Research Institute of Animal Health, Assiut,
Director Prof. Dr. S.M. Nashed.

STUDIES ON BOVINE UDDER INFECTION WITH BACILLUS CEREUS

(With 3 Tables)

By
SABAH MOUSTAFA and NAGAH SAAD*

(Received at 2/1/1989)

دراسات عن عدوى الضرع فى الأبقار بالباسيللس سيريوس

صباح مصطفى ، نجـــــاح سعـــــــــد

لدراسة مدى تواجد ميكروب الباسيللس سيريوس فى ضرع الأبقار تم جمع عدد ١٦٠ مائة وستون عينة من الألبان ممثلة لعدد ٤٠ أربعون من الأبقار الحلوب بمزارع الألبان بأسيوط . تم إختبار عينات الألبان بإستخدام الكاليفورنيا ماستيتس لإكتشاف مدى التهاب الضرع فى هذه الأبقار وبالعزل الميكروبيولوجى لميكروب الباسيللس سيريرس ثبت تواجد هذا الميكروب فى ضرع الأبقار الحلوب بنسبة ٣٠٪ وفى عينات الألبان تم جمعها بنسبة ٩٫٣٨٪ ولقد وجد أن ٦٠ ٪ من الخمسة عشر عينة الموجبة لهذا الميكروب موجبة أيضا لاختبار الكاليفرونيا لدراسة مايلعبه براز الحيوانات فى عدوى الضرع بميكروب الباسيللس سيريوس تم جمع عدد٤٠ أربعون عينة براز من نفس الأبقار التى تم أخذ عينات ألبان منها لعزل هذا الميكروب بإستخـــدام نفس وسائل العزل البكتيرية المتبعة فى عزله من الألبان ولقد وجد أن ٣٥٪ من براز هـــذه الحيوانات يحتوى على ميكروب الباسيللس سيريوس. ومما هو جدير بالذكر أن عدد ٦ ستـة من الأربعين المستخدمة لهذه الدراسة (١٥٪) كانت تحتوى على ميكروب الباسيللس سيريوس فى كل من الألبان والبراز ممايشير إلى أهمية براز الحيوانات فى إنتقال العدوى مسببة التهاب الضرع . وقد تم إختبار حساسية عترات الباسيللس سيريوس المعزولة من الألبان وعددهـــا ١٥ وكذا المعزولة من البراز وعددها ١٤ لبعض المضادات الحيوية ولقد وجد أن العترات المعزولة من الألبان حساسة للتتراسيكلين والاستربتومايسين بنسبة ٩٣٫٣٪ ، ٧٣٫٣٪ أمـــا العتـرات المعزولة من البراز فكانت حساسة للجنتامايسن ، التتراسيكلين والاستربتومايسين بنســـبة ٨٥٫٧٪ ، ٨٥٫٧٪ ، ٧٨٫٦٪ ولم يكن للامبيسيلين أى تأثير على العترات المعزولة من البـــراز وقد تم مناقشة أهمية ميكروب الباسيللس سيريوس كمسبب لإلتهاب الضرع فى الأبقار مـــن ناحية وكمسبب للتسمم الغذائى من ناحية أخرى ومايجب إتباعه لدرء خطر هذا الميكروب .

SUMMARY

A total of 160 quarter milk samples from 40 dairy cows were obtained from Assiut dairy farms under sterile conditions to study the association of Bacillus cereus with bovine mastitis. Collected milk samples were tested with California Mastitis test (CMT). Bacillus cereus was recovered from 15 (9.38%) of the tested quarters of which 60% were CMT positive. 30% of the dairy

* Dept. of Food Hygiene, Fac. of Vet. Med., Assiut Univ.

cows udder's were positive for B. cereus, whereas the organism was detected in the faeces of 35% of the examined dairy cows. 6 of 40 cows (15%) harboured B. cereus in their udders and gastrointestinal tracts. B. cereus organisms isolated from milk (15 strains) and faeces (14 strains) were tested for their antibiotic sensitivity and there was variance of resistance. Most of the isolated B. cereus strains from milk were sensitive to tetracycline (93.3%) and streptomycin (73.3%). Recovered strains from faeces were sensitive to gentamicin (85.7%), tetracycline (85.7%) and streptomycin (78.6%), but all the strains were resistant to ampicillin. The significance of B. cereus as a cause of mastitis and foodborne illness was discussed.

INTRODUCTION

Bacillus cereus has a wide distribution in nature. In addition to causing illness, B. cereus is also capable of causing mastitis, systemic infection, gangarene and other clinical problems (BROWN and SCHERER, 1957; TURUBULL et al. 1979, JOHNSON,1984).

In 1957, the first report of B. cereus as a cause of bovine mastitis was recorded in the United States by BROWN and SCHERER. On the other hand, herd outbreaks of B. cereus mastitis have occurred in connection with infusion of udders with commercial antibiotic preparations later proved to be contaminated with spores of B. cereus. Furthermore, accidental exposure of a large number of quarters to B. cereus infection allowed observations of response to infection under a variety of circumstances which varied from nonclinical infection to severe and even gangarenous mastitis and occurred in both nonlactating and lactating quarters. However, B. cereus spores can lie dormant in the udder for some weeks before causing mastitis (JASPER, et al. 1972).

JONES and TURNBULL (1981) reviewed 28 cases of bovine mastitis and one of caprine mastitis apparently caused by B.cereus and found that 5 cases of mastitic animals were fatal, while others ranged from gangarenous to mild. The source of infection in some cases were brewers grains or bedding contaminated with faeces. Besides, isolation of B.cereus from 8 of 68 quarters before treatment with a dry cow mastitis preparations and from two after calving which had been negatice before calving was reported by HEER, et al. (1987).

The rarity of mastitis due to B.cereus and the modest reactions from experimental inoculations into quarters suggest that the organism is not a natural pathogen for the udder and that special circumstances must prevail if mastitis is to result from exposure (JASPER, et al. 1972). In this respect six cows developed signs of toxicosis after intracisternal inoculation with different doses of a bovine mastitis strains of B.cereus. Symptoms disappeared within 24 hours then an acute parenchymal mastitis developed in the affected udder quarter which included an increase in the cell count, chloride ion concentration with the appearance of mucous, floccules and clots. Then the quarter

gradually atrophied and milk secretion ceased, while the organism appeared in the inoculated quarter in decreasing numbers usually for a week as described by HORVATH, et al. (1986).

B.cereus has long been associated with milk and identification of the organism is of interest. B.cereus spores may gain enterance to milk during milking or storage of the milk on the farm or those entering during operations at the dairy. However, most of the contamination originates from straw bedding and soil and enter milk from inadequately washed teat surfaces, milking machinery or aerial contamination (DAVIES and WILKINSON, 1972; JOHNSON, 1984).

MARTIN, et al. (1962) reported the presence of B.cereus in 37% of raw milk samples from Ohio farms, whereas AHMED, et al. (1983) revealed the occurrence of B.cereus in 9% and 35% of the examined raw and pasteurized milk samples. Moreover, NAGAH SAAD (1985) found that the recovery rate of B.cereus in raw milk was 50%.

Milk was known to be contaminated with B.cereus before the organisms pathogenicity was well established. It causes a defect known as bitty cream or sweet curdling. The source of the milk contamination has been traced to cans of milk that were allowed to stand after emptying and to mastitic cows (BILLING and CUTHBERT, 1958; DONVAN, 1959; OVERCAST and ATMARAM, 1974; JONES and TURNBULL, 1981; JOHNSON, 1984).

From the literature publised it apears that the effective antibiotics against B. cereus are aureomycin, dihydrostreptomycin, chloramphenicol. erythromycin, gentamicin, kanamycin, neomycin, novobiocin and tetracycline (BROWN and SCHERER, 1957; JASPER, et al. 1972; CHMIELOWSKI, 1979; HEER, et al. 1987).

As there is scarce of information in Egypt about the incidence of bovine mastitis caused by B.cereus, therefore this study was initiated to show the prevalence of B.cereus in cow's udders and its association with mastitis.

MATERIAL and METHODS

Milk samples from 160 teats, of 40 apparently healthy dairy cows were aseptically collected from Assiut dairy farms in sterile flasks after discarding the first 2-3 strips of milk. About 30 ml of milk were drawn aseptically from each quarter. Individual quarter milk samples were screened by the California Mastitis test (CMT) according to the recommended methods developed and outlined by SCHALM and NOORLANDER (1957) and the APHA (1978) using CMT reagent (Dairy Research Products, Inc. Speneerville, Indiana, USA).

All milk samples were cultured for B.cereus by standard methods using B.cereus agar base (Oxoid) supplemented with Oxoid B.cereus supplement (SR 99) and egg yolk emulsion (SR 47). Inoculated plates were incubated at 37°C for 24 h. Colonies resembling those of B.cereus were Gram-stained and Gram-positive rods were identified biochemically according to the procedures described by BAILEY and SCOTT (1974) and HARMON

and GOEPFERT (1984).

Bacteriological examination of faeces collected from the same 40 dairy cows tested in this survey for the presence of B.cereus was done as described before.

Antibiotic sensitivity test of B.cereus strains isolated from both udders of cows (15 strains) and faeces (14 strains) was carried out according to the recommended manufacturer's instructions using the following antibiotics : Ampicillin 10 mcg, Carbencillin 100 mcg, Cephalothin 30 mcg, Chloramphenicol 30 mcg, Clindamycin 2 mcg, Erythromycin 15 mcg, Gentamicin 10 mcg, Neomycin 30 mcg, Piperacillin 100 mcg, Streptomycin 10 mcg, Tetracycline 30 mcg and Tobramycin 10 mcg per disc (Difco Laboratories, Deteriot Michigan, USA).

RESULTS

Results demonstrating the occurrence of B.cereus in cows udders and faeces are recorded in Table 1 and 2. 12 of the 40 examined dairy cows (30%) proved to be carrier of B.cereus in their udders. Of the 160 examined quarters, B.cereus was recovered from only 15 (9.38%) of the quarters. Furthermore, B.cereus was recovered from 60% of 15 quarters whose initial CMT scores were 1, 2 or 3 as presented in Table (1).

The isolation rate of B.cereus from faeces of cows was 35% as shown in Table (2). The present study revealed that 6 of 40 cows (15%) harboured B.cereus in their udders and the gastrointestinal tracts.

Table (3) shows the sensitivity of B.cereus isolated from milk and faeces to different antibiotics. tetracycline and streptomycin were effective against B.cereus recovered from milk, while those isolated from faeces were sensitive to gentamicin, tetracycline and streptomycin.

DISCUSSION

The fact that B. cereus spores are common in a cow's environment while B. cereus mastitis is relatively uncommon suggests that factors predisposing to mastitis are important (JONES and TURNBULL, 1981). The number of B.cereus in infected quarters must vary widely, often being low. The organism may at times be chiefly in the spore form and, therefore, less active in the udder indicating the rarity of mastitis caused by B.cereus (JASPER, et al. 1972).

In this study, the presence of B.cereus in 35% of the faecal samples collected from dairy cows suggests that faeces are the likely source of organisms invading the udder. This substantiate what have been reported by JONES and TURNBULL (1981) who revealed that the occurrence of B.cereus in faeces in large numbers might suggest proliferation in the alimentary tract. However, B.cereus may be more likely to establish residence in a normal quarter even though it may not always cause serious problems (JASPER, et al. 1972).

B. CEREUS MASTITIS

Two types of illness have been attributed to consumption of food contaminated with B.cereus. The first and best known type is characterized by abdominal pain and diarrhea, it has an incubation period of 4 to 16 hours and symptoms which last 12 to 24 hours. The second type is characterized by an acute attack of nausea and vomiting which occurs one to 5 hours after a meal, diarrhea is not a common feature in this type of illness (HARMON and GOEPFERT, 1984).

Gentamicin, streptomycin and tetracycline were effective against B.cereus recovered from milk and faeces of examined dairy cows, while ampicillin had no effect on the tested B.cereus strains isolated from faeces. In this respect, effective antibiotics against B.cereus mastitis are aureomycin, dihydrostreptomycin, tetracycline, bacitracin (BROWN and SCHERER, 1957), neomycin, dihydrostreptomycin, kanamycin,novabiocin, erythromycin, gentamicin and chloramphenicol (CHMIELOWSKI, 1979), neomycin and framycetin (HEER, et al. 1987).

However, slight inhibition was observed with neomycin, cloxacillin, ampicillin and penicillin (CHMIELOWSKI, 1979). BROWN and SCHERER (1957) found no inhibition of B.cereus by penicillin or polymxin, while cloxacillin, penicillin and triple sulfonamides were ineffective against B.cereus mastitis (JASPER, et al. 1972).

Careful sanitary procedures coupled with proper sanitation and low temperature storage of the finished product should preclude any problems with sporeforming organisms.

REFERENCES

Ahmed, A.A-H.; Moustafa, M.K. and Marth, E.H. (1983): Incidence of Bacillus cereus in milk and some milk products. J. Food Prot. 46: 126-128.

A.P.H.A. (1978): Standard Methods for the Examination of Dairy Products. 14th Ed. Marth, E.H. ed. Washington, D.C. American Public Health Association.

Bailey, W.R. and Scott, E.G. (1974): Diagnostic Microbiology. A text book for the isolation of pathogenic microorganisms. 4th Ed. The C.V. Mosby Company, Sanit Louis.

Billing, E. and Cuthbert, W.A. (1958): Bitty cream: The occurrence and significance of Bacillus cereus spores in raw milk. J. Appl. Bacteriol. 21: 65-78.

Brown, R.W. and Scherer, R.K. (1957): A report on two cases of acute mastitis caused by Bacillus cereus. Cornell Vet. 47: 226-240.

Chmielowski, W. (1979): Antibiotic resistance of Bacillus cereus strains isolated from food products and alimentary poisoning. Med. Weter. 35: 276-277. Cited by Johnson, 1984.

Davies, F.L. and Wilkinson, G. (1973): Bacillus cereus in milk and dairy products. In the Microbiological Safety of Food. Hobbs, B.C. and Christian, J.H.P. (eds). Academic Press, London, New York.

Donovan, K.O. (1959): The occurrence of Bacillus cereus in milk and on dairy equipment. J. Appl. Bacteriol. 22: 131-137.

Harmon, S.M. and Goepfert, J.M. (1984): Bacillus cereus. In the Compendium of Methods for the Microbiological Examination of Foods. 2nd Ed. Speck, M.L. (ed.). Washington, D.C. American Public Health Association.

Heer, A.; Ewald, C.; Moll, G.; Heuking, L.; Labomh, R. and Benner, M. (1987): Mastitis due to Bacillus cereus in cows treated with a dry cow antibiotic preparation II. Prophylaxis during the dry period in cows with suspected infection. Praktische Tierarzt 68-44. Vet. Bull. 57, 6 (1987).

Horvath, G.; Toth-Marton, E.; Meszaros, J.M. and Quarini, L. (1986): Experimental Bacillus cereus mastitis in cows. Acta Veterinaria Hungarica, 34: 29-35.

Jasper, D.E.; Bushnell, R.B.; Dellinger, J.D. and Stang, A.M. (1972): Bovine mastitis due to Bacillus cereus. J. Am. Vet. Med. Assoc. 160: 750-756.

Johnson, K.M. (1984): Bacillus cereus foodborne illnes-An update. J. Food Prot. 47: 145-153.

Jones, T.O. and Turnbull, P.C.B. (1981): Bovine mastitis caused by Bacillus cereus. Vet. Rec. 108: 272-274.

Martin, J.H.; Stahly, D.P.; Harper, W.J. and Gould, I.A. (1962): Sporeforming microorganisms in selected milk supplies. XVI International Dairy Congr. C. (VIII:1): 295-304.

Nagah Saad, M. (1985): Occurrence of Bacillus cereus in milk and milk products in Assiut City. M.V.Sc. Thesis. Fac. of Vet. Med., Assiut University.

Overcast, W.W. and Atmaram, K. (1974): The role of Bacillus cereus in sweet curdling of fluid milk. J. Milk Food Technol. 27: 233-236.

Schalm, O.W. and Noorlander, D.O. (1957): Experiments observations leading to development of CMT. J. Am. Vet. Med. Assoc. 130-199.

Turnbull, P.C.B.; Jorgenson, K.; Kramer, J.M.; Gilbert, R.J. and Parry, J.M. (1979): Severe clinical conditions associated with Bacillus cereus and the apparent involvement of exotoxins. J. Clin. Pathol., 32: 289-293.

Table (1)
Relationship of CMT and culture status of examined cows and quarters

No. positive / No. tested (%)		
	Quarters	
Animals	Quarters with B.cereus	Positive quarters for CMT and B.cereus
12/40 (30%)	15/160 (9.38%)	9/15 (60%)

Table (2)
Prevalence of B.cereus in udder and faeces of examined cows

No. of animals positive / No. tested (%) in		
Udder	Faeces	Udder and faeces
12/40 (30%)	14/40 (35%)	6/40 (15%)

Table (3)
Percent antibiotic sensitivity (S) of isolated B.cereus from milk and faeces of dairy cows

Type of sample	No. of isolates	Ampicillin S%	Carbencillin S%	Cephalothin S%	Chloramphenicol S%	Clindamycn S%	Erythromycin S%	Gentamicin S%	Neomycin S%	Piperacillin S%	Streptomycin S%	Tetracycline S%	Tobramycin S%
Milk	15	4 (26.7)	4 (26.7)	5 (33.3)	8 (53.3)	6 (40)	8 (53.3)	7 (46.7)	8 (53.3)	7 (46.7)	11 (73.3)	14 (93.3)	6 (40)
Faeces	14	0 (0)	1 (7.1)	3 (21.4)	7 (50)	6 (42.9)	7 (50)	12 (85.7)	7 (50)	4 (28.6)	11 (78.6)	12 (85.7)	7 (50)