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ATYPICAL PNEUMONIA IN COWS AFTER TRANSPORTATION

Teodora Popova, Vasil Manov

University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
E-mail: dr_tpopova@abv.bg

ABSTRACT

Atypical pneumonia was proved in cetacean cows which died in the village of Boeritsa near Ihtiman after import from Austria. Fibrinous-purulent changes were detected both in lungs and pleura. Pathological anatomical findings were characteristic of pasteurellosis, but no *Pasteurella* bacteria were identified.

Serratia marcescens and small amounts of *Staphylococcus xylosus, Streptococcus pneumoniae, Enterococcus faecalis* and *Candida albicans* were isolated after the microbiological examination of the material from the lungs. Antibiotic poly-resistance was detected in vitro. This indicated that they are most likely selected in the animals after frequent treatment with such agents by almost all groups to suppress conditionally pathogenic infections, possibly due to hygiene weaknesses in breeding and feeding in the farm they had inhabited prior to their import into Bulgaria. The stress during the long transportation and adaptation to the new living conditions is a prerequisite for their multiplication, which in vivo is accompanied by an increased virulence and development of fatal pneumonia.

Key words: atypical pneumonia cows, transport, *Serratia marcescens, Staphylococcus xylosus, Streptococcus pneumoniae, Enterococcus faecalis, Candida albicans*.

Introduction

Transportation stress is a factor that increases the morbidity and mortality of cattle, including respiratory infections. One of the prerequisites is the significant reduction in total antioxidant capacity of blood serum during transport (Chirase et al., 2004). This creates conditions for developing pneumonias by conditionally pathogenic microorganisms from the normal animal microflora. Natural respiratory tract resistance is suppressed under stress and lung cells can be colonized by bacterial pathogens, leading to pneumonia (Caswell, 2013).

Due to rapid development of antimicrobial resistance, *Serratia marcescens* is among the most significant conditionally pathogenic bacteria today. It is also an object for sensitivity testing of new antibacterial products (Chockalingam et al., 2007; Cohen et al., 2008). *Staphylococcus xylosus* also belongs to the pathogens with increasing antibiotic resistance. It belongs to the group of coagulase-negative Novobiocin-resistant staphylococci, which are part of the normal microflora of the skin, skin glands and mucous membranes of mammals and birds. Although these microorganisms display low virulence, they are related to the etiology of various infections in humans and animals. as the latter are important pathogens with increasing tendency of developing antibiotic resistance over the last decade. Coagulase-negative staphylococci are often isolated from cattle, goats and sheep as well as from milk and dairy products (Novakova et al., 2006). Nagase et al. (2001) isolated *S. xylosus* mainly from cows, horses, pigs, and dogs and less frequently from humans.

The aim of the present study was to establish pathomorphological findings and causes of fatal atypical pneumonia in cows developed immediately after their import from Austria and to determine sensitivity to antimicrobial agents *in vitro*.

Materials and methods

Animals. Corpses of cows of simmental breed died in the village of Boeritsa near Ihtiman after import from Austria were autopsied and examined.

Histopathological examination. Samples from lungs were fixed in 10% neutralised buffered formalin, processed by routine histological technique and included in paraffin. Slices (5 μm) were stained with Haematoxylin-Eosin.

Microbiological studies. Materials from altered lungs were used for isolation. Cultivation was carried out on elective and selective growth media for microorganisms manufactured by Antisel (Sharlau Chemie S. A., Spain): ● agars – Mueller – Hinton (for non-demanding aerobic species), Endo (for Gram-negative aerobic bacteria), Cetrimide (for *Pseudomonas* spp.), Chapman-Stone (for staphylococci), Czapek Dox and Sabouraud (for fungi); ● broths – dextrose and Tarozzi (for strict anaerobes). Cultivation was carried out at 37 °C for 24–72 hours for bacteria and for 5–7 days at 22 °C for fungi.

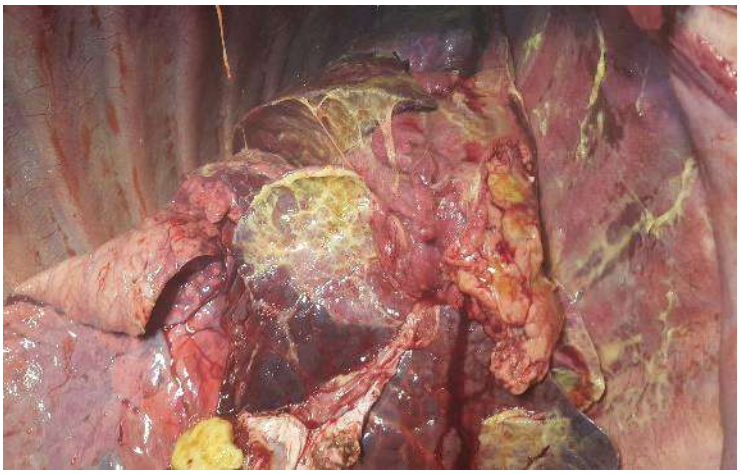
Identification of the isolated microorganisms was performed by microscopic examination of Gram stained preparations, rendering into account the cultural features on solid and liquid media and biochemical properties with Polymicrotest (NCIPD – Sofia) and tests for oxidase, catalase and others. The isolation and identification of the bacteria was performed in accordance with International Bergey's Determiner (Holt et al., 1994).

Determination of sensitivity to antimicrobial agents of isolates was performed using the classic agar-gel diffusion method of Bauer et al. (1966). Standard discs with antibiotics were used (NCIPD – Sofia), also prepared by us, after inoculation of bacterial suspensions in exponential growth phase at a concentration of 2.10^6 cells/ml on Mueller-Hinton agar. Cultivation was carried out at 37 °C for 24 hours under aerobic conditions. The results were interpreted by the three-step system of Bauer et al. (1966) after measuring the diameters of the inhibitory zones in millimetres.

Results

Clinical picture. Cows were imported from Austria during the summer months. After transportation a decline in their health was observed. It was manifested by fever, lack of appetite, heavy breathing, lurch, apathy, drowsiness, and death.

Pathological anatomical findings. On external observation of carcases purulent leaks from the nasal openings were found. By internal examination changes were observed in the lining of nasal cavity and the trachea, the lungs, both pleural surfaces, and the heart. The upper respiratory tract mucosa was oedematous and flushed. Mucinous purulent deposits were found in some areaswith discrete bleedings and erosions underlying. Visceral and parietal pleura were red and lined in places with grey-yellowish fibrinous masses adhering to each other (Fig. 1). The diffuse lesions in the lungs covered the pulmonary lobes. Lobes were enlarged with a red colour, thick consistency and relatively dry cut surface. There were many small and larger grey and white hearths that were surrounded by red peripheral zones. The perilobular interstitial tissue was thickened and clearly visible. Under pressure from cutting surface a scarce amount of purulent fluid emerged. On epicardium and parietal pleura, petechial haemorrhages were observed. Materials for pathohistological and microbiological examinations were taken aseptically from the affected parts of the lungs.



a

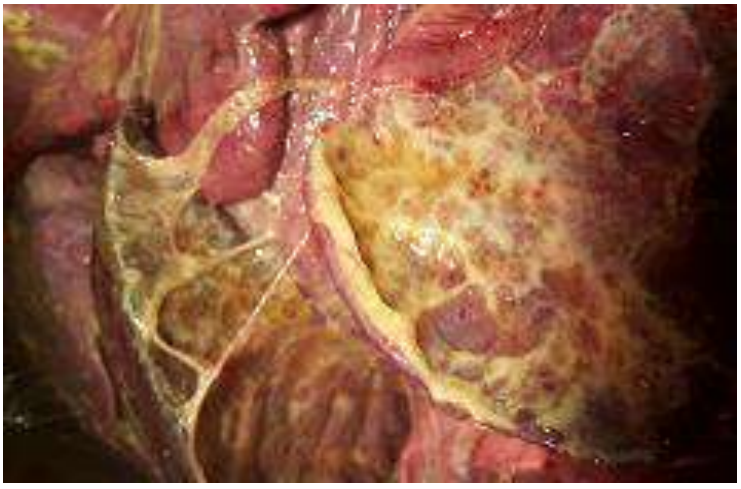


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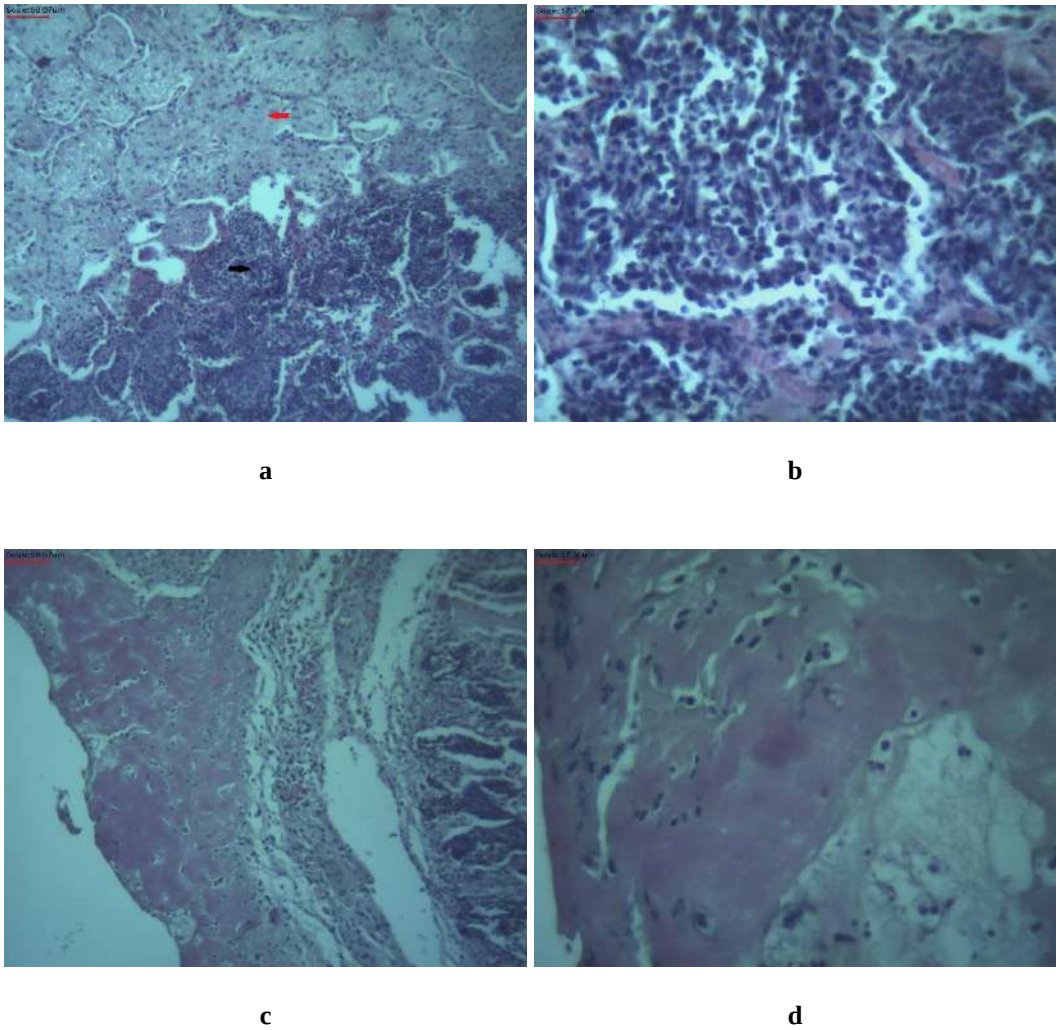
Figure 1: Pathological anatomical changes: (a) hyperaemia, petechiae, ecchymosis, and accumulation of fibrin on the visceral and parietal thoracic pleura; (b) part of "Figure 1 a": fibrin coating of an inflamed lung.

Histopathological findings. The histological examination of the material taken from the lungs revealed erythrocyte accumulation of the interstitial and interalveolar blood vessels. Alveolar, perivascular and peribronchial interstitial spaces were filled with an exudate containing pale pink strands intertwined in different directions. On some places, fibrinous matter was admixed with multiple desquamated epithelial cells, hyper segmented neutrophilic leukocytes, alveolar macrophages, lymphocytes, and plasmatic cells. (Fig. 2a). Changes found in the bronchial and bronchiolar mucosa were similar. Mucus dystrophy, necrosis and desquamation of the epithelium were observed. Whole segments of the lung structures were filled with degenerated neutrophilic leukocytes and alveolar macrophages as well as necrotized bronchial and alveolar structures surrounded by inflammatory cells (Fig. 2b). On microscopic examination of the lesions (found in the parietal and visceral lung pleura) fibrinous masses mixed with fragments of degenerated cells, hyper segmented neutrophilic leukocytes and pleural macrophages (Fig. 2c) were observed.



a **b**

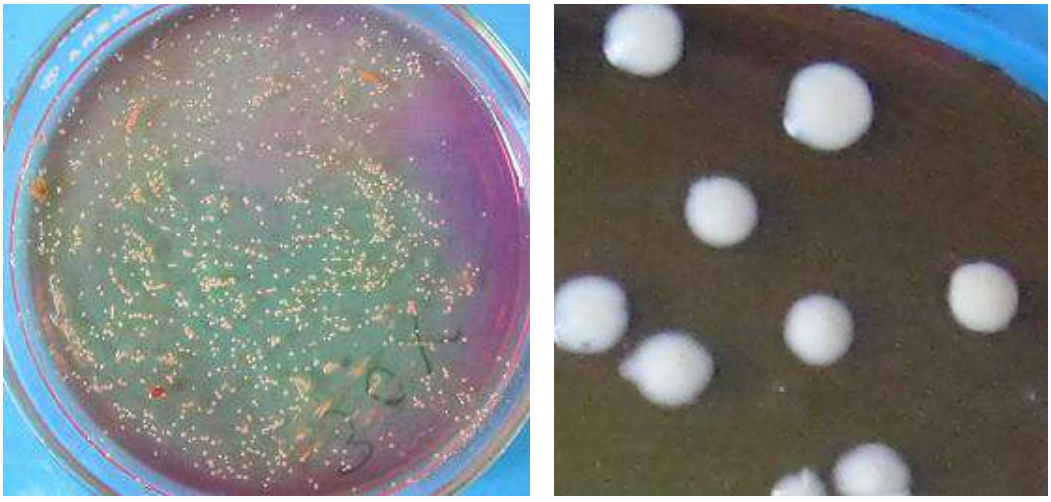
c **d**

Figure 2: Pathohistological finding in lungs of the studied cows, characteristic of diffuse purulent-fibrinous pleuropneumonia: a) a clearly identifiable boundary between alveolar spaces filled mainly with fibrinous exudate (black arrow) and alveolar spaces filled with hyper segmented neutrophilic leukocytes); b) alveolar structures filled with degenerated inflammatory cells; c) a massive layer of fibrinous material covering the visceral pleura; d) part of "Figure 2 c" – degenerated cellular structures scattered in fibrinous matter.

Microbiological finding. *Serratia marcescens* (Fig. 3) and small amounts of *Streptococcus pneumoniae*, *Staphylococcus xylosus* (Fig. 4a), *Enterococcus faecalis* and *Candida albicans* (Fig. 4b) were isolated from the tested materials. The results of biochemical identification of the isolated *Serratia marcescens* in Polymicrotest are presented in Fig. 5.

a **b**

Figure 3: Colonies of the isolated *Serratia marcescens* on Endo agar (a) and on MacConkey agar (b).



a **b**

Figure 4: Colonies of the isolated *Staphylococcus xylosus* on Chapman-Stone agar (a) and of *Candida albicans* on Sabouraud agar (в).

Figure 5: Results of biochemical identification of the isolated *Serratia marcescens* in Polymicrotest.

Sensitivity to antimicrobial agents. The results are presented in Table 1 and Fig. 6. High poly resistance of the isolated bacteria was observed. In vitro sensitivity only to certain aminoglycosides (gentamicin, amikacin, kanamycin) and quinolones (enrofloxacin, ciprofloxacin) as well as an intermediate susceptibility to cefuroxime was found. Full resistance was established to cephalosporins, even to those of third and fourth generation, as well as to penicillins. the same high level of resistance was also found to broad spectrum antibiotics such as amphenicols, tetracyclines and potentiated sulfonamides.

Table 1: Sensitivity of the isolated bacteria to antimicrobial means *in vitro*

Antimicrobial means	Disc content (µg)	Inhibitory zones in mm and sensitivity of the strain	
		S. marcescens	***S. xylosus***
Thiamphenicol	30	6 (R)	16 (I)
Tetracycline	30	6 (R)	9 (R)
Lincomycin	15	6 (R)	14 (R)
Clindamycin	2	9 (R)	12 (R)
Penicillin G	10 u	15 (R)	11 (R)
Oxacillin	1	6 (R)	8 (R)
Amoxycillin+Clavulanic acid	10	8 (R)	12 (R)
Cefuroxime	30	15 (I)	15 (I)
Cefotaxime	30	10 (R)	12 (R)
Cefepime	30	10 (R)	13 (R)
Novobiocin	30	12 (R)	12 (R)
Gentamicin	10	27 (S)	25 (S)
Amikacin	30	23 (S)	20 (S)
Kanamycin	5	22 (S)	21 (S)
Ciprofloxacin	5	25 (S)	30 (S)
Enrofloxacin	5	26 (S)	28 (S)
Sulfamethoxazole+Trimethoprim	23,75/1,25	6 (R)	10 (R)

S – sensitive; I – intermediate; R – resistant

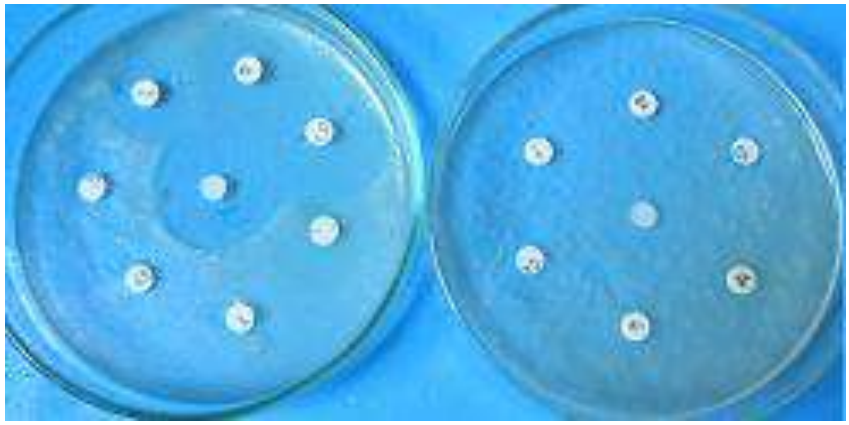


Figure 6: Sensitivity of the isolated bacteria *Serratia marcescens* to antimicrobial agents.

Discussion

Depite viruses such as bovine respiratory syncytial virus (BRSV) and infectious bovine rhinotracheitis virus (IBRV), the main causes of respiratory infections in cattle are in most cases *Mannheimia haemolytica*, *Pasteurella multocida* and *Mycoplasma* spp. (Peek, 2011). Although the pathological findings in the cows examined were characteristic of pasteurellosis, bacteria of genus *Pasteurella* were not isolated. This was an evidence for microbiological research to assist accurate diagnosis. The precise choice of the most appropriate means of antibiotic therapy was another reason to perform the study.

The atypical course of infections today is not uncommon, especially in those with more than one cause, as is the case with the studied animals. Transportation stress is one of the significant factors that provoke the development of diseases caused by conditionally pathogenic microorganisms (Chirase et al., 2004; Caswell, 2013). Combination infections, as in this case, are characterized by a heavier course and often unfavourable prognosis, especially when the agents are polyresistant to antimicrobials. The infection was complicated with development of *C. albicans*, probably as a result of antibiotic therapy prior to transportation. Materials were taken aseptically immediately after death of the animals, so it was not a post-mortem contamination. The immunosuppressive action of *C. albicans* is a factor contributing to the severity of disease and lethal outcome. To avoid morbidity and mortality losses after transportation of animals, prophylaxis is of particular importance. It includes careful transportation without exhaustion or overheating the animals during the summer months, high level ofhygiene and complete nutrition. The health before transport is also important.

Bacteria isolated at the present study were from groups that easily develop polyresistance to antibiotic treatment, especially *S. marcescens*, as evidenced by the data for sensitivity to antimicrobials. The same was also observed in *S. xylosus*, but to less extent. Similar results of susceptibility study to antimicrobials in both major bacteria isolated give us the reason to assume that these strains have evolved together as a part of the animal microflora. The results of the sensitivity tests to antibiotics confirmed the trend of increasing prevalence of polyresistant strains. Antibiotic selection should be done after in vitro antimicrobial susceptibility testing. In recent years, however, in Japan, Ohnishi et al. (2011) found a significantly higher sensitivity of *S. marcescens* isolated from cows compared to our results. Obviously, in Europe, the problem of excessive use of antibiotics in animals is greater and has its consequences.

S. marcescens, *S. pneumoniae*, *S. xylosus* and *C. albicans*, isolated by us, obviously were representatives of the normal upper airway microflora of the tested animals. Their high resistance to antibiotics indicated that they were most likely selected after frequent treatment with such agents of almost all chemical groups. This was probably done to suppress the development of conditionally pathogenic infections, due to weaknesses in hygiene of the breeding farm. The established resistance to cephalosporins of second third and fourth generations (not sensitive to β-lactamases) could also be accepted as an evidence. Obviously, these microorganisms produce broad-spectrum of β-lactamases, which is the trend today. Most producers of such enzymes are resistant to a number of cephalosporins and penicillins. Many are also resistant to non-β-lactam agents such as fluoroquinolones, trimethoprim, and aminoglycosides (gentamycin, etc.) due to concomitant plasmid expression or other co-expressed mechanisms of resistance (Pallett and Hand, 2010). The stress experienced during the long-term transportation and adapting to new living conditions was a prerequisite for their multiplication, which *in vivo* was accompanied by an increase in their virulence. Isolation of *E. faecalis* can be considered as an indicator of poor hygiene of the animals before transportation.

Conclusion

Atypical pneumonia was established in simmental cows that died after importation from Austria with signs of pasteurellosis.

Microbiological study of lung materials revealed a combined infection with Serratia marcescens and small amounts of Staphylococcus xylosus, Streptococcus pneumoniae, Enterococcus faecalis, and Candida albicans.

Poly-resistance to antibiotics of the isolated bacteria was identified in vitro. This indicated that the resistant strains were most likely selected in the animals after frequent treatment with broad spectrum of antibacterial agents to suppress conditionally pathogenic infections. The reason possibly was due to weaknesses in breeding and feeding hygiene of the animals in the farm that they inhabited prior to their import in our country.

References

1. Bauer, A. W., W. M. Kirby, J. C. Cherris, M. Truck. (1966). *Antibiotic susceptibility testing by a standardized single disk method.* The Am. J. of Clin. Pathol., Vol. 45–49: 493–496.
2. Caswell, J. L. (2013). *Failure of Respiratory Defenses in the Pathogenesis of Bacterial Pneumonia of Cattle.* Veterinary Pathology, Vol. 51(2): 393–409.
3. Chirase, N. K., L. W. Greene, C. W. Purdy, R. W. Loan, B. W. Auvermann, D. B. Parker, E.l F. Walborg Jr, D. E. Stevenson, Y. Xu, J. E. Klaunig. (2004). *Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle.* American Journal of Veterinary Research, Vol. 65(6): 860–864. https: //doi.org/10.2460/ajvr.2004.65.860.
4. Chockalingam, A., D. S. Zarlenga, and D. D. Bannerman. (2007). *Antimicrobial activity of bovine bactericidal permeability–increasing protein–derived peptides against gram-negative bacteria isolated from the milk of cows with clinical mastitis.* American Journal of Veterinary Research, Vol. 68(11): 1151–1159.
5. Cohen, A. L., A. Ridpath, J. Noble-Wang, B. Jensen, A. M. Peterson, M. Arduino, D. Jernigan, A. Srinivasan. (2008). *Outbreak of Serratia marcescens bloodstream and central nervous system infections after interventional pain management procedures.* Clin J Pain. Vol. 24(5): 374–380. doi: 10.1097/AJP.0b013e31816157db.
6. Holt, J. G., N. R. Krieg, P. H. A. Sneart, J. T. Staley, S. T. Williams. (1994). *Bergey's manual of determinative bacteriology.* Ninth edition, Williams & Wilkins.
7. Nagase, N., A., Sasaki, K.. Yamashita, A. Shimizu, Y. Wakita, S. Kitai & J. Kawano. (2001). *Isolation and species distribution of staphylococci from animal and human skin.* J Vet Med Sci, Vol. 64: 245–250.
8. Novakova, D., I. Sedlacek, R. Pantucek, V. Stetina, P. Svec and P. Petras. (2006). *Staphylococcus equorum and Staphylococcus succinus isolated from human clinical specimens.* Journal of Medical Microbiology, Vol. 55: 523–528.
9. Ohnishi, M., T. Sawada, K. Hirose, R. Sato, M. Hayashimoto, E. Hata, C. Yonezawa, H. Kato. (2011). *Antimicrobial susceptibilities and bacteriological characteristics of bovine Pseudomonas aeruginosa and Serratia marcescens isolates from mastitis.* Veterinary Microbiology, Vol. 154(1–2): 202–207.
10. Pallett, A., K. Hand. (2010). *Complicated urinary tract infections: practical solutions for the treatment of multiresistant Gram-negative bacteria.* J. Antimicrob. Chemother., Vol. 65(Suppl 3): 25–33.
11. Peek, S. (2011). *Pneumonia in adult dairy cattle (Proceedings).* CVC in Kansas City Proceedings, Aug 01, 2011. http: //veterinarycalendar.dvm360.com/pneumonia-adult-dairy-cattle-proceedings.