasteurella haemolytica* from pneumonic lungs of sheep in northern Nigeria. Small Rumin Res, 2003; 48: 239-243.
13. **Quaglia, N.C., Dambrosio, A., Normanino, G., Parisi, A., Firinu, A., Lorusso, V., Celano, G.V.:** Survival of *Helicobacter pylori* in artificially contaminated ultrahigh temperature and pasteurized milk. Food Microbiol, 2007; 24(3): 296-300.
14. **Takeda, S., Arashima, Y., Kato, K., Ogawa, M., Kono, K., Watanabe, K., Saito, T.:** A case of *Pasteurella haemolytica* sepsis in a patient with mitral valve disease who developed a splenic abscess. Scand J Infect Dis, 2003; 35(10): 764-765.
15. **Wenz, J.R., Barrington, G.M., Garry, F.B., McSweeney, K.D., Dinsmore, R.P., Goodell, G., Callan, R.J.:** Bacteremia associated with naturally occuring acute coliform mastitis in dairy cows. J Am Vet Med Assoc, 2001; 219(7): 976-981.
16. **Watanabe, T., Sato, M., Abe, T., Oda, Y.:** Bacterial croup caused by *Pasteurella haemolytica*. Acta Paediatr Jpn, 1998; 40(4): 360-361.
17. **Yaneza, A.L., Jivan, H., Kumari, P., Togoo, M.S.:** *Pasteurella haemolytica* endocarditis. J Infect, 1991; 23(1): 65-67.