Tuberculosis in cattle caused by IS*901*+ *Mycobacterium avium* subsp. *avium* – a case report

M. Ocepek[1], M. Pate[1], M. Zolnir-Dovc[2], Z. Cvetnic[3]

[1]Veterinary Faculty Ljubljana, Ljubljana, Slovenia
[2]University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia
[3]Croatian Veterinary Institute, Zagreb, Croatia

ABSTRACT: In a small cattle herd an eight-year-old cow showed a reaction to bovine tuberculin in two consecutive skin tests. The animal showing clinical signs related to tuberculosis was slaughtered for diagnostic purpose. The lungs were completely covered with disseminate tubercles of different sizes and the mediastinal lymph nodes were enlarged. *Pneumonia granulomatosa tuberculosa* and *lymphadenitis hyperplastica chronica* were diagnosed histologically. *Mycobacterium avium* subsp. *avium* of IS*901*+ and IS*1245*+ genotype was isolated from lungs and from the lymph node. Six weeks after the affected animal has been eliminated from the herd, the skin test in other animals on the farm was performed: single test with avian tuberculin in extensively reared poultry (n = 12), comparative test with bovine and avian tuberculin in cattle (n = 7). Concluding from the results of negative skin testing of other animals on the farm, it is very likely that the cow did not transmit *M. a. avium* into the environment.

Keywords: mycobacteria; lung's tuberculosis; Slovenia

Mycobacterium avium subsp. *avium (M. a. avium)* of serotypes 1, 2 and 3 and of IS*901*+ and IS*1245*+ genotype is the causative agent of avian tuberculosis which is considered as a contagious disease of poultry and birds. The disease is characterized by its chronicity, death is usually inevitable. *M. a. avium* has been isolated also from a wide range of mammals, including humans. In pigs, the disease caused by isolates of *M. a. avium* of these serotypes and genotype often occurs as lymphadenitis and rarely as parenchymatous tuberculosis. In other species of domestic animals such as horses, cats, dogs and cattle, a disease due to *M. a. avium* is very uncommon (Pavlik *et al*., 2000; Mijs *et al*., 2002).

Infection is usually self-limiting, although cases of generalized disease have been reported (Thorel *et al*., 1997, 2001). Oral infection of the cattle rarely leads to lesions. If the lesions appear, they are confined to the mesenteric lymph nodes (Thoen, 1994). However, spread from the mesenteric or retropharyngeal lymph nodes is possible and involvement of the serous surfaces of body cavities might occur. The lungs, liver, portal lymph nodes, spleen, kidneys, renal lymph nodes and adrenal glands may be affected. The gross appearance of lesions is in most cases similar to lesions of *M. bovis* infection (Lesslie and Birn, 1967; Lepper and Corner, 1983).

The importance of cattle infection with *M. a. avium* lies in the development of a sensitisation to bovine tuberculin. If a single skin test is performed, the reactions in cattle, infected with *M. a. avium*, can be interpreted as false positive or paraalergic. In the comparative skin test, reactions to avian tuberculin are usually stronger than the reactions to bovine tuberculin (Thorel *et al*., 2001).

It has been reported that the isolation of *M. a. avium* from lymphoid tissue of apparently healthy cattle is possible (Lepper and Corner, 1983). The excretion of the causative agent might precede the clinical manifestations. Tuberculous yet macroscopically unchanged milk from clinically normal cows, not classified as tuberculin reactors, could be distributed commercially for human consumption. But the public health hazard is likely to be serious only in rural areas, where unpasteurised milk from individual cows is available for consumption (Lesslie and Birn, 1967).

In the case presented, an eight-year-old cow from a small cattle herd of seven animals reacted with

bovine tuberculin in two consecutive skin tests. The animal showing clinical signs (cough, enlarged subcutaneous lymph nodes) was slaughtered for diagnostic purpose. During examination in a slaughterhouse, pathologically changed lungs and one mediastinal lymph node were observed. Therefore the organs were submitted for histopathological and microbiological examination and the tissue was examined for the presence of mycobacteria.

MATERIAL AND METHODS

Material

The lungs and the mediastinal lymph node of one eight-year-old cow were examined.

Histopathology

The pathologically changed lungs and the lymph node were examined histologically by staining with Ziehl-Neelsen (Z-N) and hematoxylin-eosin.

Bacteriology

The smears from the lungs and from the lymph node were stained by Z-N technique. The samples were decontaminated with N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method and inoculated in Mycobacteria Growth Indicator Tube (MGIT, Becton Dickinson), on Löwenstein-Jensen medium supplemented with pyruvate (LJ-P), Löwenstein-Jensen medium supplemented with glycerine (LJ-G), Middlebrook 7H10 and Stonebrink growth media (Kent and Kubica, 1985).

The identification of the mycobacteria was based upon the speed of growth, growth at different temperatures, the colour and shape of the colonies and biochemical activity (Ocepek, 1996).

Molecular methods

Two standardized amplification assays, AMPLICOR® *M. avium*, *M. intracellulare, M. tuberculosis* complex tests (Roche Diagnostic Systems, Branchburg, USA) and MTD-Amplified *M. tuberculosis* Direct Test (Gen-Probe®, San Diego, USA) were performed on the clinical samples according to the instructions of the manufacturer. In addition, an in house IS*1245* polymerase chain reaction (PCR) was conducted. The isolation of the genomic DNA was performed with a commercial kit (High Pure PCR Template Preparation Kit, Roche Diagnostics, Mannheim, Germany), following the protocol of the manufacturer. Specific primers, described by Guerrero *et al.* (1995), P1 (5′ GCC-GCC-GAA-ACG-ATC-TAC 3′) and P2 (5′ AGG-TGG-CGT-CGA-GGA-AGA-C 3′) were used to amplify 427 bp fragment of IS*1245*. PCR was performed with AmpliTaq Gold Polymerase (Perkin Elmer) using the following procedure: 6 cycles (1 min at 94°C, 1 min at 66°C with the subtraction of 1°C in every cycle, 1 min at 72°C) followed by 35 cycles (30 s at 94°C, 30 s at 60°C, 30 s with the addition of 3 s in every cycle at 72°C) and by final extension (5 min at 72°C). PCR-products were analysed by electrophoresis on 2% agarose gel stained with ethidium bromide.

ACCUPROBE® *M. avium* complex**,** *M. avium*, *M. intracellulare* and *M. tuberculosis* complex culture identification test (Gen-Probe®, San Diego, California, USA) and IS*901* PCR have been performed. ACCUPROBE® assay was performed using a mix of colonies according to manufacturer's instructions. IS*901* PCR was performed – firstly from liquid medium and then from mix of colonies grown on solid media – as described previously (Kunze *et al*., 1992).

Skin test

Six weeks after the affected animal has been eliminated from the herd, the skin test in remaining animals on the farm was performed. In 6 cattle, a comparative intradermal skin test was performed, using bovine (Bovitubal, Bioveta, Czech Republic, 5000 TU per dosis) and avian (Avitubal, Bioveta, Czech Republic, 2000 TU per dosis) tuberculins. In poultry (n = 12), a single skin test with avian tuberculin of the same manufacturer was performed. Skin testing was conducted and interpreted according to Council directive 64/432/EEC. In cattle, 0.1 ml of both tuberculins was injected on the border of the anterior and middle thirds on the both sides of the neck; in poultry, 0.1 ml of avian tuberculin was injected in the wattle. The interpretation of reactions was based on clinical observations and measurement of skin-fold thickness (the swelling of the wattle, respectively) at the sites of injection 72 hours after injection of tuberculins.

RESULTS

Histopathology

The histopathological examination indicated the tuberculosis of the lungs (*pneumonia granulomatosa tuberculosa*) – the granulomata with central caseous necrosis, epitheloid cells and Langhans' giant cells were found in the sample. The lymph node showed chronic hyperplastic lymphadenitis (*lymphadenitis hyperplastica chronica*).

Bacteriology

Acid-fast rods (AFR) were found in the smear from the lungs, while the smear from the mediastinal lymph node was negative for AFR.

Growth in MGIT was noticed after five days AFR were found in the smear. On the eleventh day of incubation, the growth on LJ-P, LJ-G and Stonebrink media was detected.

Growth of the bacteria from the lymph node sample was observed on the eleventh day of incubation in MGIT. Originally, no growth on the solid media was noticed. The bacteria were then transferred from MGIT to Stonebrink medium where the growth was detected 13 days later.

Molecular methods

The AMPLICOR® assay for *M. avium*, performed on samples of the lungs and mediastinal lymph node, was positive while the AMPLICOR® tests for *M. intracellulare* and *M. tuberculosis* complex and MTD test for *M. tuberculosis* complex showed negative results. The result of the IS*1245* PCR was positive.

The ACCUPROBE® *M. avium* complex, *M. avium*, *M. intracellulare* and *M. tuberculosis* complex tests, performed on isolates, showed positive results for *M. avium* complex and *M. avium* tests and negative results for *M. intracellulare* and *M. tuberculosis* complex tests. The IS*901* PCR was positive which finally identified *M. a. avium* subspecies.

Skin test

All skin tested animals were negative with bovine and avian tuberculins.

DISCUSSION

In this paper one of the rare occasions of IS*901*+ and IS*1245*+ *M. a. avium* causing a disease in cattle is described. Chronic tuberculosis of the lungs induced the manifestation of clinical signs. The lesions were macro- and microscopically very similar to lesions caused by *M. bovis* (Nieberle and Meyn, 1938). Therefore, without performing the complete bacteriological investigation, the causative agent could be misdiagnosed.

The frequency of *M. a. avium* infections in cattle and consequent false diagnosis might not be so rare. Nassal (1961) reported the isolation of *M. a. avium* from 53.8% cattle, suspected of having avian tuberculosis; 2% of them had generalised tuberculosis. In our case *M. a. avium* has been isolated from both lungs and the lymph node. Regarding the examination of the latter, the phenomenon of growth detected in liquid medium but not in solid medium can be explained with low number of mycobacteria present in the sample. Using the molecular methods, it was possible to obtain the results even before the isolation of the causative agent. On the basis of the standardized MTD *M. tuberculosis* complex test and AMPLICOR® *M. avium*, *M. intracellulare* and *M. tuberculosis* complex assays, performed on the samples of the lungs and the lymph node, it was possible to exclude *M. bovis* as the cause of the disease.

The rapidity and the accuracy of making the diagnosis are of utmost importance when a suspicion of a dangerous contagious disease, especially zoonosis, arises. However, even though the AMPLICOR® assay for *M. avium* and the IS*1245* PCR gave positive results, the isolation of the causative agent still represents a gold standard in making the diagnosis. The cultures were identified also by classical and molecular determination. The ACCUPROBE® assay gave identical results as AMPLICOR® assay (positive for *M. avium*, negative for *M. intracellulare* and *M. tuberculosis* complex) and the IS*901* PCR was also positive. Concluding from the results of skin testing of other animals on the farm, it is very likely that the cow did not transmit *M. a. avium* into the environment.

CONCLUSIONS

The interference of the reactions in the single skin test and the similarity of lesions caused by

two different species of mycobacteria (*M. a. avium* and *M. bovis*) can be very misleading when making the diagnosis. The diagnosis of bovine tuberculosis consequently calls for radical measures. This indicates the importance of identification of the causative agent before any conclusions are made, based solely upon the results of the skin test and histopathological examination.

Acknowledgements

We thank Milojka Setina for providing technical support.

REFERENCES

Guerrero C., Bernasconi C., Burki D., Bodmer T., Telenti A. (1995): A novel insertion element from *Mycobacterium avium*, IS*1245*, is a specific target for analysis of strain relatedness. J. Clin. Microbiol., *33*, 304–307.

Kent P.T., Kubica G.P. (1985): Public health mycobacteriology: A guide for level III laboratory. U.S. Department of Health and Human Services. Centers for Disease Control, Atlanta.

Kunze Z.M., Portaels F., McFadden J.J. (1992): Biologically distinct subtypes of *Mycobacterium avium* differ in possession of insertion sequence IS*901*. J. Clin. Microbiol., *30*, 2366–2372.

Lepper A.W.D., Corner L.A. (1983): Naturally occurring mycobacterioses of animals. In: Ratledge C., Stanford J. (eds.): The biology of the mycobacteria. 1st ed., Vol. 2. Academic Press, London. 427–435.

Lesslie I.W., Birn K.J. (1967): Tuberculosis in cattle caused by the avian type tubercle bacillus. Vet. Rec., *80*, 559–564.

Mijs W., De Haas P., Rossau R., van der Laan T., Rigouts L., Portaels F., van Soolingen D. (2002): Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp. *avium* to bird-type isolates and *M. avium* subsp. *hominissuis* for the human/porcine type of *M. avium*. Int. J. Syst. Evol. Microbiol., *52*, 1505–1518.

Nassal J. (1961): Ergebnisse und Probleme der aviären Tuberkulose des Rindes. Berl. Münch. Tierärztl. Wschr., *74*, 210–214.

Nieberle K., Meyn A. (1938): Tuberkulose und Fleischhygiene. 1st ed. Gustav Fischer Verlag, Jena. 36–58.

Ocepek M. (1996): Antigenic structure of *Mycobacterium avium* complex, isolated from domestic animals in Slovenia in the period from 1989 to 1993. Zb. Vet. Fak., *33*, 245–253.

Pavlik I., Svastova P., Bartl J., Dvorska L., Rychlik I. (2000): Relationship between IS*901* in the *Mycobacterium avium* complex strains isolated from birds, animals, humans and environment and virulence for poultry. Clin. Diag. Lab. Immunol., *7*, 212–217.

Thoen C.O. (1994): *Mycobacterium avium* infections in animals. Res. Microbiol., *145*, 173–177.

Thorel M.F., Huchzermeyer H.F., Weiss R., Fontaine J.J. (1997): *Mycobacterium avium* infections in animals. Literature review. Vet. Res., *28*, 439–447.

Thorel M.F., Huchzermeyer H.F., Michel A.L. (2001): *Mycobacterium avium* and *Mycobacterium intracellulare* infection in mammals. Rev. Sci. Tech. Off. Int. Epiz., *20*, 204–218.

Received: 02–10–07
Accepted afte correction: 03–02–17

Corresponding Autor

Matjaz Ocepek, PhD., Veterinary Faculty Ljubljana, Gerbiceva 60, 1000 Ljubljana, Slovenia
Tel. +386 1 477 92 52, e-mail: matjaz.ocepek@vf.uni-lj.si