Veterinary Medicine (Groups Hathor and Apis)

Bericht über parasitologische Forschung in Ägypten

Prof. Dr. M.-A. Hasslinger

Ehemals Institut für Vergleichende Tropenmedizin und Parasitologie der Tierärztlichen Fakultät der LMU München
Kaulbachstr. 37, D-80539 München

Acht Kurzzeitdozenturen an vier Tierärztlichen Fakultäten in Ägypten (Beni Suef, Cairo, Edfina, Kafr El-Sheik) wurden dazu genutzt, neben der Lehre zusätzlich mit verschiedenen ägyptischen Kollegen eine Reihe von parasitologischen Forschungsprojekten durchzuführen. In diesem Bericht wird allgemein aufgezeigt, wie sich eine fruchtbare Zusammenarbeit zwischen verschiedenen Personen und Institutionen letztendlich darstellen sollte.

Bei **Equiden** war uns die Verbreitung von Gastrodiscus aegyptiacus wichtig, einem im Darm lokalisierten Trematoden, der weitgehend nur in afrikanischen Ländern und auf Madagaskar vorkommt. Für Dictyocaulus arnfieldi hat sich in Untersuchungen von Norden (Kaluobyia) nach Süden (Aswan) bestätigt, dass der Esel den Hauptwirt darstellt und dieser Lungenwurm im Maultier sowie Pferd weniger anzutreffen ist. Kenntnisse zum Befall von Eseln und **Wiederkäuern** (Rind, Wasserbüffel) mit Fasciola gigantica erschienen uns deshalb relevant, weil in Ägypten Leberegel-Infektionen beim Menschen bis zu 43% vorkommen sollen. Bezüglich des Infektionsrisikos für die Bevölkerung hielten wir weiterhin bei **Fleischfressern** vergleichende Untersuchungen (Ägypten, Türkei) an Hunden über Toxocara canis (Larva migrans visceralis) und Ancylostoma caninum (Larva migrans cutanea) für sinnvoll. Der Magenwurm der Katze, Ollulanus tricuspis, war in Ägypten nicht bekannt. Er wurde aber nur übersehen und von uns vor 15 Jahren erstmals bei Katzen in Ägypten bzw. auf dem afrikanischen Kontinent entdeckt; inzwischen ergab sich bei streunenden Katzen (Cairo, Behara) eine Infektionsrate von 27,1%.

Detaillierte Daten findet man bereits in verschiedenen Publikationen dokumentiert, die der speziell daran Interessierte der nachfolgenden Aufstellung entnehmen kann:

1. HASSLINGER, M.-A., H. M. OMAR and A. HASSAN (1984): The occurence of Ollulanus tricuspis, the stomach worm of the cat, in Egypt. Vet. Med. J. 32, 73-75
2. HASSLINGER, M.-A. (1987): The importance of parasites in stray cats in Egypt. J.Egypt. vet. med. Ass. 47, 743-750
3. HASSLINGER, M.-A., H. M. OMAR und M. K. SELIM (1988): Das Vorkommen von Helminthen in streunenden Katzen Ägyptens und anderen mediterranen Ländern. Vet.-med. Nachr. 59, 76-81
4. HASSLINGER, M.-A. (1989): Dictyocaulus arnfieldi in equids: Present situation and future aspects. J. Egypt vet. med. Ass. 49, 445-455
5. HASSLINGER, M.-A. (1991): Toxocara canis as a cause for ‚Larva migrans visceralis'. J. Egypt. vet. med. Ass. 51, 507-517
6. EL-SEIFY, M. A. and M.-A. HASSLINGER (1991): Natural infections of equids with D. arnfieldi in Egypt. J. Egypt. vet. med. Ass. 51, 519-528

7. HASSLINGER, M.-A., Th. M. M. EL-ASSALY and M. K. SELIM (1992): Comparative studies on coprologic results of carnivorous animals in Zoological Gardens in Giza / Egypt and Munich / Germany. Assiut vet. med. J. 26, 102-109
8. HASSLINGER, M.-A., K. I. ASHMAWY and S. A. ABU EL-WAFA (1993): A contribution to Gastrodiscus aegyptiacus (Cobbold, 1876). J. Egypt vet. med. Ass. 53, 299-304
9. HASSLINGER M.-A., A. BURGU, M. A. EL-SEIFY und Th. EL-ASSALY (1993): Vergleichende Untersuchungen zum Helminthenstatus bei streundenden Hunden und seine Bedeutung für die menschliche Gesundheit. Tierärztl. Umsch. 48, 596, 603-606
10. AHMED, Z. G., G. A. HOSSNY, M.-A. HASSLINGER and M. A. EL-SEIFY (1994): Tissue reactions to the Dictyocaulus arnfieldi in Egyptian dunkeys. Beni Suef vet. med. Res. 4, 76-85
11. HASSLINGER, M.-A. und M. A. EL-SEIFY (1996): Eine Studie zum Paramphistomiden-Befall bei Equiden in Ägypten. Berl. Münch. tierärztl. Wschr. 109, 224-226
12. HASSLINGER M.-A., M. A. EL-SEIFY, Th. M. M. EL-ASSALY and N. MOWAFY (1997):Distribution of trematode infections in cattle and buffalo with special reference to liver fluke in human beings. J. Egypt. vet. med. Ass. 57, 617-632

Fate of enterohaemorrhagic Escherichia Coli O157:H7 in buffalo's milk and some of its manufacturing products

El-Kosi O. H. R.
Abdel-Hakiem E. H.
Saad A. H.

Summary

The present study was conducted to trace the survivability of *E. coli* O157:H7 in raw and pasteurized buffaloes' milks, yoghurt and Damietta cheese. The results revealed that the organism populations in raw milk of 8.34-$\log_{10}$ cfu/ml initial inoculum stored at 22±2°C increased slightly through the first day, then decreased gradually from 9.5-$\log_{10}$ cfu/ml at the 2nd day to 2.35- $\log_{10}$ cfu/ml at the 10th day. It could survive at higher counts in the refrigerated raw milk achieving 5.74-$\log_{10}$ cfu/ml by the end of the same holding period. In the pasteurized milk, the tested organism undergone continuous regular decrease from 7.86-$\log_{10}$ cfu/ml at 0 time to 1.4-$\log_{10}$ cfu/ml by the end of the 30 days holding period. In yoghurt, the pathogen survived for 8 days at refrigerated

storage following its preparation. However, its populations reduced from 8.23-$\log_{10}$ cfu/ml at inoculation time to 1.8-$\log_{10}$ cfu/ml at the 8th day. The pH of the product was 4.12 and reached 4.06 through the corresponding period. Concerning the three manufactured cheese varieties (LS, MS and HS), the organism populations decreased sharply during the curd formation, started to increase during the draining period and through the first 4 days of storage at 22±2°C and returned to decrease again through the rest of storage term achieving 2.76-, 3.4 and 0.0-$\log_{10}$ cfu/ml in LS, MS and HS cheeses, respectively. For cheeses held at 4±1°C, the organism persisted at considerably higher counts achieving 7.11-, 4.08 and 4.15-$\log_{10}$ cfu/ml in the 3 cheese varieties, respectively. The inhibitory effect of the high salt percentage on EHEC was more pronounced than that arising from the lowered pH values in all the 3 cheeses. The public health significance of the tested pathogen as well as suggestions for its exposure avoidance via consumption of foods, particularly dairy products, were discussed.

Introduction

Since 1920s, *Escherichia coli (E. coli*) has been isolated from dairy products with variable incidences and counts. Some of its serotypes have been involved in outbreaks of human gastroenteritis (Aureli et al., 1992). As the organism is commonly present in gastrointestinal tract of animals and man, many types of foods including meat products, fish, milk and dairy products, vegetables, baked products, and water would be hazardous if exposed to direct or indirect contamination with faecal materials carrying it, along with improper storage temperature and inadequate heat treatment. As a result, these foods have been associated with gastroenteritis of *E. coli* origin in many countries (Kornacki and Marth, 1982 and Garvani, 1987). The term enteropathogenic *E. coli* has been used rather indiscriminately in the past for strains of *E. coli* which cause infantile diarrhea by different mechanisms (Moon et al., 1979). More recently, it was shown that diarrheogenic *E. coli* strains are now classified on the basis of clinical symptoms, mechanisms of pathogenesis and in some instances, biochemical and serological markers into five categories: enterotoxigenic (ETEC), enteroinvasive EIEC), enterohaemorrhagic (EHEC), enteropathogenic (EPEC) and enteroaggregative-diffuse adherent (EAgg-DAEC) (Donnenberg and Karper, 1992; Gomez et al., 1989 and Giron et al., 1991).

EHEC (O157:H7) has received recently a considerable attention, as it was implicated in several outbreaks of gastroenteritis, with several cases developing haemolytic ureamic syndrome (HUS), haemorrhagic colitis (HC) and thrombotic thrombocytopenic purpura (TTP). Raw milk (Martin et al., 1986), yoghurt (Morgan et al., 1993) and acidic foods such as mayonnaise (Keene et al., 1994) were among the food vehicles incriminated in the EHEC outbreaks. The organism can affect all ages, requires a low inocula (50 viable cells) and can cause death (Neil, 1994). The virulence factor of EHEC is the production of shiga-like toxins (SLTs) or verotoxins (VTs) which have two types, the SLT-I and SLT-II (Konowalchuck et al., 1977 and 1978a & 1978b). The organism and its verotoxins are destroyed by adequate cooking or pasteurization (Verman and Evans, 1991).

In the last decade, efforts were conducted by several investigators on the detection of EHEC in dairy products. In USA, Padhye and Doyle (1991) and Chapman and Wright (1993) similarly isolated EHEC O157: H7 from 10% of the examined raw milk samples. In Germany, Montag (1994); Bockemuehl and Karch (1996) and Perlberg (1996) found that 2.63%, 4% and 3.95% of the examined raw milk samples, respectively, contained the pathogen. On the other hand, Knappstein et al. (1996) detected *E. coli* O157: H- in 9.2% and 59% of the examined heated and raw milk

cheeses, respectively. Abdel-Hakiem et al. (1998) isolated the pathogen of SLT-I and SLT-II producer from a yoghurt sample representing 0.81% of the examined dairy products. Literature on the behaviour of EHEC (O157:H7) in dairy products are nearly still lacking. However, Sharpe et al. (1995) found the organism survived well in refrigerated raw milk and dairy products, as well as low pH products. Reitsma and Henning (1996) reported that the pathogen survived in Cheddar cheese along the manufacuring course and remained viable for 102 days thereafter.

In Egypt, in some instances, milk produced by individual owners in small farms that lack of proper sanitation. In addition, some dairy products as soft cheeses, cream and butter are manufactured under local conditions from raw milk without addition of lactic acid starters or colourant. Such products, if consumed fresh or after being preserved in refrigerated conditions, represent a major source of foodborne illness with any of the virulent *E. coli* serotypes. Therefore, the present investigation is conducted to trace the pathway and fate of EHEC (O157:H7) when being inoculated in raw buffalo's milk, laboratory pasteurized milk, and the milk used in manufacturing of yoghurt and Damietta cheese.

Material and Methods

Preparation of EHEC culture

EHEC (O157:H7) strain of SLT-I and SLT-II producer was obtained from Dr. Aman, I. M, Faculty of Veterinary Medicine, Tanta University to be used in this investigation. The strain was subcultured overnight in trypticase soya broth at 30°C, centrifuged at 1200 rpm for 10 min at 4°C and the cells were washed with sterile saline 2-3 times with centrifugation. Finally, the gathered cells were suspended in sterile saline to be used for inoculation.

Viability of EHEC in raw milk

Two liters of freshly drawn buffaloes' milk were obtained from the dairy farm of the Faculty of Veterinary Medicine, Suez Canal University. Before being inoculated, the milk was analyzed for naturally occurring *E. coli* by streaking 0.1 ml from the prepared tenth fold dilutions over sorbitol MacConkey agar (SMA) plates as described in A.P.H.A. (1992). The milk was then inoculated with the prepared cultures of the chosen organisms to obtain an initial inocula of ca 10^8 cells/ml. The inoculated milk was distributed aseptically after thorough mixing into two sterile stoppered-bottles. The first was placed in refrigerator (4±1°C), while the second was left at room temperature (22±2°C). Then, they were examined at 0 time and daily thereafter for EHEC counts, total bacterial counts (background microflora) and pH values.

Viability of EHEC in pasteurized milk

The laboratory pasteurized buffaloes' milk (63°C for 30 min followed by immediate cooling to <10°C within 5 min) was inoculated with the prepared cultures of the chosen organisms to provide an initial inocula of ca 10^8 cells/ml. The inoculated milk was held in refrigerator (4±1°C) and examined for EHEC counts, total bacterial counts (background microflora) and pH values during the 30 days storage period. Examination was performed daily during the first 5 days, every other day until the 15^{th} day and every third day until the 24^{th} day.

Viability of EHEC in yoghurt

Yoghurt was prepared according to Lampert (1975) from previously heated (90°C) buffaloes' milk. After being cooled to 42°C, the milk was inoculated at a rate of ca 10^8 cells/ml with the prepared cultures of EHEC. Then the starter culture (fresh commercial product made from pasteurized milk having a minimum of 15 days shelf-life) was added at a rate of ca 0.2%. The inoculated milk was distributed in sterile, stoppered glass bottles (150 ml capacity) and incubated at 42°C until the formation of the desired curd. The prepared yoghurt was store in the refrigerator (4±1°C). Samples were taken before the addition of the starter and EHEC cultures, after their addition, after curd formation, daily until the 9^{th} day and every other day until the end of the 15 days investigation period to be examined for EHEC counts and pH values.

Viability of EHEC in Damietta cheese

Three lots of Damietta cheese were prepared from partially raw and heated (90°C) buffaloes' milk according to the traditional method of making such type of cheese in Egypt; the procedure described by Fahmi and Sharara (1950). The milks were inoculated with the test organism at a rate of ca 10^8 cfu/ml. Sodium chloride was added at concentrations of 1% (light salted "LS"), 3% (medium salted "MS") and 5% (high salted "HS"). Rennet extract was added according to the manufacturer's direction at a rate of 0.2%, and the formed curd was left to drain its whey for 24 h at room temperature. Each lot of prepared cheese, with its whey, was divided into two equal portions, one of which was left at room temperature (22±2°C) while the other was kept in refrigerator (4±1°C). Samples to be examined for EHEC and pH values were taken before inoculating the test organism, immediately after inocultion, after setting and curd formation, after draining the whey and periodically through the storage period of every type of the prepared cheeses.

Enumeration of EHEC and total bacterial count (TBC)

A quantities of 0.1 ml of tenth-fold serial dilutions of milk and milk products were streaked on sorbitol MacConkey agar (SMA) and incubated at 32±1°C for 24 hours. When the counting procedure fail to find the characteristic colonies of EHEC from the dilutions as well as from the original sample, isolation trials using trypticase soya broth (TSB) overnight cultures were performed (A.P.H.A., 1992).

Total bacterial counts (TBC) of raw and pasteurized milk were determined according to A.P.H.A. (1992).

Measurement of pH

The pH of the tested samples was measured using Jenway 3051 pH meter supplied with standard combination glass electrode. The apparatus was calibrated before each measure using standard buffer solutions pH 4.00 and pH 7.00 at 25°C.

Results and Discussion

Viability of EHEC in raw milk

Populations of the EHEC (O157:H7) test strain in the raw buffaloes' milk held at room temperature (22±2°C) and in refrigerator (4±1°C) are shown in Fig. 1. For milk left at room temperature (Fig. 1/A), it is very obvious that, EHEC O157:H7 populations remained unchanged during the first 5 days of incubation although the milk is curdled at the second day (pH 4.55). At the end of the holding period (10 days), the counts showed ca 5-$\log_{10}$ decrease. Such decrease can be assumed to be due to both competitive microbial growth, evidenced by the very high counts of background microflora achieving ca 14-$\log_{10}$ cfu/ml at the end of the 8th day, and the very low pH; reached 3.96 by the end of the holding period.

Concerning the refrigerated milk, it is easy to declare from Fig. 1/B that there was a somewhat regular slow reduction in the numbers of the inoculated organism along the term of incubation. However, the decline rate was greater during the first day, achieving ca 1.8-$\log_{10}$. Such decrease in EHEC populations was accompanied by a corresponding regular increase in the background bacteria from 9.9-$\log_{10}$ cfu/ml at 0 time to 12.5-$\log_{10}$ cfu/ml by the end of the holding period. These findings lie in a very close agreement with those reported by Sharpe et al. (1995) and Heuvelink et al. (1998) upon testing the survivability of *E. coli* O157:H7 in raw cow's milk. Unfortunately, from such obtained results one can expect that raw milk, whatever its type, may play a significant role in disseminating *E. coli* O157:H7 among consumers. This is particularly true within the circumference of underdeveloped countries where the tradition of consuming milk at its raw state still common.

Viability of EHEC in pasteurized milk

Populations of the *E. coli* O157:H7 test strain in the inoculated pasteurized buffaloes' milk held in refrigerator (4±1°C) are shown in Fig. 2. It is very clear from the data assembled in the figure that, *E. coli* O157:H7 populations survived for the whole test period (30 days). They showed gradual regular decrease from the beginning (7.82-$\log_{10}$ cfu/ml) until the end of the holding period (1.4-$\log_{10}$ cfu/ml). The pH of the milk did not altered significantly through whole storage term as it was 6.6 at 0 h and achieved 6.28 at the 30th day. The background microflora increased at first achieving 9.32-$\log_{10}$ cfu/ml through the 3rd day after which they undergone a marked decrease along the rest of the investigation period. This decline in both *E. Coli* O157:H7 and total bacterial counts is, of course, due to the unfavourable low temperature of incubation. At such temperature the majority of microorganisms loss their ability to grow and only what is called "psychrotrophs" can survive. Indeed, in order to produce pasteurized milk free from the pathogen in concern, properly pasteurized product protected from any sort of post pasteurization contamination is an essential requirement.

Viability of EHEC during preparation and storage of yoghurt

Careful inspection of the results shown in Fig. 3 reveals that *E. coli* O157:H7 populations have undergone considerable reduction (ca 2 $\log_{10}$) by the end of yoghurt preparation. Such reduction occurred during the 3 hours incubation at 42°C. Simultaneously, the pH of the product has decreased sharply from 6.16 upon adding the starter culture to 4.12 by the end of curd formation, explaining why did such decline in EHEC counts has taken place. There was continuous regular decrease in the numbers of the pathogen from day to day for upto the 9th day, after which 3 successive, every other day, isolation trials have failed to find the organism. This was certainly because it can no longer persist in the product. The pH of the yoghurt continued to de-

crease during its refrigerated storage, but to a much lesser extent than before. By the end of the investigation period, the pH of yoghurt has reached 3.96. These findings support what has been reported by Hudson et al. (1997), Abdel-Ghany and Hosny (1998) and El-Hawary and Aman (1998). A faster disappearance of *E. coli* O157:H7, occurring just after the curd formation of yoghurt, was recorded by Dineen et al. (1998). Such difference could be attributed to the smaller initial inoculum (10^5 cfu/ml milk) added upon processing the yoghurt and/or variability in the virulence among the tested strains. However, the same authors established that *E. coli* O157:H7 was recovered at <10 cfu/g at 12 days from commercial yoghurt (pH 4.0) inoculated by the same organism at a rate of 10^3 cfu/g. So, they concluded the presence of *E. coli* O157:H7 cells in yoghurt is more likely to reflect post processing contamination than survival of the organism through the yoghurt fermentation process. Indeed, the obtained findings suggest that both ways can lead to its presence in yoghurt during the few days of its refrigerated storage following processing. The persistence of the pathogen in yoghurt at low pH for such period confirmed the implication of yoghurt in an outbreak of HC in London as reported by Morgan et al. (1993).

Viability of EHEC during manufacturing and storage of Damiette cheese

With regard to the cheeses stored at room temperature assembled in Fig. 4/A, one can recognize that *E. coli* O157:H7 is considerably inhibited as the salt content of the cheese increased. In the LS cheese, populations of the inoculated pathogen remained unchanged for as long as 28 days storage. The organism appeared as if unaffected by the lowered pH (3.59) of the cheese at that time. Populations of *E. coli* O157:H7 showed a marked decrease during the rest of the storage term from 7.08-$\log_{10}$ cfu/g at the 28th day to 2.76-$\log_{10}$ cfu/g at the 70th day. In MS cheese, the organism behaved more or less similarly as in LS one. Concerning HS cheese, the effect of the high salt content on the test organism was very pronounced from the first moment. It was reflected by a ca 3.5-$\log_{10}$ cfu/g decrease in comparison with the former ones (LS & MS) during course of manufacturing and storage. The test organism could not survive until the end of its storage term. Actually, we have to point out here that there were a marked fungal growth (unpresented data) in all the 3 types during the last stages of storage (40th -70th day). Perhaps, this phenomenon (competitive growth) was the reason of the higher survivability of *E. coli* O157:H7 in LS and MS cheeses than in HS one, because it was more pronounced in the former than in the later two cheeses.

Concerning the refrigerated cheeses (Fig. 1/B), the organism populations in the LS cheese showed a regular gradual slight increase during the first 4 weeks after which they started to decrease. There were 8.5-$\log_{10}$ cfu/g at the beginning of the refrigerated storage, 9.7- $\log_{10}$ cfu/g at the 28th day and reached to 7.1- $\log_{10}$ cfu/g by the end of the storage term. In correspondance, the pH has undergone a steady gradual decrease from ca 5.5 at the first day to ca 4.5 by the end of the storage term. A somewhat lower EHEC counts and higher pH values were registered through the whole refrigerated storage term of the MS type of cheese. Therefore, the inhibitory effect of the higher salt concentration upon EHEC in the MS cheese overcomed that of the lower pH values in the LS one. In the HS cheese, EHEC populations undergone a significant decrease through the manufacturing course, but remained a more or less unchanged during the refrigerated storage term. In comparison with the other 2 cheese types, HS cheese appeared to be the most inhibitory one to the tested strain, although its pH was relatively higher. In general, such obtained results revealed that the higher the salt content in the cheese, the higher the degree of inhibition of EHEC regardless of the product pH. These findings to have some degrees of similarity with

those reported by Reitsma and Henning (1996) and Ramsaran et al. (1998) upon their tested cheese varieties. However, Glass et al. (1998) recorded a comparably lower *E. coli* O157:H7 in the inoculated processed cheese slices. The obtained results also revealed that the storage temperature had a marked effect upon the survival rate of the tested strain. If the temperature is high, it favours the growth of lactic acid producing bacteria resulting in high death rate of the organism.

In conclusion, these findings gave us a clear idea about the high possibility with which dairy products contaminated by such powerful pathogen be hazardous to consumers due to its longevity therein. The most important factor in the prevention of gastroenteritis in human by pathogenic *E. coli* is to prevent contamination of food and water, directly or indirectly, by faecal matters. This can be achieved by developing effective sanitation in water supplies, and treatment and disposal of sewage. Another factor to be considered is the prevention of contamination of foods due to poor personal hygiene by people who are shedding the pathogen. Here, in Egypt, we are in great need to stop the manufacturing of dairy products from raw milk or inadequately heat treated one. Finally, we have to recognize that if the pathogen is present in very small initial numbers in a food, temperature abuse can facilitate multiplication of cells to high levels necessary for disease symptoms. Thus foods, including, of course, milk and various types of dairy products should be refrigerated or eaten quickly (Kornacki and Marth, 1982 and Garvani, 1987).

References

Abdel-Ghany, S. and Hosny, I. M. (1998): Fate of *Escherichia coli* O157:H7 in yoghurt and as affected by nisin or pH of synthetic medium and milk. Arab Universities J. of Agricult. Sci. 6 **(1)** 211-221.

Abdel-Hakiem, E. H.; Leyk, W. and Neumann, H. (1998): Prevalence of *Escherichia coli* with special reference to enterohaemorrhagic *E. coli* (EHEC) in some foods and water. In proceeding-1 of the 8th Sci. Cong. 15-17 Nov., Fac. Vet. Med. Assiut, Egypt. pp 98-117.

A.P.H.A. (1992): Standard Methods for the Examination of Dairy products. American Public Health Association 16th Ed. Washington, DC.

Aureli, P. A.; Costantini, L.; Fenicia, M. and Rainaldi, L. (1992): Occurrence of pathogenic *Escherichia coli* other than O157:H7 from patients in Germany. Zbl. Bakt. 276: 189-195.

Bockemuehl, J. and Karch, H. (1996): Zur aktuellen Bedeutung der enterohemorrhagischen *Escherichia coli* (EHEC) in Deutschland. Bundesgesundheitsblatte 290-296.

Chapman, P. A. and Wright, D. J. (1993): Untreated milk as a source of verotoxigenic *Escherichia coli* O157:H7. The Vet. Record 14: 171-172.

Dinnen, S. S.; Tackeuchi, K.; Soudah, J. E. and Boor, K. J. (1998): Persistence of *Escherichia coli* O157:H7 in dairy fermentation systems. J. Food Prot. 61 **(12)** 1602-1608.

Donnenberg, M. S. and Karper, J. B. (1992): Minireview: Enteropathogenic *Escherichia coli*. Infect. Immun. 60: 3953- 3961.

El-Hawary, I. I. and Aman, I. M. (1998): Survival characteristics of *Escherichia coli* serotype O157:H7 in ice cream and yoghurt. In proceeding-1 of the 8TH Sci. Cong. 15-17 Nov., Fac. Vet. Med. Assiut, Egypt. pp 75-82.

Fahmi, W. H. and Sharara, A. H. (1950): Studies on Egyptian Damietta cheese. J. Dairy Res. 17: 312-327.

Garvani, R. B. (1987): Food science facts. Dairy Food Environ. Sanitation 7: 20-26.

Giron, J.A.; Jones, T.; Velasco, F., Munoz, E. Zarte, L.; Karper, J.P. Shoolink, G.K. and Riley, L.W.(1991): Diffuse adhering *Escherichia coli* (DAEC) as a putative pathogen in Mayan children in Mexico. J. Infect. Immun. 163: 507-513.

Glass, K. A.; Kaufman, K. M. and Johnson, E. A. (1998): Survival of bacterial pathogens in pasteurized process cheese slices stored at 30°C. J. Food Prot. 61 **(3)** 290-294.

Gomez, T.A.T.; Blake, P.A. and Trabulsi, L.R. (1989): Prevalence of *Escherichia coli* strains with localized, diffuse and enteroaggregative adherence to HeLa cells in infants with diahrrea in matched control. J. Clin. Microbiol. 27: 266 - 269.

Heuvelink, A. E.; Bleumink, B.; van den Biggelaar, F. L. A. M.; Te Giffel, M. C.; Beumer, R. R. and de Boer, E. (1998): Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157:H7 in raw cow's milk in the Netherlands. J. Food Prot. 61 **(12)** 1597-1601.

Hudson, L. M.; Chen, J.; Hill, A. R. and Griffiths, M. W. (1997): Biolumiscence: A rapid indicator of *Escherichia coli* O157:H7 in selected yoghurt and cheese varieties. J. Food Prot. 60 **(8)** 891-897.

Keene, W. E.; McAnulty, J. M.; Hoesly, L. P. and Fleeming, W. D. (1994): A swimming associated outbreak of haemorrhagic colitis caused by *Escherichia coli* O157:H7. N. Engl. J. Med. 331 579-584.

Knappstein, K.; Hahn, G. and Heeschen, W. (1996): Untersuchungen zum Vorkommen von Verotoxinbidenen *Escherichia coli* (VTEC) in Weichkaese mit Gensonden. Arch. fuer Lebensmittelhygiene. 47: 57-60.

Konowalchuck, J.; Dickie, N.; Stavric, S. and Speirs, J. L. (1978 a): Comparative studies of five heat-labile toxic products of *E. coli* cytotoxin. Infect. Immun. 22: 644-648.

Konowalchuck, J.; Dickie, N.; Stavric, S. and Speirs, J. L. (1978 b): Propertiies of an *E. coli* cytotoxin. Infect. Immun. 20: 575-577.

Konowalchuck, J.; Stavric, S. and Speirs, J. L. (1977): Vero response to a cytotoxin of *E. coli*. Infect. Immun. 18: 775-779.

Kornacki, J. L. and Marth, E. H. (1982): Foodborne illness caused by *Escherichia coli*: a review. J. Food Prot. 45: 1051-1061.

Lampert, L. M. (1975): Modern Dairy Products. Third Ed. Chemical Publishing Company, Inc. New York.

Martin, M.L.; Shipman, L.D.; Wells, J.D.; Potter M.E.; Hedberg, K.; Wachmuth, I.K.; Tauxe, R.V.; Davis, J.P. and Tilleli, J. (1986): Isolation of *E.coli* O157:H7 from dairy cattle associated with two cases of hemolytic uremic syndrome. Lancet. 11: 1403.

Montag, M. (1994): Zur Haeufigkit Shiga-like Toxin bildner Enterobacteriaceae in Lebensmitteln in Nord-deutschland, Untersucht mit der Polymerase-kettenreaktion (PCR). Dissertation, Universitaet Hamburg.

Moon, H. W., Isaacson, R. E. and Pohlenz, J. (1979): Mechanism of association of enteropathogenic *Escherichia coli* with intestinal epithelium. Am. J. Clin. Nutr. 32: 119-127.

Morgan, D.; Newman, C. B.; Hutchinson, D. N.; Walker, A. M.; Rowe, B. and Majid, F. (1993): Verotoxin producing *Escherichia coli* O157: H7 infections associated with consumption of yoghurt. Epidemiol. Enfect. 111: 181-187.

Neil,. M.A. (1994): E.coli O157:H7 time capsule: What do we know and when did we know it. Dairy Food Environ. Saintation. 14: 374-377.

Padhye, N. V. and Doyle, M. P. (1991): Rapid procedure for detecting enterhaemorrhagic E. coli O157: H7 in foods. Appl. Environ. Micobiol. 57: 2693-2698.

Perlberg, K. W. (1996): Persoenliche Mitteilung. (cited after Kuntze et al. 1996. Arch. fuer Lebensmittelhygiene. 47: 141-144).

Ramsaran, H.; Chen, J.; Brunke, B. Hill, A. and Griffiths, M. W. (1998): Survival of bioluminescent *Listeria monocytogenes* and *Escherichia coli* O157:H7 in soft cheeses. J. Dairy Sci. 81 **(7)** 1810-1817.

Reitsma, C. J. and Henning, D. R. (1996): Survival of enterohaemorrhagic *Escherichia coli* O157:H7 during the manufacture and curing of Cheddar cheese. J. Food Prot. 59: 460-464.

Sharpe, J. M.; Reilly, W. J.; Coia, J. E.; Curnow, W. B. and Synge, B. A. (1995): Escherichia coli O157:H7 infection in Scotland; an epidemiological overview 1984-1994. PHLS Microb. Dig. **12** 134-140.

Verman, A. H. and Evans, M. G. (1991): Foodborne Pathogenes. Wolfe Publshing Ltd., England.

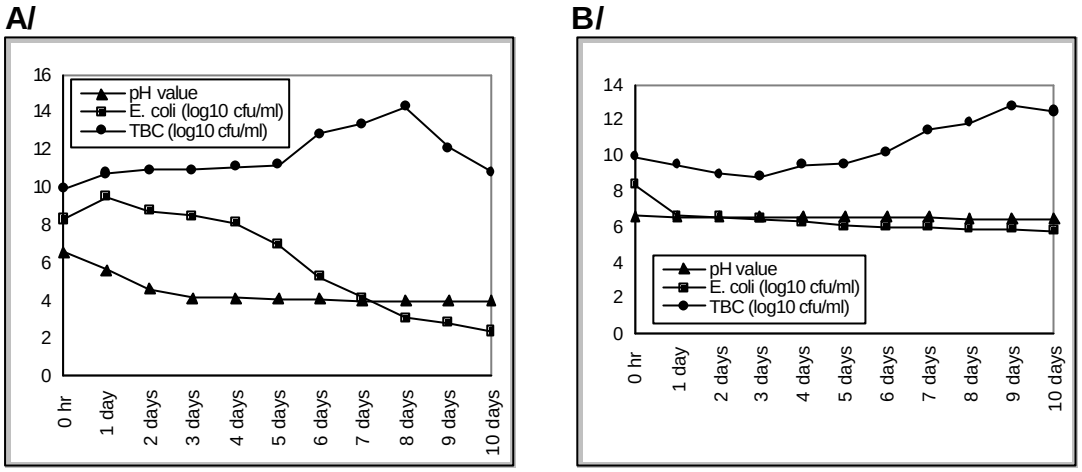

Fig.1 Survival period of EHEC in raw buffalo's milk held at:
A/ 22±2°C (room temperature)---B/ 4±1°C (refrigerator)

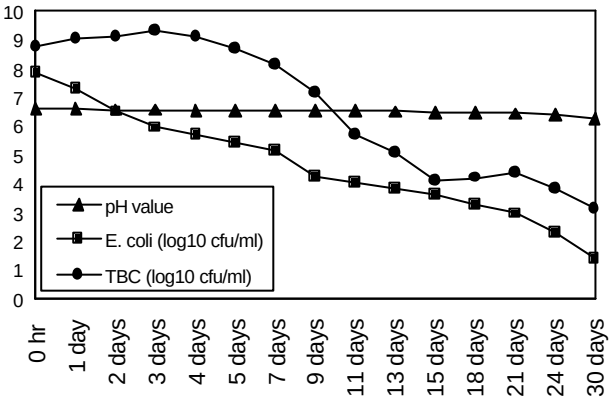

Fig.2 Survival period of EHEC in pasteurized milk held at (4±1°C).

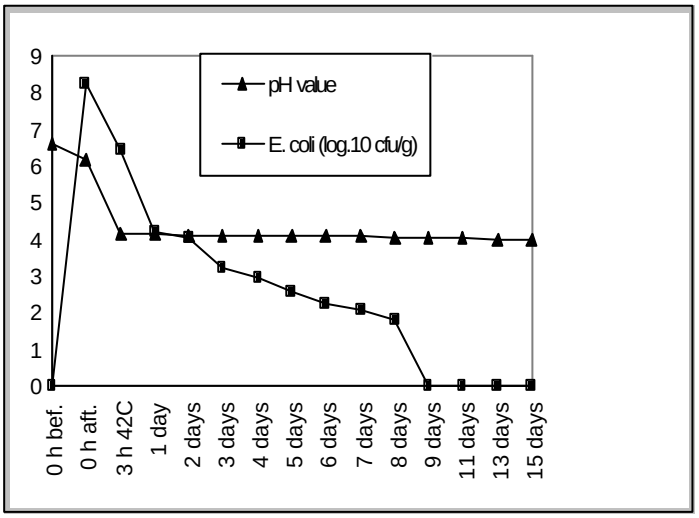

Fig.3 Survival period of EHEC during preparation and storage of yoghurt
0 h bef.= before inoculation of EHEC and starter
0 h aft.= after inoculation of EHEC and starter
3 h 42C= after 3 hours incubation at 42°C

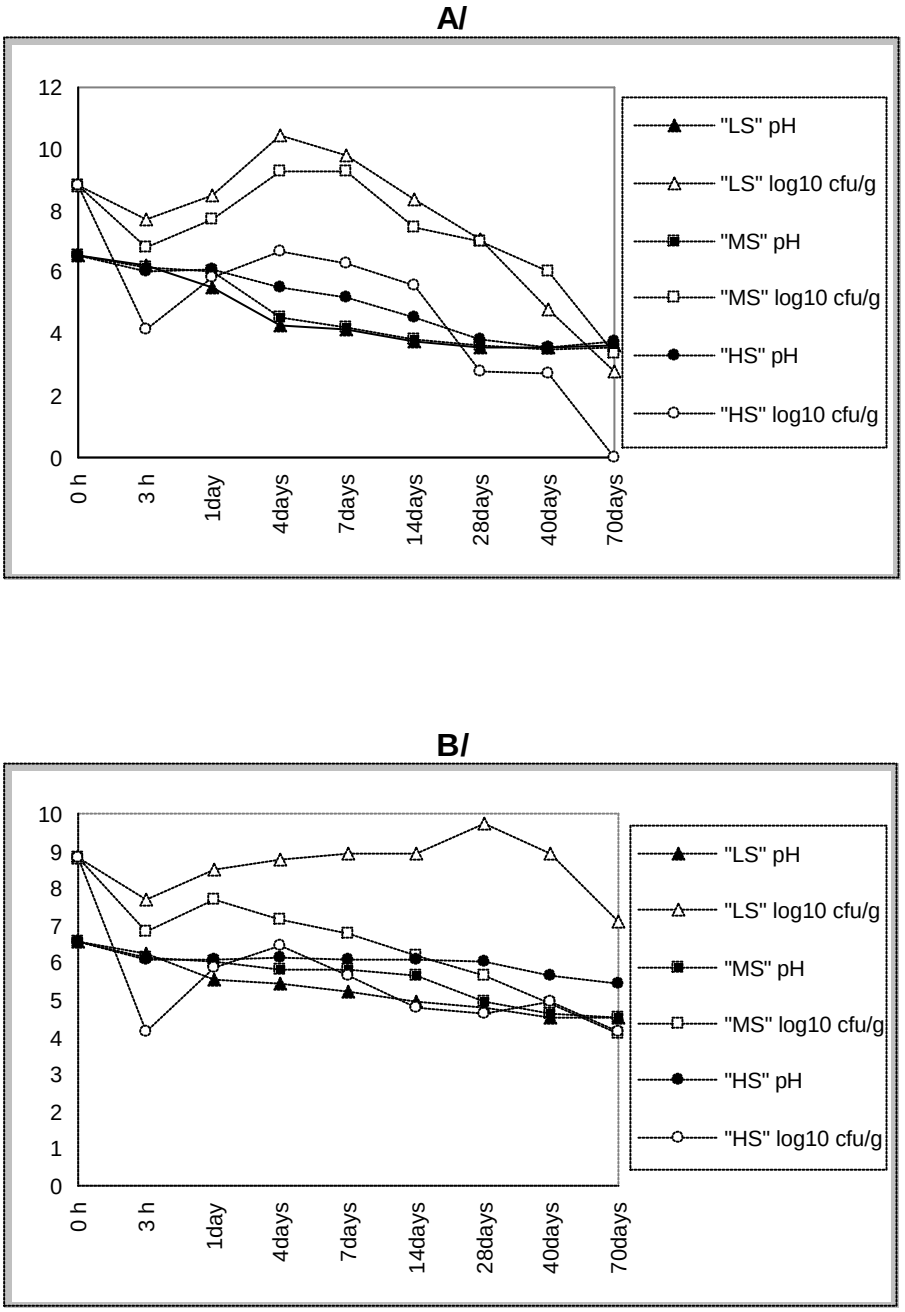

Fig.4 Survival period of EHEC during preparation and storage of Damietta cheese at: A/ 22±2°C (room temperature)---B/ 4±1°C (refrigerator)

0 h = from the milk just after adding the prepared culture

3 h = from the formed curd 3 hours later at 32°C

1 day = from the prepared cheese after draining the whey 24 hours later at room temperature.

Studies on the *Pseudomonas aeruginosa* isolated from fleece rot in sheep suffering from dermatitis in northern & middle Jordan

Dr. Saeb N. El-Sukhon

Faculty of Pharmacy and Medical Technology
Al-Zaytoonah University
Amman – Jordan

Abstract

A total of 162 sheep fleece samples (7%) were bacteriologically examined. Seventy nine sheep of them showed clinical signs in which exudation and abcessation were recorded in 21 (13%) sheep, while fleece coloration was observed in 18 (11 %) and mixed clinical signs in 7 (4 %). The rest 83 (51 %) sheep showed no obvious clinical signs. Seventeen *Pseudomonas aeruginosa* isolates were cumulated. The isolates were identified biochemically and their susceptibility toward 16 commonly used antibiotics was determined. The resistance toward amikacin, ciprofloxacin and the norfolaxcin was determined in 11.7%, 23.4 % and 29.4%, respectively while the knanmycin, streptomycin, tetracycline, amoxycillin, erythromycin and the co-trimoxazol were ineffective against more than 90% of the *P. aeruginosa* isolates. Intermediate resistance against each of the norfloxacin, ciprofloxacin, enrofloxacin, kanamycin, streptomycin, tetracyclin, amocycillin, erythromycin, ampicillin, neomycin, doxicyclin, nalidixic acid, amikacin, nitrofuantoin was recorded among 6, 7, 5, 5, 2, 1, 1, 1, 2, 3, 7, 1 and 2 of the examined *P. aeruginosa* isolates.

Fourteen (82%) isolates were recorded as serum resistant and their count after 1-3h incubation in the sheep and calf sera, increased by 2-2.9 and 2.5-3.5 logs, respectively.

Introduction

Sheep fleece rot is an exudative bacterial dermatitis associated and influenced by the low hygienic raising conditions such as prolonged wetting of the skin (2, 8). *P. aeruginosa* seems to be the main causative organism of fleece rot and it was concluded that it contributes in the disease complex. The importance of *P. aeruginosa* which was associated with increased severity of fleece rot and subsequent flystrike can not be underestimated and warrants consideration for inclusion it in fleece rot diagnosis and control trials such as vaccination (8). Experimentally, the sheep which were inoculated cutaneously with *P. aeruginosa* developed rapidly a green coloration while the animals which were kept dry, showed no signs of dermatitis (2). This coloration was associated with an outpouring of serous exudates in the fleece rot lesion and with the formation of microabscesses (2).

On the other side, although the *P. aeruginosa* was evaluated as possible cause, in part, to the disease complex, its role and severity in the fleece rot should not be underestimated (8). It should also be taken in consideration in the diagnosis of the fleece rot as well as in its control trials e.g. vaccination (8).

Serologically, the sheep which were wetted and inoculated with *P. aeuginosa*, unlike the dry animals, showed a serologic reactivity (2). Such seroreactivity was against the outer rather the inner membranous protein of the bacterium (2).

P. aeruginosa is among the bacteria, which show a remarkable resistance toward many antibiotics (1, 5). Clinically, significant *P. aeruginosa* strains were found resistant to eight relatively new antibiotics (1). Such a resistance ranged from 9.1% to 31.9% toward the individual antibiotics (1). Combination of two or more antibiotics is likely to be needed to assign the antibiotic treatment protocol. For example, combinations of the gentamicin, lincomycin, spectinomycin and tylosin or the combination of penicillin, streptomycin, lincomycin, spectinomycin and minocycline were tried but yet *P. aeruginosa* recovered by *in-vitro* testing of such combination (5, 6).

Serum-resistance which counteracts the bactericidal effect of the complement system is an important factor in the pathogenicity of *P. aeruginosa* (12). Thirty seven percent of clinical isolates were found to be serum resistant (13). However, such a resistance was attributed to certain serotypes (12) where the serotyping, along with the isolation site, antibiotic resistance, and beta-lactamase production were approached to determine the profiles of *P. aeruginosa* from clinical isolates (7, 14, 12).

Since, in Jordan, no information is available about this affection, this study aims to determine the prevalence of fleece rot dermatitis among sheep herds in the northern and middle sectors of Jordan, to isolate the bacterial causation with special reference to the *Ps. aeruginosa* and to determine the antibiotic sensitivity and the serum resistance patterns of the isolates

Materials and Methods

A total of 162 wool samples were aseptically collected from 32 sheep herds(2273 heads) . Collection of the samples was reconsidered, as possible, to be from animals showing abnormal skin problems especially from those having skin/wool discoloration or associated with an exudates and abscesses.

The samples were processed, promptly. They were cultivated on different enriching / differential media, namely: *Pseudomonas* selective agar, Sheep Blood Agar, Mac-Conkey Agar, Tryptic Soy Agar and Tryptic Soy Broth. All of the media were from OXOID. The media were incubated at 37^{o}C for 24-48 h under aerobic conditions.

A total of 17 (10.5%) isolates showing the general characteristics of the *Pseudomonas aeruginosa,* specially the production of greenish/bluish pigmentation and/or the characteristic fruity odor, were cumulated.

For confirmatory diagnosis and for characterization the isolates, different biochemical reactions were determined, namely: oxidase production, growth at low (5^{o}C) and high (42^{o}C) temperature, citrate utilization, OF (glucose fermentation), sugar fermentation (fructose, mannitol and xylose), nitrate reduction, gelatin liquefaction, urease production, tween hydrolysis and the motility reactions according to Koneman (9) and Cowan & Steel (4). Sensitivity to antibiotics was conducted by determination of the minimum inhibitory concentration in solid medium according to the guidelines of the National Committee for Clinical Laboratory Standards (11).

For determination of their serum resistance, blood of 6 healthy sheep as well as of 3 healthy young calves was collected and the serum of each species was pooled. To assay (15) the serum resistance of the *P. aeruginosa* isolates, 80% of each untreated and heat treated (56^{o}C/30 min) serum was used. The serum heating aims to inactivating the complement bactericidal components. An 18h old tryptic soy broth culture of each isolate was subcultured into a tryptic soy broth and was grown to the mid-log

phase. Cells (approximately 3×10^7/ ml) were added directly to treated and untreated sera and incubated at 37°C for certain intervals. After elapsing of 0, 1, 2 and 3 hours, the viable bacterial count was determined, in triplicate, by ten folds dilution (15).

Results

A total of 162 samples (7%) were examined (Table 1) from which, 17 *P. aeruginosa* isolates were cumulated. The samples were collected almost equally from both sexes but the majority (127 samples) were from elder sheep (1-2 years old). Clinical signs could be observed in 79 sheep. The exudation and abcessation were recorded in 21 (13%), the coloration in 18 (11 %) and mixed clinical findings were recorded in 7 (4 %). The majority, 83 (51 %) of the examined sheep showed no obvious indicative clinical signs.

The antibiotic resistance patterns of the 17 *P. aeruginosa* isolates against 16 antibiotics are displayed in Table 2. The recent antibiotics like the amikacin, ciprofloxacin and the norfolaxcin were not fully effective where resistance against them was determined in 11.7%, 23.4 % and 29.4%, respectively. On the other side, the antibiotics of old generation like the knanmycin, streptomycin, tetracycline, amoxycillin, erythromycin and the co-trimoxazol which were ineffective against more than 90% of the tested *P.aeruginpsa* examined strains. However, intermediate resistance against each of the norfloxacin, ciprofloxacin, enrofloxacin, kanamycin, streptomycin, tetracyclin, amocycillin, erythromycin, ampicillin, neomycin, doxicyclin, nalidixic acid, amikacin, nitrofuantoin was recorded among 6, 7, 5, 5, 2, 1, 1, 1, 2, 3, 7, 1 and 2 of the examined *P. aeruginosa* isolates.

Figures 1 and 2, show the serum resistance patterns of the 17 *P. aeruginosa* strains. By using the heated (inactivated) sheep serum (Fig. 1), the count of all 17 isolates increased by 2 - 3.1 logs while the count only of 3 isolates incubated in the untreated serum decreased by 0.7 - 1.3 logs. The other 14 (82%) strains were recorded as resistant and their count increased in 2-2.9 logs. By using the calf serum, almost the same pattern among the 17 isolates was recorded. However, the same previously notified 14 (82%) resistant strains showed, relatively, higher count which was ranging from 2.5-3.5 logs (Fig. 2) while the count of sensitive ones decreased in 1-1.8 logs.

Discussion

Wool production is a strategic target of sheep raising. Certain factors may predispose the skin affection which results to dermatitis and consequently to lowering of the fleece quality (2, 8). In this study, about 49% of the examined sheep showed various skin/fleece denaturations (table 1) from which about 13% were having clinical signs in form of exuadation and abcessation. The incidence of fleece rot increases in humid environment and/or in wet skin (2, 8) especially among the unvaccinated (8) herds. In this study, the *P. aeruginosa* was isolated in 10.5% from the examined samples which indicates its remarkable role (2) in sheep dermatitis and subsequently in the development of fleece rot in Jordan. This is strongly predicted, especially that 13 out of the 17 (82%) isolates were cumulated in the period between December-April months which are wet and/or humid months in Jordan (2, 8). Indeed, in March-April months in which the atmospheric temperature starts to increase, 6 (35%) isolates were cumulated and the role of flies in spreading of the infection among herds (8) can not be excluded.

In Jordan, lack of vaccination attempts (8) for competing such affections, may add some other factor in this sheep health problem. On the other hand, unlike the sex, the age can be counted as another predisposing factor since the *P. aeruginosa* was mostly isolated from the B and C age groups i.e. elder sheep (table 1).

P. aeruginosa is well known in its resistance against a wide spectrum of antibiotics. In this study, the antibiotic susceptibility of the isolated *P. aeruginosa* strains was tested toward 16 different antibiotics which were comprising recent as well as antibiotics of old generations (Table 2). In the UK, about 18.5% of the *P. aeruginosa* were resistant, or had reduced susceptibility, to some recent antibiotics (3). Although the new antibiotics used in this study, namely: amikacin, ciprofloxacin and the norfolaxcin were effective in about 82%, 77% and 71%, respectively and a remarkable number of the susceptible isolates showed, however, an intermediate susceptibility. These results indicate the diminishing of efficacy of these antibiotics against the *P. aeruginosa*. Such findings, indeed, ring the bell about the merging and the developing of some resistance against these new antibiotics, especially that the antibiotic misuse is problematic to the health authorities/enterprises in the world including this country. Synergistic combination of two or more antibiotics is commonly, *in-vitro*, investigated (10). The ciprofolaxcin combined with the imipenem did not result to improving the effect of the ciprofolaxcin on the *Pseudomonas sp.* while the addition of a third rifampicin antibiotic, results to remarkable synergistic effect (10). In this study, some isolates displayed a high (< 90%) resistance against the majority of the old antibiotics. However, the rest 10% of isolates, which were recorded as susceptible ones, were, indeed, having an intermediate susceptibility. Such findings were obviously, recorded toward the tetracycline, amoxicillinm, erythromycin and ampicillin (Table 2).

The bactericidal effect of the serum is mainly referred to the complement effect. In this study, serum resistance of the isolated *P. aeruginosa* was determined against sera of two animal species, namely: the sheep from which the *P. aeruginosa* was isolated and the calf as a foreign species. Although the 14 serum resistant strains showed the same resistance patterns toward the sera of both sources, their count in the calf serum was determined, relatively, with higher logs (2.5-3.5 logs) than that in the sheep serum (2.5 - 2.9 logs). On the other side, the viable count of each of the 3 (18%) serum sensitive isolates was decreased and was determined in lower 1 - 1.8 logs when they were exposed to the untreated calf serum.

This is the first study deals with sheep dermatitis/fleece rot problem in Jordan. The obtained results indicate the importance of further investigation to explore the different circumstances affecting such sheep infection especially that sheep migration between Jordan and the neighbouring countries is not uncommon and does undergone deficient control / quarantine measures.

References

1) Bonfiglio-G, Carciotto-V, Russo-G, Stefani-S, Schito-GC, Debbia-E, Nicoletti-G, 1998. Antibiotic resistance in *Pseudomonas aeruginosa,* An Italian Survey. J. Antimicrob. Chemotherapy, l 41,(2), 307-310

2) Chin-JC, Dai-Y, Watts-JE, 1995. Antibody-response against *Pseudomonas aeruginosa* membrane-proteins in experimentally infected sheep. Vet. Mic., l 43, (1), 21-32

3) Chen-HY, Yuan-M, Livermore-DM, 1995. Mechanisms of resistance to beta-lactam antibiotics amongst *Pseudomonas aeruginosa* isolates collected in the UK in 1993. J. Med. Microbio.,43, (4), 300-309,

4) Cowan & Steel, 1975. Manual for identification of medical bacteria. 2nd ed., Cambridge University Press, Cambridge.
5) Eaglesome-MD, Garcia-MM, 1995. Comparisons of antibiotic combinations to control *Pseudomonas aeruginosa* in bovine semen; Can. J. Vet. Res.Rev., 59(1), 73-75
6) Gerceker-AA and Gurler-B,1995. *In-vitro* activities of various antibiotics, alone and in combination with amikacin against *Pseudomonas-aeruginosa.* J. Antimicrob. Chemotherapy. 36, 707-711
7) Gogolewski-RP, Nicholls-PJ, Mortimer-SI, Mackintosh-JA, Nesa-M, Ly-W, Chin-JC, 1996. Serological responses against *Pseudomonas aeruginosa* in merino sheep bred for resistance or susceptibility to fleece rot and body strike. Austr. J. Agri, 47(6),917-926
8) Kingsford, N.M. and Raadsma, H.W., 1997. The occurrence of *Pseudomonas aeruginosa* in fleece washings from sheep affected and unaffected with fleece rot. Vet. Microbiol., 54 (3-4), 275-285
9) Koneman, 1983. Color Atlas and Textbook of Diagnostic Microbiology, Lippincott, Philadephia,
10) Kumar A, Wofford-McQueen R, Gordon RC, 1989.Ciprofloxacin, imipenem and rifampicin: in- vitro synergy of two and three drug combinations against *Pseudomonas cepacia.* J Antimicrob Chemother., 23(6):831-5
11) National Committee for Clinical Laboratory Standards, 1984. Performance standards for antimicrobial disk susceptibility tests, ed. 3.
12) Nippon Seikeigeka, 1991. Study on serum-sensitivity of *Pseudomonas aeruginosa*. Gakkai Zasshi, 65:909-17
13) Pajdak E, Szkarlat A, Kobylarz K, 1990. Characteristics of clinical isolates of *Pseudomonas aeruginosa*. I. Resistance to serum bactericidal effect and production of proteolytic enzymes. Arch Immunol Ther Exp (Warsz), 38, 309-14
14) Ravaoarinoro-M, Mohapatra-S, Shore-J, Rawal-S, Omri-A; Yaghi-J, Oriol-F.,1996. Serotyping clinical isolates of *Pseudomonas aeruginosa* in relation to infection site antibiotic susceptibility and beta-lactamase production. Int. J. Antimicrob. Agents., 7 (1), 65-68
15) Stacy M. Burns, and Sheila I. Hull, 1998. Comparison of Loss of Serum Resistance by Defined Lipopolysaccharide Mutants and an Acapsular Mutant of Uropathogenic Escherichia coli O75:K5. Infection and Immunity, 66, 4244- 4253

Histometric Studies of the Equine Hoof Wall in Normal and Laminitic Horse

MOSTAFA, M.B. and EL - GHOUL, W.S.

Department of Surgery, Anaesthesiology and Radiology
Faculty of Veterinary Medicine
Cairo University, Egypt, Giza - 12211

Summary

The study was carried on 10 clinically normal horses and 10 horses subjected to experimental laminitis. Histometric examination of hoof specimens was done, after staining, using micrometer eyepiece. The results showed a significant ($p<0.05$) decrease in the number of horny tubules / mm^2, the maximum transverse diameter of the tubule, the thickness of the cortex, the quotient value of the thickness of the cortex / maximum transverse diameter of the tubule in horses affected by laminitis. Whereas, the maximum transverse diameter of the medulla, the quotient value of maximum transverse diameter of the medulla / thickness of the cortex, the quotient value of maximum transverse diameter of the medulla / maximum transverse diameter of the tubule, total surface area of the medulla, the quotient value of medullary surface area / tubular surface area were significantly ($p< 0.05$) increased in laminitis. It was concluded that the histometric changes in the microstructure of the tubular and intertubular horn play an important role in the production of poor quality hoof horn during laminitis.

Introduction

The hoof wall is composed of three layers: stratum externum, stratum medium and stratum lamellatum (Dellman, 1981). The stratum medium forming the bulk of the wall and consists of tubular and intertubular horn. The horny tubule consists of cortex and medulla. The cortex has three zones; the inner, middle and outer zones containing keratinized cells oriented around the medulla in a fairly tight coils.

The medulla contains loose elements (Wilkens, 1963; Stump, 1967; Steven, 1981 Dirks, 1985 and Christopher, 1995)

Equine laminitis is an inflammation of the dermo-epidermal junction especially in the dorsal hoof wall (Wintzer, 1982 and Stashak, 1987). Histopathological studies in horses and cattle affected by laminitis revealed disturbances in the epidermal keratinization process (Obel, 1948; Roberts et al, 1980 and Ekfalck et al, 1988).

Histopathological changes in the stratum medium during experimental laminitis in the horse showed cylindrical, oval or atrophied horny tubules associated with multiple cyst formation. The intertubular layers and the onychogenic fibers appeared swollen and fragmented (Mostfa, 1986). The developing horny cells are remarkably small and their intercellular space is widened (Marks and Budras, 1987).

Meyer (1985) concluded that the horn quality of the hoof is influenced by its microstructure which in turn can be determined by variable histometric parameters. The quality of the hoof horn depend on the number of tubules, the boundaries of tubules with the intertubular horn and the percentage of tubules to the intertubular horn (Tscherne, 1910; Dietz et al, 1971; Fuchs, 1976 and Kastner, 1976).

There is little literature concerning the histometric changes in tubular and intertubular horn during laminitis in horse. The aim of the present work was to describe the changes in the histometric parameters of the microstructure of the hoof wall in normal and in horses affected by laminitis to gain a basis for diagnosing the frequent pathological changes of the equine hoof.

Materials and Methods

Histometric examination of the normal hoof was done on specimens collected from euthanasized 10 horses. The hoof was obtained and splitted longitudinally at the middle of the toe by means of a saw. A specimen 1 cm x 1 cm including the structures of the whole thickness of the hoof was obtained from the upper, middle and lower parts of the hoof wall.

Laminitis was experimentally induced in horses by overfeeding of concentrated carbohydrate diet which consisted of 17 gm/kg b.w. of corn starch and 1.2 gm/kg of very fine saw dust (Mostafa, 1987). Hoof specimens were taken, at different times (24 and 48 hr., 6, 10, 20, 30, 40 and 75 days). The samples were preserved in 10% formal-saline solution and sectioned using freezing microtome at - 30 C into thin sections about 5 μm. Staining was done with haematoxyline and Eosin stain, periodic acid schiff reaction and Van Giesson stain (Carleton, et al, 1967).

The hoof sections were examined for the following histometric parameters: counting the number of horny tubules / mm^2 according to Geyer (1980), Hartel (1985) and El-Ghoul (1991); measurement of the maximum transverse diameter of the horny tubules and its medulla (Hofstetter,1985); calculation of the thickness of the cortex (Empel et al, 1982); calculation of the maximum transverse diameter of the medulla / thickness of the cortex (Dietz, 1976); calculation of the maximum transverse diameter of the medulla / maximum transverse diameter of the tubule (Hofstetter, 1985); calculation of the thickness of the cortex / maximum transverse diameter of the tubule (Empel et al, 1981); measurement of the total surface area of the medullary and horny tubules; calculation of the medullary surface area / tubular surface area (Hofstetter, 1985) and calculation of the surface density (Hartel, 1985).

Statistical analysis of the data were done by *t*- test and ANOVA using SPSS (Statistical Product & Service Solutions) (Kuehl, R., 1994). All data were presented as mean ± standard error, and $p< 0.05$ was considered significant.

Results

The results of the histometric examination revealed that, the average number of horny tubules, the maximum transverse diameter of the tubule, the thickness of the cortex and the ratio of the cortex thickness / tubular diameter in laminitis were significantly lower ($p< 0.05$) than that of the clinically healthy horses (table 1 and figures 1, 2 and 3).

The maximum transverse diameter of the medulla, the ratio of the medullary diameter / cortex thickness, medullary diameter / tubular diameter, medullary surface area / tubular surface area and the total surface area of the medulla were significantly higher ($p<0.05$) in laminitis than that of the clinically healthy horse (table 1 and figures 4, 5 and 6).

Insignificant differences were found in the total surface area of the tubule and the surface density between normal and laminitic horses (table 1).

Table 1: Histometric parameters in normal and laminitic horse.

Histometric parameters	Normal hoofs	Laminitic hoofs
	mean ± SE	**mean ± SE**
Number of horny tubules /mm^2	11.634 ± 0.196	10.477± 0.091**
Maximum transverse diameter of the medulla (µm)	15.241 ± 0.819	18.581 ± 0.989**
Maximum transverse diameter of the tubule (µm)	67.517 ± 4.351	56.706 ± 1.796**
Thickness of the cortex (µm)	25.732 ± 2.226	18.642 ± 0.952**
Maximum transverse diameter of the medulla / thickness of the cortex	0.806 ± 0.127	2.759 ± 0.550**
Maximum transverse diameter of the medulla / maximum transverse diameter of the tubule	0.261 ± 0.024	0.363 ± 0.021**
Thickness of the cortex / maximum transverse diameter of the tubule	0.368 ± 0.012	0.318 ± 0.011**
Total surface area of the medulla	198.255 ± 25.930	533.622 ± 86.719**
Total surface area of the tubule	3591.503 ± 407.41	3483.866 ± 212.44
Medullary surface area / tubular surface area	0.078 ± 0.014	0.179 ± 0.023**
Surface density	39076.6 ± 5609.2	35193.7 ± 2145.1

The histometric parameters in the hoof wall at different times from the onset of laminitis revealed a significant decrease ($p<0.05$) in the number of horny tubules, thickness of the cortex and surface density which starting at 2 and 6 days after appearance of signs of laminitis (table 2 and figures 7,8 and 9). The maximum transverse diameter of the medulla, total surface area of the medulla and the tubule, the ratio of medullary diameter / tubular diameter, medullary diameter / cortex thickness and medullary surface area / tubular surface area were significantly ($p< 0.05$) increased at 2 and 6 days after appearance of signs of laminitis. Inconstant significant changes were observed in the histometric parameters from 10 to 75 days (table 2 and figures 10 and 11).

Discussion

Poor hoof horn quality generally manifests clinically as hoof affections (Eustace, 1994). The horn quality of the hoof is influenced by its microstructure which can be judged by histometric examination (Meyer, 1985). Many authors established certain parameters for evaluation of the horn quality (Dietz, 1976; Hofstetter, 1985 and El-Ghoul, 1991).

Histometric studies of normal equine hoof in this study revealed that the number of horny tubules is 11.634 tubules / mm^2. Whereas Tscherne (1910) counted 5.0 tubules / mm^2 and Rossner (1940) found 9.8 tubules / mm^2 in the normal hoof wall. This may be attributed to the breed differences which influence the number of tubules as mentioned by Sedlacek (1933) who stated that the number of horny tubules is higher in hot - blooded horses than in cold - blooded one.

During this study, histometric evaluation of the hoof microstructure showed that the number of horny tubules / mm^2, the maximum transverse diameter of the tubule, the thickness of the cortex, the quotient value of the thickness of the cortex / maximum transverse diameter of the tubule in laminitis were significantly ($p<0.01$) lower than normal horse.

The number of the horny tubules determines the hardness of the hoof. The higher the number of tubules the harder the hoof horn. A horn with low number of tubules, means more intertubular surface (Dietz, 1976 and Empel et al, 1982). The increase in the thickness of the cortex and the quotient value of the thickness of the cortex / maximum transverse diameter of the tubule means increase in hoof horn hardness. That is because the tubular cortex is formed of cells of high quality which surround the medulla and give the tubule the first rate stabilization character (Geyer, 1980). The maximum transverse diameter of the tubule determines the hardness of the hoof, the wider the diameter, the softer is the hoof horn (Distal et al, 1981).

Consequently our findings showed poor horn quality of the hoof during experimental laminitis. Defects in the hoof horn reduce the functional integrity and are a major cause of reduced performance of the horse (Kempson, 1990). Therefore, decrease in the number of the horny tubules and the thickness of the cortex and increase in the tubular diameter reduced the quality of the hoof horn during laminitis.

The results of the maximum transverse diameter of the medulla, the quotient value of maximum transverse diameter of the medulla / thickness of the cortex, the quotient value of maximum transverse diameter of the medulla / maximum transverse diameter of the tubule, total surface area of the medulla, the quotient value of medullary surface area / tubular surface area in laminitis were significantly higher ($p< 0.01$) than the normal horse.

Distal et al (1981), Hofstetter (1985) and Zincker (1995) concluded that the increase in the maximum transverse diameter of the medulla , the total surface area of the medulla and tubule and their ratios indicates the increase in hoof horn softness. That is because the medulla is formed from decayed cells of poor quality.

The obtained results revealed a significant decrease in the number of horny tubules, surface density and thickness of the cortex, meanwhile a significant increase in the diameter and surface area of the medulla starting at 2 and 6 days after appearance of signs of laminitis. The results of the histometric parameters indicated that, the changes in the hoof microstructure require a time for appearance than the changes in the microcirculation of the hoof corium which occur directly following induction of laminitis (Nilsson, 1963).

There is association between the hoof affected with laminitis and poor hoof horn quality. As weak, poor quality hoof horn appears to lose its natural water - proofing properties, more prone to environmental influences, cannot protect against concussion and generally manifests clinically as hoof affections (Kempson, 1990; Eustace, 1994 and Zincker, 1995).

The findings of histometric parameters determined in this study indicated that, laminitis leads to formation of poor quality horn which make the hoof more susceptible to affections as mentioned by Kempson (1990). Furthermore, these results potientiate the hypothesis that laminitis is associated with changes in the epidermal parts of the hoof (Obel, 1948).

In conclusion histometric studies of the microstructure of the hoof during laminitis are accompanied with changes in the quality of horn material. The decreased in the number of the horny tubules and the thickness of the cortex coincide with the increase in diameter and surface area of the medulla and tubules will produce poor hoof horn quality.

References

Carleton, M.; Drury, R.; Wallington, E. and Cameron, H. (1967): Carleton's Histological technique.
Christoffer, C. (1995): Color atlas of the horse`s foot. First edition, Mosby - Wolfe, Times mirror international publishers limited.
Dellman, H.D. and Brown, E.M. (1981): Text book of veterinary histology. 2 nd ed. Lea and Febiger. Philadelphia. pp.:405-408.
Dietz, O. (1976): Probleme der Gliedmassen - und Klauengesundheit. Tierhygiene - Information Eberswalde - Finow 8, Sonderheft.
Dietz, O.; Gangel, H. and Koch, K. (1971): Die Erhaltung der Gliedmassen - und Klauengesundheit unter modernen Produktionsbedingungen. Monatsh. Veterinarmed. 26, 241 - 246.
Dirks, C. (1985): Makroskopische, licht- und elektronenmikroskopische Untersuchungen über den Rückenteil der Rinderklaue. Berlin, Freie univ. Fachber.Veterinarmed., Diss.
Distal, O.; Krausslich, H. and Graf, F. (1981): Commission on cattle production: Genetic variation in morphological, histological and electrophoretical parameters of claw horn and correlations between the parameters. 32 nd. Annual meeting of the European Association for Animal Production (EAAP).
El - Ghoul, W. S. (1991): Clinical importance of the horn quality of the bovine claw in native and foreign breeds. M.V.Sc. (Vet. Surgery), Cairo University.
Ekfalck, A. and Martinez, H. (1990): Cultivation of tissue from the matrix of the stratum medium of the equine and bovine hoof walls. Am.J.Vet.Res., 51:1852-1856.
Empel, W.; Distl, O.; Krausslich, H.; Strandberg, P. and Thysen, I. (1982): Claw quality in cattle. Symposium international sur les affections du pied des ruminants. Ecole nationale veterinaire d`Alfort.
Eustace, R. (1994): Factors affecting equine hoof horn growth rate and quality. In practice, May:129 - 131.
Fuchs, G. (1976): Aspekte zum Begriff der Grossanalagentauglichkeit - Klauengesundheit. Monatsh. Veterinarmed., 31:930 - 935.
Geyer, H. (1980): Zur mikroskopischen Anatomie der Epidermis der Schweineklaue. Zbl. Vet. Med. C 9, 337 - 360.
Hartel, M. (1985): Histometrische Untersuchung, Wasser und Asche- gehaltsbestimmung am Klauenhorn von Mastschweinen. Hannover, Tierarztl. Hochsch., Diss.
Hofstetter, B. (1985): Contribution a l`etude histologique a but pratique de la corne des onglons des bovins. Schwiz. Arch. Tierheilk. 127, 417-432.
Kasapi, M. and Gosline, J. (1997): Design complexity and fracture control in the equine hoof wall. J.Exp. Biol., 200: 1639 - 1659.
Kastner, D. (1976): Untersuchungen zur Klauenhornhistologie als Qualitätsparameter beim Landschwein. Berlin, Humboldt - Univ., Biowiss. Fak., Diss.
Kuehl, R. (1994): Statistical Principles of Research Design and Analysis. Belmont, CA: Duxbury Press.
Marks, G and Budras, K. (1987): Licht- und elektronmikroskopische Untersuchungen über die akute Hufrehe des Pferdes. Berl. Münch. Tierärztl. Wschr., 100:82 - 88.
Meyer, B.D. (1985): Histometrische Untersuchungen, Bestimmung des Wasser- und Aschegehalts sowie der Klauenhärte am Klauenhorn von Mastschweinen bei unterschiedlicher Bodenbeschaffenheit. Hannover, Tierärztl. Hochsch. Diss.
Mostafa, M.B. (1986): Studies on experimental laminitis in the horse. Ph.D. Thesis Cairo University.

Mulling, C.; Bragulla, H.; Budras, K. and Reese, S. (1994): Structural factors influencing the horn quality and predilection sites for diseases at the bottom surface of the bovine hoof. Schweiz. Arch. Tierheilkd., 136: 49 - 57.
Nilsson, S.A. (1963): Clinical, morphological and experimental studies of laminitis in cattle. Ph.D.Thesis, Stockholm.
Obel, N. (1948): Studies on the histopathology of the acute laminitis. Almquist & Wiksells Boktryckeri AB, Uppsala, Schweden.
Roberts, E.D.; Ochoa, R. and Haypes, P.F. (1980): Correlation of dermal - epidermal laminar lesions of equine hoof with various disease conditions. Vet. Pathol., 17: 656 - 666.
Rossner, G. (1940): Untersuchungen über die Verteilung von Hornröhrchen und Zwischenhorn in der Schutzschicht bei verschiedener Wandstellung am Huf des Pferdes. Hannover, Tierärztl. Hochsch. Diss.
Sedlacek (1933): Das Verhältnis der Hornröhrchen und der Hornmasse in der Hufhornwand. Zver. Rozpravy, 7:33 - 36.
Stashak, S. (1987): Adam's Lameness in Horses. Fourth edition. Lea & Febiger. Philadelphia. pp:486-489.
Steven, D. (1981): Functional anatomy of the horse`s foot. In practice, 22 - 27.
Stump, J.E. (1987): Anatomy of the normal equine foot, including microscopic features of the laminar region. J.A.V.M.A., 151:1589-1598.
Tscherne (1910): Über die Beziehungen der Qualitat des Wandhornes der Pferdehufe zur histologischen Einrichtung derselben. Leipzig, Univ. medizin. Fak. vet. med. Diss.
Wilkens, H. (1963): Zur makroskopischen und mikroskopischen Morphologie der Rinderklaue mit einem Vergleich der Architektur der Klauen und Hufröhrchen. Habilschrift, Hannover, Tierärztl, Hochsch.
Wintzer, H.J. (1982): Krankheiten des Pferdes. Verlag Paul Parey, Berlin und Hamburg.
Zenker, W.; Josseck, H. and Geyer, H. (1995): Histological and physical assessment of poor hoof horn quality in Lipizzaner horses and a therapeutic trial with biotin and a placebo. Equine Vet. J., 27: 183 - 191.

Relationship between claw disorders and metabolic disturbances in dairy cattle[1]

W. El-Ghoul [2]; W. Hofmann [1]; Y. Khamis [2] and A. Hassanein [2]
Klinik fuer Klauentiere der Freien Universitaet Berlin/Deutschland [1]
und Dept. of Surgery, Anaesthesiology and Radiology, Faculty of Vet. Med.
Cairo Univ.,Egypt [2]

Summary

The study was performed on a total number of 143 dairy cows affected by both claw disorders and metabolic disturbances. It was found that, in cows affected by claw disorders associated with alkalosis, the average proportion of segmented neutrophils, CK activity, GLDH activity and TP were significantly higher. In cows associated with primary acetonaemia, the proportion of eosinionphils and the CK activity were significantly higher. In hypocalcemia the haematocrit value and Ca level was significantly lower, whereas the total leukocytic count, segmented neutrophils, and urea were significantly higher than normal. In hypophosphatemia the average P was significantly lower, whereas the average CK and bilirubin were significantly higher. In cows associated with hypomagnesemia the proportion of segmented neutrophils, CK activity, GLDH, TP and bilirubin were significantly higher, whereas the average Mg was significantly lower. In cows affected by fat cow syndrome the proportion of segmented neutrophils, GLDH activity and TP value was higher than normal. It was concluded that, the metabolic and microcirculatory changes associated with ruminal acidosis, acetonaemia, fatty liver, hypocalcemia, hypophosphatemia and hypomagnesemia leading to ischemic necrosis and degeneration of the horn producing structures and destruction of the connection between claw horn and corium predisposing to claw affections.

Introduction

The horn quality of the claws appear to be affected very much by the animal's metabolism. Metabolic changes may predispose to the occurrence of chronic necrotic pododermatitis (Greenough, 1962).

The trigger mechanism is thought to be in the digestive tract. Absorption of toxic fermentation products into the circulation brings the action to the burdened pododerm. Damage of this tissue manifests itself in a latter stage in the claw sole (Peterse, 1987).

Laminitis arising from a systemic disorder due to a wide spectrum of probably largely interdependent aetiological factors. These varies from systemic influences such as metabolic and digestive disorders, calving or severe inflammatory processes (e.g. endometritis or mastitis) to localized influences in the claw. However, all have something in common; in a first phase, predominantly vasoactive substances which have been released into the circulatory system, may trigger pathological mechanisms which ultimately cause degenerative changes in the epidermal-dermal junction of the claw (Baggott, D.G., 1982 and Ossent, P. and Lisher, C., 1994).

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The following three factors were suggested to be important in triggering the changes in the claws: (a) endotoxin released from inflammatory foci and endotoxaemia, (b) lactic acid in relation to ruminal acidosis and (c) histamine, released in allergic reactions are absorbed from the gut (Bossman, 1990 and Vermunt, 1994).

The aim of the present study is to search in the causes of claw affections combined with metabolic disturbances in dairy cows through examination of total blood picture, biochemical blood parameters, acid - base balance, blood clotting profile, urine and ruminal fluid.

Material and methods

The study was performed on a total number of 143 cows affected by both claw disorders and metabolic disturbances. From these cows 110 one were examined for total blood picture, biochemical blood parameters, urine and ruminal fluid. Another 21 cows examined for blood clotting profile and another 12 cows for acid-base balance. All cows were examined for claw status.

Claw examination:

To determine the site of lesion, the claw was examined routinely by cleaning, manual palpation, pressure test, percussion, paring the horn and probing (Rosenberger et al, 1979). Dignosis of claw affections was based on the clinical signs and symptoms as defined in literature particulary the observations of Weaver et al (1981).

Blood examination:

Blood samples were taken in two tubes one containing EDTA for whole blood collection, the other being for serum.

Total blood picture: haemoglobin, haematocrit, erythrocytic and total leukocytic count were determined in an automatic microprocessor - based haematology analyzer. The proportion of eosinophils, basophils, unsegmented, segmented, juvenile and immature neutrophils, lymphocytes and monocytes were calculated manually by counting 100 leukocytic cells in zigzag manner in different microscopic fields and calculate the percentage of each type.

Blood biochemical parameters: Serum Ca and Mg were determined in an atomic absorption spectrophotometer. Serum sodium and potassium were determined, quantitatively, in an Electrolyte Analyzer. Serum inorganic phosphorus, aspartate aminotransferase, creatin kinase, glutamat-dehydrogenase, gamma glutamyl transferase, total protein, bilirubin and urea values were determined in an auto - analyzer.

Blood clotting profile: Two blood samples were taken , one containing sodium citrate and the other whole blood. The blood platelets was counted in an automatic microprocessor-based haematology analyzer. The Coagulometer was used for determination of recalcification time, partial plasma thromboplastin time (PTT), plasma thromboplastin time (TPT), plasma thrombin time (TT) and fibrinogen. Recalcification time was determined chemically (200 µl citrated blood (1 : 9), 1 minute prewarming and with 200 µl 0,025 molar Calcium chloride mixing). The Thromboplastogram reaction time (TEGr), clot formation time (TEGkt) and maximum amplitude (TEGma) were determined in Thromboplastograph-D.

Acid - base balance: Blood samples were taken on heparin and transfered in ice. Acid -base balance parameters were determined in an automatic blood gas analyzer. The examined parameters are: hydrogen ion concentration (pH), carbon

dioxide tension *(p*CO2), oxygen tension *(p*O2) concentration of hydrogen carbonate (HCO3-) and actual base excess (ABE).

Urine examination:

Urine was examined for colour, specific gravity, pH, protein, ketone bodies, glucose, bile pigments and haemoglobin and red blood cells (Combur test).The judgment of the examined parameters depends on the colour of the reaction (Rosenberger et al, 1979).

Ruminal fluid examination:

The ruminal fluid was examined directly for colour, odour, viscosity, pH, sedimentation and floatation activity, methylene blue reduction test and infusoria (Rosenberger et al, 1979).

Statistical analysis of the data was done by Analysis Of Variance (ANOVA) using Statistical Analysis Systems (SAS Institute Inc., 1992). The data were presented as mean ± standard error and the difference considered significant at $p<0.05$ and Highly significant at $p<0.01$.

Results

The encountered claw affections diagnosed with the different metabolic disturbances are pododermatitis aseptica diffusa (11), pododermatitis circumscripta non-purulenta (28), pododermatitis circumscripta purulenta (30), pododermatitis septica profunda (14), subclinical laminitis (12), podoarthritis (4), os pedis necrosis (4), phlegmona interdigitalis (7), dermatitis digitalis (4), dermatitis interdigitalis (8), hyperplasia interdigitalis (4), erosio ungulae (8), vertical crack (3), toe ulcer (3) and overgrown claws (3).

The results of the total blood picture showed that, in cows affected by alkalosis (abomasal displacement) associated with claw disorders, the proportion of segmented neutrophils was significantly ($p<0.05$) higher. In primary acetonaemia, the proportion of segmented neutrophils and eosinophils were significantly ($p<0.05$) higher. In hypocalcemia, the average haematocrit value, erythrocytic count and proportion of lymphocytes were significantly ($p<0.05$) lower. In hypophosphatemia, the average proportion of segmented neutrophils was significantly ($p<0.05$) higher whereas, the average haematocrit value was significantly ($p<0.05$) lower. In hypomagnesemia, the average total leukocytic count and the proportion of segmented neutrophils were significantly ($p<0.05$) higher whereas the average haematocrit value was significantly ($p<0.05$) lower. In fatty cow syndrome, the average total leukocytic count and the proportion of segmented neutrophils were significantly ($p<0.05$) higher whereas, the average haematocrit value was significantly ($p<0.05$) lower (table 1).

Table 1: Total blood picture.

Parameter	Alkalosis (N= 66)	Primary Acetonaemia (N= 16)	Hypoc-alcemia (N= 2)	Hypopho-sphatemia (N= 5)	Hypoma-gnesemia (N= 8)	Fat Cow Syndrom e (N= 6)	*Normal range*
Hb (g/l)	100 ±3.14	107.4 ±7.56	94.5 ±9.65	111.8 ±13.25	101.4 ± 17.41	96.5 ± 6.87	*80 - 120*
Hkt (l/l)	0.3 ±0.04	0.323 ±0.21	0.265 ± 0.05*	0.284 ±0.08	0.287 ± 0.06	0.29 ±0.04	*0.30 - 0.40*
RBCs (T/l)	5.96 ±2.52	6.114 ±2.01	4.88 ± 1.08	5.952 ±1.02	6.027 ±2.47	5.86 ±3.21	*5 - 8*
WBCs (G/l)	9.81 ±4.12	9.187 ±3.04	13.2 ± 3.01*	8.32 ±2.14	10.86 ±3.45	10.92 ±4.78	*5 - 10*
Eos. (%)	2.621 ±0.56	6.125 ± 1.56 *	2 ±0.01	0.2 ±0.03	2.375 ±1.98	1.5 ±0.74	*0 - 3*
Baso. (%)	0.06 ±0.001	0.25 ±0.23	0	0	0.125 ±0.74	0	*0 - 5*
Unseg. (%)	2.469 ±1.02	3.75 ±1.94	2 ±0.1	1.4 ±0.04	4.75 ±1.96	2.333 ±0.58	*0 - 5*
Seg. (%)	53.53 ± 6.32 *	43.44 ±8.41	58.5 ± 11.4*	44.4 ±4.79	48.63 ± 7.96*	54.83 ± 9.45*	*20 - 40*
Lym. (%)	40.98 ±4.56	46 ±9.54	37.5 ±8.64	54 ±14.32	43.75 ±13.24	41.17 ±15.41	*40 - 80*
Mono. (%)	0.196 ±0.21	0.321 ±0.01	0	0	0.375 ± 0.08	0.166 ±0.07	*0 - 5*
Juv. (%)	0.06 ±0.01	0.125 ±0.02	0	0	0	0	*0 - 5*

Biochemical blood analysis revealed that, in cows affected by alkalosis (abomasal displacement) associated with claw disorders, the average CK, GLDH and TP values were significantly ($p<0.05$) higher. In primary acetonaemia, the average CK and GLDH values were significanatly ($p<0.05$) higher. In hypocalcemia, the average urea was significantly ($p<0.05$) higher whereas, the average Ca was significantly ($p<0.05$) lower. In hypophosphatemia, the average CK, TP and bilirubin values were significantly ($p<0.05$) higher whereas, the average P was significantly ($p<0.05$) lower. In hypomagnesemia, the average CK, GLDH, TP and bilirubin values were significantly ($p<0.05$) higher whereas, the average serm Mg value was significantly ($p<0.05$) lower. In fatty cow syndrome, the average AST, GLDH and TP values were significantly ($p<0.05$) higher (table 2).

Table 2: Blood serum biochemical parameters.

Parameter	Alkalosis (N= 69)	Primary Acetonaemia (N= 16)	Hypoc-alcemia (N= 3)	Hypopho-sphatemia (N= 6)	Hypomag-nesemia (N= 10)	Fat Cow Syndrome (N= 6)	*Normal range*
Ca (mmol/l)	2.249 ± 0.45	2.205 ±1.4	1.75 ± 0.57*	2.168 ±1.07	2.222 ±0.97	2.376 ±1.02	*2 - 3*
P (mmol/l)	1.576 ±0.67	1.64 ±0.94	1.93 ±0.34	0.803 ± 0.54*	1.433 ±0.56	1.511 ±0.84	*1.30 - 2.20*
Mg (mmol/l)	0.825 ±0.16	0.838 ±0.24	0.726 ±0.27	0.786 ±0.34	0.519 ± 0.14*	0.696 ±0.34	*0.60 - 1.30*
Na (mmol/l)	138.7 ±15.42	137.8 ±24.57	136.2 ±20.89	142.5 ±30.47	139 ±25.10	137.7 ±14.79	*130 - 150*
K (mmol/l)	3.982 ±1.90	3.945 ±1.27	4.12 ±1.65	4.59 ±0.98	3.765 ±1.02	3.843 ±1.56	*4.0 - 5.0*
AST (IU/l)	72.09 ±6.57	54.63 ±6.78	52.33 ±8.79	71.33 ±8.97	79.6 ±9.24	106.2 ±18.75	*0 - 100*
g-GT (IU/l)	20.41 ±3.45	14.14 ±5.89	18.91 ±6.54	12.5 ±3.78	17.22 ±7.03	24.5 ±12.23	*0 - 25*
CK (IU/l)	161.5 ± 20.14*	165.8 ± 23.78*	143.5 ±22.47	247.7 ± 30.87*	176.3 ± 19.65*	54 ±14.65	*0 - 60*
GLDH (IU/l)	12.51 ±6.45*	9.575 ± 2.47	7.32 ±3.58	5.9 ±2.47	11.8 ± 7.21*	19.3 ± 8.21*	*0 - 7*
TP (g/l)	101.8 ± 9.85*	78.48 ±10.11	75.41 ±6.45	80.15 ± 9.84	88.28 ± 8.94*	102.1 ± 16.94*	*60 -.80*
Bili (mmol/l)	8.424 ±3.57	6.468 ±1.98	6.133 ±3.89	10.42 ± 4.21*	14.83 ±4.0*	7.65 ±3.05	*0.30 - 8.50*
Urea (mmol/l)	5.185 ±3.12	4.1 ±1.45	17.3 ± 6.47*	3.15 ±0.94	3.2 ±1.07	3.2 ±1.7	*0 - 8.0*

Concerning the blood clotting profile, the results showed that the recalcification, plasma thrombin, plasma thromboplastin and thromboplastogram reaction times were significantly ($p<0.01$) higher, Whereas, plasma fibrinogen was significantly ($p<0.01$) lower than the normal range (table 3).

Table 3: Blood clotting parameters.

The blood gases analysis revealed insignificant differences in all the examined parameters (table 4).

Table 4: Blood gases parameters.

The urine colour was light or darck yellow in 33.75%, light yellow or colourless in 28.75% or gold yellow in 35% of cows.The specific gravity was lower than 1.020 in all cows. The pH was lower than 7.0 degree in 27.71%, between 7.0 and 8.0 degree in 39.75% and more than 8.0 degree in 32.53%. The protein concentration was less than 15 mg / dl in 24.39%, between 15 and 20 mg / dl in 8.53%, 30 mg / dl in 47.56% and 100 mg / dl in 18.85% of cows. The ketone bodies concentration was lower than 15 mg / dl in 83.52%, 40 mg / dl in 4.71%, 80 mg / dl in 5.88% and 160 mg / dl or more in 4.71% of cows.The glucose concentration was less than 100 mg / dl in 91.76%. The bile pigments were abscent or only traces in 98.75%. The haemoglobin and red blood cells were abscent in 78.75%, 25 erythrocytes / µl in 8.75%, 80 erythrocytes /µl in 5% and 200 erythrocytes /µl in 7.50% of cows (table 5).

Table 5: Urine examinations.

The ruminal fluid colour was brownish green in 85.71% and grey olive in 14.28% of cows. The odour was aromatic in 95.77% and acidic in 4.22%. The ruminal fluid was viscous in 51.42% and watery in 47.14%. The pH values were between 6.2 and 7.2 degree in 47.82% and more than 7.2 in 52.17% of cows. The sedimentation and flotation activity was normal in 30%, abscent in 32.85% and abscent of flotation and rapide sedimentation in 37.14%. The methylene blue reduction time was shorter than 3 min. in 53.62%, longer than 3 min. in 36.23% and longer than 6 min. in 10.14% of cows. The infusoria was abundent in 22.85%, moderate in 38.57%, few in 22.85% and abscent in 15.71% of cows (table 6).

Table 6: Ruminal fluid examinations.

Discussion

The encountered metabolic disturbances associated with claw disorders in this study were: alkalosis, primary acetonaemia, hypocalcemia, hypophosphatemia, hypomagnesemia and fat cow syndrome.

In the present study, significant neutrophilia, increase in the levels of creatin kinase, glutamate dehydrogenase and total protein were associated with alkalosis. Significant eosinophilia and increase in the creatin kinase activity were associated with primary acetonemia. Leukocytosis, neutrophilia, decrease in haematocrit value, and increased urea level were associated with hypocalcemia. Significant increase in the creatin kinase and bilirubin values were found in hypophosphatemia. Neutrophilia, increased creatin kinase, glutamate dehydrogenase, total protein and bilirubin values were associated with hypomagnesemia. Neutrophilia, increased activity of glutamate dehydrogenase and level of total protein were associated with fat cow syndrome. Meanwhile, Nilsson (1963) found that in cows affected by acetonaemia associated with claw disorders, the erythrocytic and total leukocytic count, magnesium and sodium were insignificantly higher, whereas, the proportion of basophils, immature neutrophils, monocytes, calcium and phosphorus were significantly low. Similar findings were recorded by Maclean (1965 / 1966 / 1970) who found that in cows affected by claw disorders associated with acetonaemia the erythrocytic count, the total leukocytic count, proportion of basophils, immature neutrophils, monocytes and aspartate aminotransferase were within normal.

It has been suggested that hypocalcemia may cause adrenal cortical hyperactivity resulting in leukocytosis and neutrophilia. The increased bilirubin level in cases of hypophosphatemia indicate the presence of hepatopathy. Cows with fatty liver have increased blood concentration of liver-specific enzymes (Radostitis et al, 1994).

Acetonaemia is often associated with fatty infiltration of the liver or liver degeneration and this tissue damage might contribute a focus for increased histamine formation which predisposes to claw affections. The claw disorders and the associated acetonaemia may be due to abnormal ruminal bacterial fermentation which often occurs in connection with acetonaemia caused by a protein rich diet with unbalanced feeding. In this condition there are overgrowth of the physiological ruminal flora by bacteria of the coli and proteus strains which occur in alkaline pH and toxic products are formed (Nilsson, 1963 and Dirksen,1983).

Also, the fatty acids formed in case of chronic latent ruminal acidosis may be shifted in favor of the proportion of butyric acid which can transformed into beta-hydroxybtyric acid resulting in subclinical ketosis. Ruminal acidosis due to ration rich in easily digestible carbohydrates leads to many metabolic disorders such as metabolic acidosis, subclinical ketosis and fat cow syndrome. These metabolic changes can be considered in this respect as the primary factor in the pathogenesis of claw disorders. In case of claw disorders associated with ruminal acidosis, the toxic feed decomposition products absorbed from the rumen seem to cause severe circulatory disturbances in the claw corium with separation of the horny capsule from the third phalangeal bone (Dirksen, 1983). Meanwhile, Modrakowski (1978) found that metabolic and circulatory disturbances diminished blood supply and nutrition to claw corium leading to the manifestation of pododermatitis circumscripta.

The changes in the blood clotting profile indicate that there are marked microcircultory disturbances in the claw corium. Nilsson (1963) and Boosman (1990) found that activation of the clotting system resulted from endotoxaemia, reflected by thrombocytopenia, increased PT, PTT and circulating soluble fibrin monomers could possibly lead to blockage of the claw microcirculation leading to laminitis. This supports the findings of Hofmann (1992) who described that in all forms of laminitis there is microcirculatory disturbances in the claw corium which may be due to allergic agents, endotoxin (histamine, lactic acid), bacterial toxins, blood acidosis. All these factors lead to direct damage of the endothelial cells or diffuse intravascular clotting and both lead to disturbances in the permeability of the capillaries resulting in destruction of the connection between the claw horn and the claw corium.

Regarding the concentrations of blood gases insignificant differences in the average oxygen tension, hydrogen carbonate concentration in plasma and actual base excess have been encountered. On the other hand Boosman (1990) found significant difference in the actual base excess in laminitic cows. This result means that there was no marked variations in blood gases parameters in relation to claw disorders.

The urine analysis indicated the presence of proteinuria with alkaline pH whereas ketone bodies has been encountered. Apparently, there was no marked specific changes in the urine parameters in relation to claw disorders. The changes of the ruminal fluid were more or less within the physiological range.

In conclusion it can be said that, the occurrence of claw affections in association with metabolic disturbances may be attributed to the metabolic and circulatory changes associated with ruminal acidosis, acetonaemia, fatty liver, hypocalcemia, hypophosphatemia and hypomagnesemia. These metabolic changes lead to the production of histamine, ketone bodies and lactic acid which causing severe microcirculatory disturbances in the claw corium. The mirocirculatory disturbances leading to ischemic necrosis and degeneration of the horn producing structures and destruction of the connection between claw horn and corium predisposing to claw affections.

References

Baggott, D. (1982): Hoof lameness in dairy cattle. In Practice, 4, 133 - 141.
Boosman, R. (1990): Bovine laminitis. Histopathologic and arteriographic aspects, and its relation to endotoxaemia. Ph.D. Thesis, Utrecht, The Netherlands.
Dirksen, G. (1983): Indigestions in cattle. Schnetztor-Verlag GmbH Konstanz, West Germany.
Greenough, P.R. (1962): Observations on some of the diseases of the bovine foot part II. Vet. Rec. 74, 53 - 62.
Hofmann, W. (1992): Rinderkrankheiten. Band 1 : Innere und chirurgische Erkrankungen. Verlag Eugen Ulmer Stuttgart.
Maclean, C.W. (1965): Observations on acute laminitis of cattle in South Hampshire. Vet. Rec. 77, 662 - 672.
Maclean, C.W. (1966): Observations on laminitis in intensive beef units. Vet. Rec. 78, 223 - 231.
Maclean, C.W. (1970): The haematology of bovine laminitis. Vet. Rec., 86, 710-714.
Modrakowski, A. (1978): Some aspects of the incidence of pododermatitis circumscripta in bulls kept at insemination centers. Report on the second symposium on bovine digital disease, Veterinary institute , Sept., 1978, Skara, Sweden.
Nilsson, S.A. (1963): Clinical, morphological and experimental studies of laminitis in cattle. Ph.D. Thesis, Stockholm.
Ossent, P. and Lischer, C.J. (1994): Theories on the pathogenesis of bovine laminitis. Eighth International symposium on disorders of the ruminant digit and international conference on bovine lameness, June, 1994, Banff, Canada.
Peterse, D.J. (1987): Aetiology of claw disorders in dairy cattle. Cattle housing systems, lameness and behaviour. Proceedings of a seminar on the influence of the design of housing systems for cattle on lameness and on behaviour, Brussels at the Commission of the European Communities. Martinus Nijhoff Publishers.
Radostits, O. M.; Blood, D.C.; Gay, C.C.and McDaniel (1994): Veterinary Medicine. A textbook of the Diseases of Cattle, Sheep, Pigs,Goats and Horses. Eighth Edition, Bailliere Tindall, London.
Rosenberger,G; Dirksen, G.; Grunder, H.; Grunert,E.; Krause, D. and Stober, M. (1979): Clinical examination of cattle. Verlag Paul Parey, Berlin and Hamburg.
SAS Institute Inc. (1992): Doing More with SAS / ASSIST Software, Version 6. First Edition.
Vermunt, J.J. (1994): Predisposing causes of laminitis. Eighth international symposium on disorders of the ruminant digit and international conference on bovine lameness, June, 1994, Banff, Canada.
Weaver,A.; Andersson, L.; Banting,A.; Demerzis,P.; Knezevic,P.; Peterse,D. and Sankovic,F. (1981): Review of disorders of the ruminant digit with proposals for anatomical and pathological terminology and recording. Vet.Rec.,108:78-82.

Anschrift der Verfasser:
Konigsweg 65
D 14163 Berlin
Deutschland