VETERINARSKI ARHIV 81 (1), 67-76, 2011

Mycobacterium avium subsp. *hominissuis* in wild boar (*Sus scrofa*) in the Republic of Croatia

Željko Cvetnić[1]*, Silvio Špičić[1], Josip Tončić[2], Ivana Račić[1], Sanja Duvnjak[1], and Maja Zdelar-Tuk[1]

[1]*Croatian Veterinary Institute, Zagreb, Croatia*

[2]*Institute of Forestry, Jastrebarsko, Croatia*

CVETNIĆ, Ž., Š. ŠPIČIĆ, J. TONČIĆ, I. RAČIĆ, S. DUVNJAK, M. ZDELAR-TUK: *Mycobacterium avium* subsp. *hominissuis* in wild boar (*Sus scrofa*) in the Republic of Croatia. Vet. arhiv 81, 67-76, 2011.

ABSTRACT

During the research period (2001-2005), the lymph nodes of 123 wild boars from seven locations in the Republic of Croatia were tested for the presence of mycobacteria. Mycobacteria were isolated from the lymph nodes of 15 (12.2%) wild boars from five locations. Specific hybridization showed that 11 (8.9%) isolates belonged to *M. avium*, 2 (1.6%) isolates were typed as *M. fortuitum*, and 2 (1.6%) isolates belonged to *M. gordonae*. All 11 isolates belonging to *M. avium* sp. were tested by means of the specific primers P1 FR300 and P2 FR300. Product size in all isolates was 300 bp, which confirms that *M. avium* subsp. *hominissuis* was present in all of them. As in swine from intensive farming, *M. avium* subsp. *hominissuis* is a dominant mycrobacteria type in wild boar in Croatia. The results lead to the conclusion that the most important source for wild boar is an environment suitable for the development and survival of *M. avium* subsp. *hominissuis*, as confirmed in earlier research studies by other authors.

Key words: wild boar, *Mycobacterium avium* subsp. *hominissuis*, Croatia

Introduction

There is no mycobacterial species specific to pigs such as *Mycobacterium (M.) tuberculosis* in humans, *M. bovis* in bovides or *M. avium* in poultry and birds. However pigs are susceptible to infections caused by members of the *M. tuberculosis* and *M. avium* complex as well as opportunistic mycobacterial species such as *M. fortuitum* and *M. chelonae* (CVETNIĆ et al., 1998; MATLOVA et al., 2004). Being widespread in the environment, potentially pathogenic mycobacteria are a frequent cause of mycobacteriosa in bovides, swine and humans. The environment (water, soil, sawdust, feedstuff, birds

*Corresponding author:

Prof. Dr. Željko Cvetnić, PhD, DVM, Croatian Veterinary Institute, Savska cesta 143, 10000 Zagreb, Croatia, Phone: + 385 1 6123 602; Fax: + 385 1 6190 841; E-mail: cvetnic@veinst.hr

ISSN 0372-5480
Printed in Croatia

etc.) is a risk factor for human and animal infections caused by the *M. avium* complex (CVETNIĆ et al., 1998; MARTIN and SCHIMMEL, 2000; REED et al., 2006; MATLOVA et al., 2004; FISCHER et al., 2006). Considering its virulence for birds, the *M. avium* complex is divided in three groups. The first group, *M. avium* subsp. *avium* includes serotypes 1, 2 and 3 and is very pathogenic for birds and it has insertion sequence (IS*901*) (PAVLIK et al., 2000; MIJS et al., 2002). The second group, *M. avium* subs. *hominissuis* includes serotypes 4-6,8-11 and 21 and does not have IS901, but does have IS1245 (RITACCO et al., 1998). The third group, *M. intracellulare* includes serotypes 7, 12 - 20, 22 and 28 and has no IS element, and is not pathogenic for birds (MIJS et al., 2002). MACHACKOVA et al. (2003) and TRCKA et al. (2006) described the spread of various types of mycobacteria in wild boar in Central Europe and the Czech Republic. Previous studies in Croatia describe the findings of *M. avium* complex and other mycobacteria in domestic pigs (CVETNIĆ et al., 1998 and 2007).

This paper presents the results of research into the presence of mycobacteria in wild boars in various regions of Croatia in the period from 2001 until 2005, and identification of mycobacteria was done by various molecular methods.

Materials and methods

During the research period of five years (2001 to 2005), samples of lymph nodes of 123 wild boars of various categories were submitted from regular hunting. The wild boars originated from 7 areas: Velika Gorica (6 samples), Sisak (18 samples), Novska (12 samples), Nova Gradiška (10 samples), Đakovo (33 samples), Vinkovci (21 samples) and Beli Manastir (23 samples).

Gross examination. For tests for the presence of mycobacteria, samples of lymph nodes were submitted (ln. submandibularis, ln. mesenterialis, ln. inguinalis, ln. mediastinalis, ln. hepaticus, ln. ileocaecales). Upon sampling, pathoanatomic inspection of lymph nodes and organs of wild boars on tuberculosis and other diseases was done. Laboratory testing was conducted on 110 samples of various lymph nodes in 2001, 219 in 2002, 94 in 2003, 88 in 2004 and 19 in 2005. A total of 530 samples from 123 wild boars were examined in period from 2001-2005.

Bacteriological examination. Lymph node smears were stained by Ziehl-Neelsen (ZN) and checked for the presence of acid-fast bacteria. Lymph nodes were homogenised, concentrated and decontaminated using NALC-NaOH and inoculated on standard nutrient media: Löwenstein-Jensen with pyruvate, Löwenstein-Jensen with glycerine, Stonebrink and Middlebrook 7H10 and incubated at 37 °C. The media were checked for growth of mycobacteria during the following two months at weekly intervals. All grown colonies were stained using ZN staining for acid-fast bacteria. Positive colonies were subcultivated

and identified by specific hybridization and PCR methods (KENT and KUBICA, 1984; HANCE et al., 1989; KUNZE et al., 1992).

Polymerase chain reaction (PCR). The fact that isolated strains belonged to the genus *Mycobacterium* was confirmed by amplification of the DNA sequence containing the gene coding 65 kDa antigen common for all mycobacteria. The primers TB1 (5'- GAG-ATC-GAG-CTG-GAG-GAT-CC-3') and TB2 (5'- AGC-TGC-AGC-CCA-AAG-GTG-TT-3') were used (HANCE et al., 1989). The amplification product size using these primers is 383 base pairs.

Isolated mycobacteria were specifically hybridised using Geno Type® Mycobacterium CM kit (Molecular Genetic Assay for identification of the clinically most relevant *Mycobacterium* species from cultured material, Hain, Germany). The test enables differentiation of 14 important mycobacterial species namely: *M. avium, M. chelonae, M. abscessus, M. fortuitum, M. gordonae, M. intracellulare, M. scrofulaceum, M. interjectum, M. kansasii, M. malmoense, M. peregrinum M. marinum/M. ulcerans, M. tuberculosis complex* and *M. xenopi*. The procedure includes several steps: isolation of DNA from cultured mycobacteria, amplification using biotinilated primers and reverse hybridisation. The hybridisation process includes chemical denaturation of the amplification product, hybridisation using one chain biotinilated product on a membrane with a probe, addition of conjugate streptavidin- alkaline phosphatase and evaluation of coloration.

Typing of isolated mycobacteria regarding integrated IS*901* was performed using primers P1 FR300 (5'- CAG- CCA- GCC- GAA- TGT- CAT- CC- 3') and P2 FR300 (5' CAA- CTC- GCG- ACA- CGT- TCA- CC- 3'). Amplification product size depends on the presence or absence of IS 901: If there is no incorporated IS*901,* product size is 300 bp (*M. avium* subsp. *hominissuis* - serotypes 4-6, 8-11 and 21) or otherwise product size is 1700 bp (*M. avium* subsp. *avium* - serotypes 1, 2 and 3) (KUNZE et al., 1992).

In both tests visualisation was done after electrophoresis in 2% agarose gel using UV transluminator and camera (Bio-Capt, Vilbert Lourmat, France).

Results

Bacteriological isolation and identification of isolated mycobacteria. Mycobacteria were isolated from 15 (12.2%) of 123 wild boars. *M. avium* was identified in 11 (8.9%), *M. fortuitum* in 2 (1.6%) and *M. gordonae* in 2 (1.6%) samples (Table 1). Mycobacteria were isolated from wild boars from 5 locations: Velika Gorica (1 isolate), Sisak (3 isolates), Nova Gradiška (1 isolate), Vinkovci (6 isolates) and Beli Manastir (4 isolates). All 15 isolates were proven to belong to *Mycobacterium* sp. (Fig. 1).

Specific hybridisation by Geno Type® Mycobacterium CM kit was used to type all 15 mycobacteria isolates of wild boar and 11 isolates marked as VG/1, BM/3, 4, 5, 6, S/7, S/8, V10,11,12,13 were found to belong to *M. avium*, 2 isolates marked DS/14 and DS/15

were typed as *M. fortuitum*, and those marked NG/2 and S/9 were of type *M. gordonae* (Table 2).

Table 1. Mycobacteria in wild boars in Croatia (2001-2005)

Year	Number of animals	Number of isolates (%)	Number of mycobacteria isolates (%)		
			M. avium	*M. fortuitum*	*M. gordonae*
2001	29	3	3	0	0
2002	49	9	6	2	1
2003	21	1	1	0	0
2004	20	2	1	0	1
2005	4	0	0	0	0
Total	123	15	11 (8.9%)	2 (1.6%)	2 (1.6%)

Table 2. Results of specific hybridisation by use of Genotype Mycobacterium CM kit

Type *Mycobacterium* sp.	Wild boar (Isolate code)
Mycobacterium sp.	15
M. avium sp.	VG/1, BM/3,4,5,6 S/7, S/8, V10,11,12,13
M. chelone	0
M. abscessus	0
M. fortuitum	V/14, V/15
M. gordonae	NG/2, S/9
M. intracellulare	0
M. scrofulaceum	0
M. interjectum	0
M. kansasii	0
M. malmoense	0
M. peregrinum	0
M.marinum /M. ulcerans	0
M. tuberculosis complex	0
M. xenopi	0

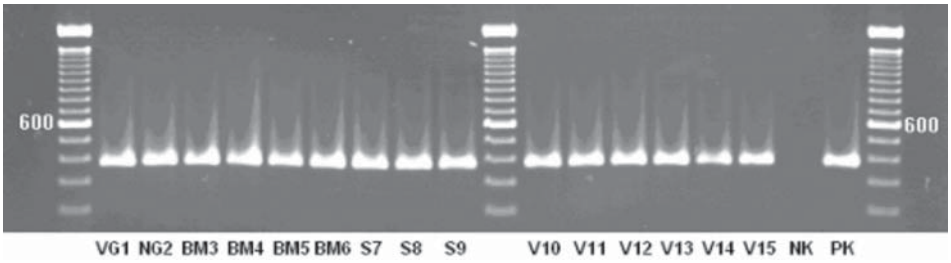

Fig. 1. Evidence of belonging to *Mycobacterium* sp. (65 kDa) of isolates from wild boar samples

Legend: 600 - amplification product size of 600 base pairs (bp); NK - negative control of amplification; PK - positive control of amplification (*Mycobacterium bovis* subsp. *caprae*); Mycobacteria isolates from wild boar were lined by number and hunting ground code VG1 - Velika Gorica (1 isolate), NG-2 - Nova Gradiška (1 isolate), BM-3,4,5,6 - Beli Manastir (4 isolates), S-7,8,9 - Sisak (3 isolates), V-10,11,12,13,14,15 - Vinkovci (6 isolates)

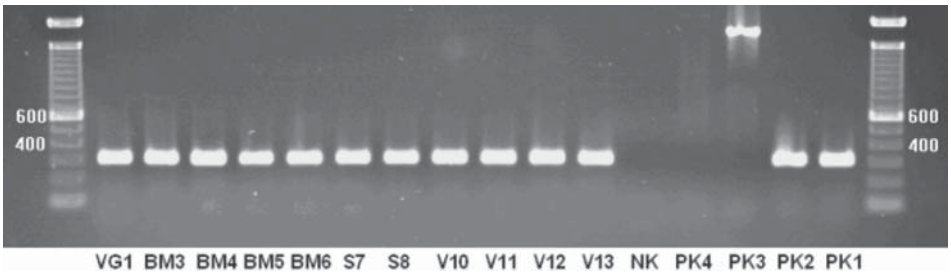

Fig. 2. Confirmation of *M. avium subsp. hominissuis* in isolated mycobacteria

Legend: 400 - amplification product size 400 base pairs; 600 - amplification product size 600 base pairs; PK1 - *Mycobacterium avium* sv. 8; PK2 - *Mycobacterium avium* sv. 4; PK3 - *Mycobacterium avium* sv. 2; PK4 - *Mycobacterium bovis;* Isolates of *M. avium* sp. from wild boar were lined by number and hunting ground code VG-1 - Velika Gorica (1 isolate), BM3,4,5,6 - Beli Manastir (4 isolates), S7,8 - Sisak (2 isolates), V10,11,12,13 - Vinkovci (4 isolates)

Furthermore, amplification product sized 300 bp was found in all isolated mycobacteria implicating that the isolated mycobacteria belonged to *M. avium* subsp. *hominissuis* sv. 4-6, 8-11 and 21 - apathogenic for poultry (Fig. 2).

Discussion

Wild animals are natural reservoirs of various types of mycobacteria, particularly those pathogenic for humans. PRODINGER et al. (2002) described the finding of *M. caprae* in deer in Austria, PRODINGER et al. (2005) in camels in a zoo in the Czech Republic, deer

in the Czech Republic, wild boar in Hungary, and PATE et al. (2006) those in camels and buffalo in a zoo in Slovenia. BIET et al. (2005) differentiated mycobacteria into 2 groups according to their significance for infection of humans and domestic animals. The first group included the *M. tuberculosis* complex of specific socio-economic and public health significance, and the second group included mycobacteria from the environment, among which the most significant are those of the *M. avium* complex. Despite extensive research in the world, the issue of mycobacterial infection with *M. avium* is still of topical interest. In Croatia, except for routine control of the spread of tuberculosis in swine and cattle in intensive farming, there has been no control of the spread of tuberculosis among wild animals, including wild boars. Most recent studies by scientists in Spain on the European wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) show that they are reservoirs of tuberculosis agents in the wild (*M. tuberculosis* complex) and the most probable vectors to other types of domestic and wild animals and humans (VICENTE et al., 2006; NARANJO et al., 2008). It was proved that the badger (*Meles meles*) is a reservoir of *M. bovis* in Great Britain and Ireland (DE LISLE et al., 2001 and 2002). In research including almost all European countries, MACHACKOVA et al. (2003) isolated *M. tuberculosis* complex from wild boar samples in 33.9% cases and 39.8% isolates had *M. bovis* complex. As for other types of mycobacteria, isolated were also *M. intracellulare* (3.8%), *M. avium* subsp. *avium* (3.8%), *M. terrae* (2.4%), *M. fortuitum* (2.2%), *M. scrofulaceum* (2.2%), *M. gordonae* (0.8%), *M. simiae* (0.5%), *M. szulgai* (0.5%), *M. xenopi* (0.5%), *M. smegmatis* (0.2%), *M. vaccae* (0.2%), fast growing, non-identified types (0.2%) and non-identified mycobacteria (8.8%). TRCKA et al. (2006) isolated mycobacteria from 8.3% examined wild boar samples.

Identification of isolates was started by polymerase chain reaction by amplification of a gene part coding 65 kD antigen (*hsp65)*, present in all mycobacteria (TORTOLI, 2003). The characteristic product of amplification of 383 bp was determined for all 15 isolates. In the following test, specific hybridisation by means of GenoType Mycobacterium CM kit (Hain Lifescience, Germany) differed primarily between the 2 most significant mycobacteria complexes: *M. tuberculosis* complex and *M. avium* complex (*M. avium* sp. and *M. intracellulare*). Hybridisation tests, in general, are a significant advancement in the identification of mycobacteria types, as they make comparative testing of isolates possible. Their specificity and sensitivity is high (80%), and non-specific reactions are very rare and mostly referred to fast growing mycobacteria or types that were not yet discovered at the time when tests were developed (TORTOLI et al., 2001; TORTOLI, 2003; TORTOLI et al., 2005; TORTOLI, 2006). In our typing we did not manage to identify any isolate as *M. Intracellulare*, while 11 isolates were found to be *M. avium* sp.

After typing with Geno Type® Mycobacterium CM Molecular Genetic Assay, all isolates belonging to *M. avium* (11 isolates) were tested by means of the specific primers

P1 FR300 and P2 FR300. They are used to prove the insertion sequence IS*901*. Where IS*901* is not integrated, the product size is 300 bp and isolates belong to *M. avium* subsp. *hominissuis* (serotypes 4 - 6, 8 -11 or 21), and where IS*901* is there, product size is 1700 bp and isolates belong to *M. avium* subsp. *avium* (serotypes 1, 2 or 3) (KUNZE et al., 1992). In the beginning, *M. avium* subsp. *avium* was considered pathogenic only for poultry (BONO et al., 1995), however later it was isolated from swine (RITTACO et al., 1998). In Slovenia, the ratio of *M. avium* subsp. *avium* in swine was 33.8% and *M. avium* subsp. *hominissuis* 60.9% (PATE et al., 2004). THEGERSTRÖM et al. (2005) isolated this type in as many as 46% swine with tuberculosis in Sweden. In our earlier investigation (CVETNIĆ et al., 2006) most mycobacteria isolated from swine belonged to *Mycobacterium avium* complex (175 or 95.7%). Other identified species were *M. fortuitum* (6 or 3.3%), *M. chelonae (*1 or 0.5%) and *M. peregrinum* (1 or 0.5%). *M. avium* subsp. *hominnissuis* dominated over *M. avium* subsp. *avium* (138 versus 37 isolates or 78.9% versus 21.1%).

Based on the typing of isolates from wild boar in Croatia, all 11 isolates belong to *M. avium* subsp. *hominissuis*. As in swine from intensive breeding, *M. avium* subsp. *hominissuis* is the dominant type of mycobacteria in swine in Croatia. The results lead to the conclusion that the most important source for wild boar is an environment (water, soil, sawdust) suitable for the development and survival of *M. avium* subsp. *hominissuis*, as confirmed by former research studies by other authors.

Acknowledgements

The presented results are based on scientific project no. 048-0481150 -1150: Molecular epizootiology of the important bacterial zoonoses conducted with support from the Ministry of Science, Education and Sport of the Republic of Croatia.

References

BIET, F., M. L. BOSCHIROLI, M. F. THOREL, L. A. GUILLOTEAU (2005): Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium-intracellulare* complex (MAC). Vet. Res. 36, 411-436.

BONO, M., T. JEMMI, C. BERNASCONI, D. BURKI, A. TELENTI, T. BODMER (1995): Genotypic characterization of *Mycobacterium avium* strains recovered from animals and their comparison to human strains. Appl. Environ. Microbiol. 61, 371-373.

CVETNIĆ, Ž., H. KOVAČIĆ, M. OCEPEK (1998): Mykobakterien in der Umwelt und im Futter von Schweinen in Kroatien. Wien. Tierärztl. Monatscrift. 85, 18-21.

CVETNIĆ, Ž., S. ŠPIČIĆ, M. BENIĆ, V. KATALINIĆ-JANKOVIĆ, M. PATE, B. KRT, M. OCEPEK (2007): Mycobacterial infection of pigs in Croatia. Acta. Vet. Hung. 55, 1-9.

DE LISLE, G. W., C. G. MACKINTOSH, R. G. BENGIS (2001): *Mycobacterium bovis* in free-living and captive wildlife, including farmed deer, Rev. Sci. Tech. Off. Int. Epizoot. 20, 86-111.

DE LISLE, G. W., R. G. BENGIS, S. M. SCHMITT, D. J. O'BRIEN (2002): Tuberculosis in free-ranging wildlife: detection, diagnosis and management, Rev. Sci. Tech. Off. Int. Epizoot. 21, 317-334.

FISCHER, O. A., L. MATLOVA, L. DVORSKA, P. SVASTOVA, M. BARTOS, R. T. WESTON, I. PAVLIK (2006): Various stages in the life cycles of syrphid files (*Eristalis tenax; Diptera*: *Syrphidae*) as potential mechanical vectors of pathogens causing mycobacterial infections in pigs herds. Folia Microbiol. 51, 147-153.

HANCE, A. J., B. GRANDCHAMP, V. LEVI-FREBAULD, D. LECOSSIER, J. RAUZIER, D. BOCART, B. GICQUEL (1989): Detection and identification of mycobacteria by amplification of mycobacterial DNA. Mol. Microbiol. 7, 843-849.

KENT, P. T., G. P. KUBICA (1985): Public Health Mycobacteriology: A Guide for Level III Laboratory. US Department of Health and Human Services, Centers for Disease Control. Atlanta, GA, USA.

KUNZE, Z. M., F. PORTAELS, J. J. MCFADDEN (1992): Biologically distinct subtypes of *Mycobacterium avium* differ in possession of insertion sequence IS*901*. J. Clin. Microbiol. 30, 2366-2372.

MACHACKOVA, M., L. MATLOVA, J. LAMKA, J. SMOLIK, I. MELICHAREK, M. HANZLIKOVA, J. DOCEKAL, Ž. CVETNIĆ, G. NAGY, M. LIPIEC, M. OCEPEK, I. PAVLIK (2003): Wild boar (*Sus scrofa*) as a possible vector of mycobacterial infections: review of literature and critical analysis of data from Central Europe between 1983 and 2001. Vet. Med. 48, 51-65.

MARTIN, G., D. SCHIMMEL (2000): *Mycobacterium avium* infections in poultry - a risk for human health or not? Dtsch. Tierärztl. Wochenschr. 107, 53-58.

MATLOVA, L., L. DVORSKA, K. PALECEK, L. MAURENC, M. BARTOS, I. PAVLIK (2004): Impact of sawdust and wood shavings in bedding on pig tuberculous lesions in lymph nodes, and IS1245 RFLP analysis of *Mycobacterium avium* subsp. *hominissuis* of serotypes 6 and 8 isolated from pig and environment. Vet. Microbiol. 102, 227-236.

MIJS, W., P. DE HAAS, R. ROSSAU, T. VAN DER LAAN, L. RIGOUTS, F. PORTAELS, D. VAN SOOLINGEN (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.

NARANJO, V., C. GORTAZAR, J. VICENTE, J. DE LA FUENTE (2008): Evidence of the role of European wild boar as a reservoir of *Mycobacterium tuberculosis* complex. Vet. Microbiol, 127, 1-9

PATE, M., I. ZDOVC, T. PIRS, B. KRT, M. OCEPEK (2004): Isolation and characterisation of *Mycobacterium avium* and *Rhodococcus equi* from granulomatous lesions of swine lymph nodes in Slovenia. Acta Vet. Hung. 52, 143-150.

PATE, M., T. ŠVARA, M. GOMBAČ, T. PALLER, M. ŽOLNIR-DOVČ, I. EMERŠIČ, W. M. PRODINGER, M. BARTOŠ, I. ZDOVC, B. KRT, I. PAVLIK, Ž. CVETNIĆ, M. POGAČNIK,

M. OCEPEK (2006): Outbreak of tuberculosis caused by *Mycobacterium caprae* in a Zoological Garden. J. Vet. Med. B. Infect. Dis. Vet. Public. Health. 53, 387-392.

PAVLIK, I., P. SVASTOVA, J. BARTL, L. DVORSKA, I. RYCHLIC (2000): Relationship beetwen IS*901* in the *Mycobacterium avium* complex strains isolated from birds, animals, humans and the environment and virulence for poultry. Clin. Diagn. Lab. Immunol. 7, 212-217.

PRODINGER, W. M., A. EIGENTLER, F. ALLENBERGER, M. SCHONBAUER, W. GLAWISCHING (2002): Infection of red deer, cattle and humans with *Mycobacterium bovis* subsp. *caprae* in western Austria. J. Clin. Microbiol. 40, 2270- 2272.

PRODINGER, W. M., A. BRANDSTÄTTER, L. NAUMANN, M. PACCIARINI, T. KUBICA, M. L. BOSCHIROLI, A. ARANAZ, G. NAGY, Ž. CVETNIĆ, M. OCEPEK, A. SKRYPNYK, W. ERLER, S. NIEMANN, I. PAVLIK, I. MOSER (2005): Characterization of *Mycobacterium caprae* isolates from Europe by mycobacterial interspersed repetitive unit genotyping. J. Clin. Microbiol. 43, 4984-4992.

REED, C., C. F. VON REYN, S. CHAMBLE, T. V. ELLERBROCK, J. W. JOHNSON, B. J. MARSH, L. S. JOHNSON, R. J. TRENSCHEL, C. HIRSBURGH (2006): Environmental risk factor for infection with *Mycobacterium avium* complex. Am. J. Epidemiol. 164, 32-40.

RITACCO, V., K. KREMER, T. VAN DER LAAN, J. E. PIJNENBURG, P. E. DE HAAS, D. VAN SOOLINGEN (1998): Use of IS*901* and IS*1245* in RFLP typing of *Mycobacterium avium* complex: relatedness among serovar reference strains, human and animal isolates. Int. J. Tuberc. Lung. Dis. 2, 242-251.

THEGERSTRÖM, J., B. I. MARKLUND, S. HOFFNER, D. AXELSSON-OLSSON, J. KAUPPINEN, B. OLSEN (2005): *Mycobacterium avium* with the bird type IS1245 RFLP profile is commonly found in wild and domestic animals, but rarely in humans. Scand. J. Infect. Dis. 37, 15-20.

TORTOLI, E., A. NANETTI, C. PIERSIMONI (2001): Performance assessment of new multiplex probe assay for identification of mycobacteria. J. Clin. Microbiol. 39, 1079-1084.

TORTOLI, E. (2003): Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. Clin. Microbiol. Rev. 16, 319-354.

TORTOLI, E., L. CHIANURA, L. FABBRO (2005): Infections due to the newly described species *Mycobacterium parascrofulaceum*. J. Clin. Microbiol. 43, 4286-4287.

TORTOLI, E. (2006): The new mycobacteria: an update. FEMS Immunol. Med. Microbiol. 48,159-178.

TRCKA, I., J. LAMKA, M. KOPECNA, V. BERAN, I. PARMOVA, I. PAVLIK (2006): Mycobacteria in wild boar (*Sus scrofa*) in the Czech Republic. Vet. arhiv 76 (Suppl.), S27-S32.

VICENTE, J., U. HÖFLE, J.M. GARRIDO, I. G. FERNANDEZ-DE-MERA, R. JUSTE, M. BARRAL, C. GORTARAZ (2006): Wild boar and red deer display high prevalence of tuberculosis-like lesions in Spain. Vet. Res. 37, 107-119.

Received: 23 November 2009
Accepted: 22 December 2009

CVETNIĆ, Ž., Š. ŠPIČIĆ, J. TONČIĆ, I. RAČIĆ, S. DUVNJAK, M. ZDELAR-TUK: *Mycobacterium avium* subsp. *hominissuis* izdvojen iz divlje svinje (*Sus scrofa*) u Republici Hrvatskoj. Vet. arhiv 81, 67-76, 2011.

SAŽETAK

Tijekom istraživanog razdoblja (2001. do 2005.) radi pretrage na prisutnost mikobakterija obrađeni su limfni čvorovi 123 divlje svinje iz sedam lokaliteta u Republici Hrvatskoj. Mikobakterije su izdvojene iz limfnih čvorova 15 (12,2%) divljih svinja iz pet lokaliteta. Specifičnom hibridizacijom dokazano je da 11 (8,9%) izolata pripada vrsti *M. avium* sp., 2 (1,6%) izolata tipizirana su kao *M. fortuitum*, a 2 (1,6%) izolata pripadali su vrsti *M. gordonae*. Svih 11 izolata koji su pripadali *M. avium* sp. testirani su pomoću specifičnih početnica P1 FR300 i P2 FR300. U svih izolata veličina proizvoda iznosila je 300 parova baza što potvrđuje da se u svih radi o vrsti *M. avium* subsp. *hominissuis*. Kao i u svinja iz intenzivnog uzgoja, *M. avium* subsp. *hominissuis* predstavlja dominantnu vrstu mikobakterija u divljih svinja u Hrvatskoj. Na temelju dobivenih rezultata može se zaključiti da je po svemu sudeći najvažniji izvor za divlje svinje okoliš koji pogoduje razvoju i opstanku *M. avium* subsp. *hominissuis*, što potvrđuju i ranija istraživanja drugih autora.

Ključne riječi: divlja svinja, *Mycobacterium avium* subsp. *hominissuis*, Hrvatska