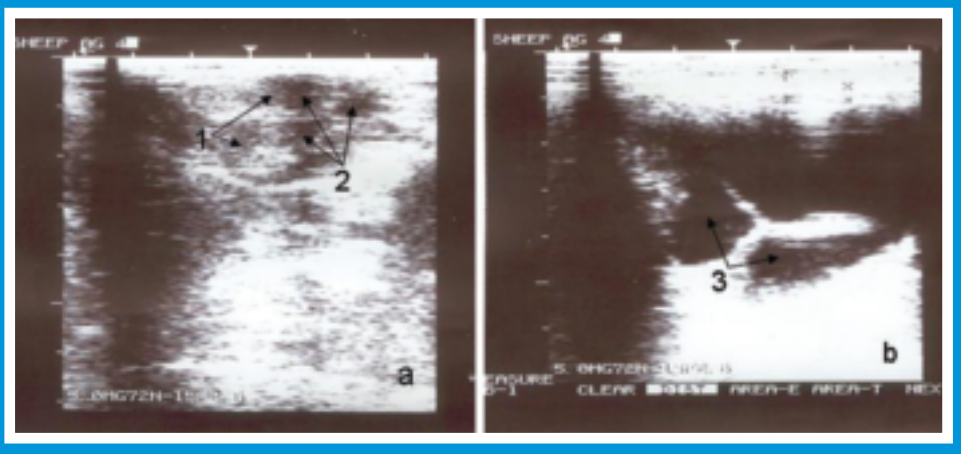
ISSN 1580-4003

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK



Slov Vet Res 2009; 46 (4): 121-58

Volume 46 4

Slov Vet Res • Ljubljana • 2009 • Volume 46 • Number 4 • 121-58

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Volume 46 4

Slov Vet Res • Ljubljana • 2009 • Volume 46 • Number 4 • 121-58

The Scientific Journal of the Veterinary Faculty University of Ljubljana

SLOVENIAN VETERINARY RESEARCH
SLOVENSKI VETERINARSKI ZBORNIK

Previously: RESEARCH REPORTS OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA
Prej: ZBORNIK VETERINARSKE FAKULTETE UNIVERZA V LJUBLJANI

4 issues per year / izhaja štirikrat letno

Address: Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia
Naslov: Veterinarska fakulteta, Gerbičeva 60, 1000 Ljubljana, Slovenija
Tel.: +386 (0)1 47 79 100, 47 79 129, Fax: +386 (0)1 28 32 243
E-mail: slovetres@vf.uni-lj.si

Sponsored by the Slovenian Research Agency
Sofinancira: Agencija za raziskovalno dejavnost Republike Slovenije

ISSN 1580-4003
Printed by / tisk: Birografika Bori d.o.o., Ljubljana
Indexed in / indeksirano v: Agris, Biomedicina Slovenica, CAB Abstracts, IVSI
Urlich's International Periodicals Directory, Science Citation Index Expanded,
Journal Citation Reports/Science Edition
http://www.slovetres.si/

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Slov Vet Res 2009; 46 (4)

Original Research Papers

Domanjko Petrič A, Cvetko S. Aortic stenosis in dogs: Clinical characteristics and survival in 80 cases 125

Škrlep M, Prevolnik M, Šegula B, Čandek-Potokar M. Association of plasma stress markers at slaughter with carcass or meat quality in pigs 133

Case Reports

Yotov S, Dimitrov D, Fasulkov I. Hydrometra in a sheep after oestrus synchronization and insemination in the anoestral season 143

Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. High prevalence of *Mycobacterium avium* subsp. *hominissuis* in a batch of quarantined pigs in Croatia 149

Subject Index Volume 46, 2009 155

Author Index Volume 46, 2009 157

Slov Vet Res 2009; 46 (4): 125-31
UDC 636.7.09:616.132:616-007.271-073:616-071

Original Research Paper

AORTIC STENOSIS IN DOGS: CLINICAL CHARACTERISTICS AND SURVIVAL IN 80 CASES

Aleksandra Domanjko Petrič*, Sanja Cvetko

Clinic for Surgery and Small Animal Medicine, Veterinary Faculty, University of Ljubljana, Cesta v Mestni log 47, 1000 Ljubljana, Slovenia

*Corresponding author, E-mail: aleksandra.domanjko@vf.uni-lj.si

Summary: Eighty cases of dogs with aortic stenosis (AS) examined at the University Small Animal Clinic in Ljubljana, Slovenia between 2002 and 2007 were analysed. Our focus was on breed predisposition, natural course of the disease and survival of dogs with different grades of AS. German Boxer was the most common breed (56 dogs), followed by 8 Golden Retrievers, 3 Newfoundlands and 2 German Shepherds. Eleven breeds had only one representative. The genders were equally represented. Syncope occurred in 22% of dogs with mild AS, 20% of those with moderate AS and 37% of those with severe AS; in the last group, syncope occurred more frequently. Heart failure was a rare consequence of AS alone (4%) – most dogs with heart failure had a concurrent cardiac disorder. Fifty-one dogs had mild, 10 had moderate and 19 had severe AS. Most of the dogs had a subvalvular type of AS (94%). A positive correlation was found between murmur intensity and AS pressure gradient. A negative correlation was found between murmur intensity, left ventricular systolic diameter and survival days, and between AS pressure gradient and aortic root diameter. Pulmonic stenosis was the most common concurrent diagnosis (35% of dogs). Average survival for all dogs (n=55) was 2053±1198 days (range 312–4619 days) and no significant differences in survival were found between groups. 29% of dogs died by the end of the study, in the severe group mostly as a consequence of AS, and in the other groups because of concurrent cardiac or noncardiac diseases. 38% of dogs with severe AS lived as long as dogs from the other two groups. A good screening program is essential for lowering the incidence of aortic stenosis.

Key word: dog diseases – congenital; heart diseases – congenital; aortic stenosis, subvalvular – ultrasonography; echocardiography; dogs

Introduction

Aortic stenosis (AS) has become the most common congenital heart disorder in dogs, and, over the past 10 years, German Boxers have proved to be the most sensitive breed (1–5). The subvalvular form – subaortic stenosis (SAS) – has been reported as that most frequently seen (in 95%) (6, 7). Aortic stenosis has been graded as 'mild', with pressure gradients (PG) either from 16 to 40 mmHg (corresponding to aortic velocities, v, of 2.3–3.2 m/sec) or from 20 to 49 mmHg (corresponding to velocities of 2.25–3.5 m/sec, 'moderate', with PG either from 40 to 80 mmHg (v=3.2–4.4 m/sec) or 50 to 80 mmHg (v=3.5–4.5 m/sec) and 'severe' with PG above 80 mmHg, corresponding to velocities over 4.5 m/sec (8, 9).

Clinical signs such as weakness, syncope and sudden death are more commonly seen in dogs with severe or moderate AS than in those with mild SAS (6, 7, 8). Dogs with mild AS rarely show any signs at all (6). Those with severe AS have a shorter life expectancy than dogs with mild AS, and often die suddenly in the first three years of life (7, 8). Careful physical examination reveals crescendo-decrescendo systolic murmur from grades 1 to 6. Final diagnosis has to be confirmed by two-dimensional and Doppler echocardiography, by which evaluation of morphologic characteristics the type of stenosis and the pressure gradient across the stenosis can be assessed (7, 8, 9). Standard or 24-hour electrocardiography can be used to detect arrhythmias that

Received: 27 February 2009
Accepted for publication: 20 September 2009

can occur in moderate or severe stenosis (6, 11). On thoracic radiographs a post-stenotic aortic dilation can be visible when present or when signs of heart failure are appreciable, especially when AS is complicated by another congenital or acquired heart disease. In this study aortic stenosis was analysed retrospectively to determine the prevalence in individual breeds, the natural course of the disease, the prevalent type of the disease, the frequency of the different grades of AS and correlations between murmurs and grades of AS. Survival was calculated for the groups of AS.

Material and methods

The study was carried out at the Clinic for Surgery and Small Animal Medicine, Veterinary Faculty of Ljubljana, Slovenia. Retrospective research included cases with AS between years 2002 and 2007. Information about health and survival was obtained from owners by phone interview with a standard questionnaire: "Is the dog active while taking walks? Does it run or only walk? Is it getting tired after physical exercise? Has it ever had syncope/fainting or weakness? If yes, when and at what occasion did syncope occur? Is the dog on any medication and/or have any concurrent disease?" Remaining information was taken from medical records and the cardiac service data base. All dogs had 9 lead ECG recorded in right recumbence, and thoracic radiographs were taken as needed. Echocardiography, including Doppler study, was performed with GE Vigmed System Five on unsedated dogs in standard right and left views. (12) Aortic velocity was measured from the left apical view with a continuous Doppler imaging probe of 1.7-3.5 MHz. (13) In many dogs a subcostal view with the same probe was used. Aortic stenosis was graded according to the latest recommendations on the basis of aortic velocity measured in the left apical view (9). Dogs were classified in the following groups: mild AS with gradients 20–49 mmHg (v =2.25–3.5 m/sec), moderate AS with gradients 50–79 mmHg (v=3.5–4.5 m/sec) and severe AS with gradients over 80 mmHg (v>4.5 m/sec).

Statistical analysis made use of SPSS 15.0 and Kaplan-Meier survival curves were obtained with MATLAB 7.5.0 software. Pearson correlation coefficients were calculated between echocardiographic parameters, murmur grade and survival. Differences in survival between the three groups of AS were calculated with independent sample T test; P values < 0.05 were considered significant.

Results

Eighty-seven dogs were diagnosed with AS between 2002 and 2007. Seven dogs from this group were not included because of possible flow murmurs or borderline aortic velocities with turbulent blood flow in the left ventricular outflow tract (LVOT) (v≤2.22 m/sec (n=4)) or dynamic AS (n=3) caused by anterior mitral valve motion (2) and thickened interventricular septum due to pulmonic stenosis (1). The 80 dogs comprised 56 German Boxers, 8 Golden Retrievers, 3 Newfoundlands, 2 German Shepherds, and 11 other breeds with only one representative each (Table 1). Affected dogs were equally distributed according to gender; 41 were males and 39 females. The median age at diagnosis was 1.6 years (range 0.17-10.25 years). Thirteen dogs were 6 months old or less at the time of examination, (the youngest was 2.4 months). Thirty-seven dogs (46%) were diagnosed at less than 1.5 years, 11 (14%) between 1.5 and 3.5 years, 17 (21%) between 3.5 and 6.5 years and the remainder (n=15; 19%) at more than 6.5 years. Of those 24 dogs that were examined at 1 year or less, 13 dogs had mild AS, four had moderate stenosis and 7 had severe disease. Only 5 of those dogs examined at one year or less, and ten dogs older at diagnosis were re-examined later. All the dogs had systolic crescendo-decrescendo murmur. The frequencies of murmur grades were: 1 dog (1%) with 1/6 murmur, 20 (25%) with 2/6 murmur, 28 (35%) with 3/6 murmur, 20 (25%) with 4/6 and 2 (3%) with grade 5/6 murmur. No murmur grade was specified in 9 dogs (11%) (Figure 1).

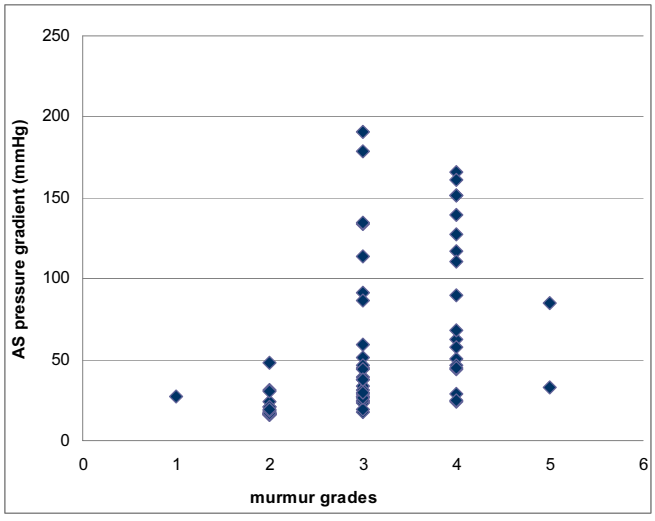


Figure 1: Murmur grades in relation to the pressure gradients

Table 1: Breeds presented with AS and concurrent cardiac disorders

breed	no. patients with AS	concurrent cardiac disorders
German Boxer	56	PS, AI, MD, TD, MR, TR
Golden retriever	8	PS, AI,
New Foundland	3	PS, AI, MR
German Shepherd	2	AI
Alaskan Malamute	1	neoplasia in right atrium
American Staffordshire Terrier	1	/
Bernese Mountain dog	1	AI, MR
French Bulldog	1	VSD, AI
Irish Setter	1	AI
Parson Russell Terrier	1	/
Labrador Retriever	1	PS, AI
Pug	1	AI
Rottweiler	1	PS
Samoyed	1	/
West Highland Terrier	1	PS, VSD

Legend: PS – pulmonic stenosis, AI – aortic insufficiency, MR – mitral regurgitation, TR – tricuspid regurgitation, MD – mitral dysplasia, TD – tricuspid dysplasia, VSD – ventricular septal defect

Seventy-five dogs (94%) had subvalvular AS and 5 (6%) valvular AS. Fifty-one dogs had mild AS, 10 had moderate AS and 19 had severe AS.

Syncope, or episodic weakness, occurred in 11/51 dogs (22%) with mild AS, 2/10 (20%) with moderate AS and 7/19 (37%) in the severe group. Syncope occurred more often in the severe group. In the group of dogs with syncope and mild AS, 2 dogs had additionally mild PS and one had tricuspid dysplasia with CHF (ISACHC II). It has to be pointed out that only in the 5 dogs with mild stenosis did syncope/episodic weakness occur before the diagnosis while, in the remainder, (6) syncope occurred some time later (information obtained form the phone interview). Syncope was defined as a transitional loss of consciousness or episodic weakness. One dog with moderate AS and syncope had additionally severe PS. Dogs with severe AS and syncope had no concurrent diseases; one of them was in CHF (ISACHC II). Congestive heart failure is a rare complication in dogs with AS. In our research there were eight such dogs that had concurrent cardiac problems such as mitral (n=4) and tricuspid regurgitation (n=1), tricuspid (n=1) and mitral dysplasia (n=1), ventricular septal defect (n=1), myxomatous mitral degeneration (n=1) and PS (n=2). Two dogs developed myocardial failure.

Table 2: Correlation coefficients (r) between murmur grade and various variables and aortic stenosis pressure gradient (Asp) and various variables

variables		r	P
murmur grade			
	ASp	0,388	<0,001
	IVSs	0,254	0,034
	LVDs	-0,354	0,003
	ASv	0,414	<0,001
	DT MVE	0,365	0,015
	survival	-0,284	<0,001
	PSp	0,566	0,001
	PSv	0,618	<0,001
ASp			
	AOd	-0,276	0,05
	PVs	0,609	0,004
	IVSs	0,412	<0,001
	IVSd	0,384	0,001
	survival	-0,041	0,703
	LVPWd	0,538	<0,001
	LVPWs	0,368	0,001

Legend: Asp – aortic stenosis pressure gradient, IVS – interventricular septum in systole, LVDs – left ventricular dimension in systole, ASv – aortic stenosis velocity, DT MVE – deceleration time of the mitral E wave, PSp – pulmonic stenosis pressure gradient, PSv – pulmonic stenosis velocity, AOd – aortic diameter, PVs – pumonic vein systolic velocity, IVSd – interventricular septum in diastole, LVPWd – left ventricular posterior wall in diastole, LVPWs – left ventricular posterior wall in systole

Correlation coefficients between echocardiographic parameters, grade of murmur and survival are listed in Table 2. Murmur intensity and AS pressure gradient were significantly correlated (r=0.388; P<0.001), as were murmur intensity and PS pressure gradient (r=0.566; P=0.001). Murmur intensity was negatively correlated with left ventricular diameter in systole (r=-0.354; P=0.003) and with survival days (r=-0.284; P<0.001). Negative correlations were observed between aortic diameter and AS pressure gradient (PG) (r=-0.276, P=0.050), and between AS PG and survival (r=-0.041, P=0.703). Positive correlations existed between AS pressure gradient and interventricular septum diameter in systole (r=0.412; P<0.001) and in diastole (r=0.384; P=0.001), AS pressure gradient and left ventricular posterior wall diameter in systole (r=0.368; P=0.001) and diastole (r=0.538; P<0.001) were correlated. Pulmonic stenosis was the most frequently concurrently diagnosed cardiac disease (n=28; 35%) with AS.

Eight dogs (10%) received pharmacological therapy, two of them with moderate AS and 6 with severe AS. The dosage of atenolol ranged from 0.46 to 1.62 mg/kg/12h. Since these dogs did not come to a recheck we cannot report on the pharmacological effect on the PG.

Arrhythmias were observed in 17 dogs (21%) with AS. Ventricular premature contractions, left bundle branch block and supraventricular tachycardia were seen in 5 dogs, atrial fibrillation in 4 dogs, atrial premature contractions in 2 dogs, and sinus bradycardia and ventricular preexcitation in one dog. Five dogs had more than single arrhythmia. Six dogs with arrhythmias were in congestive heart failure (ISACHC II and III).

Survival was calculated for the 55 dogs whose data were available. Mean survival of the whole group was 2053±1198 days (range 312-4619 days, median 2030 days or 72 months). Survival for the mild, moderate and severe AS groups did not differ significantly (Table 3, Figure 2).

23 dogs (28.8%) with AS died during our research. 12 died suddenly and 11 were euthanized. Of those that died suddenly 3 dogs had mild AS, 2 had moderate AS and 7 had severe AS. Dogs with mild AS had other cardiac diseases as follows: one dog had mild PS, one had severe tricuspid dysplasia with mild PS, one had severe PS and one had severe aortic insufficiency. One dog with moderate AS also had a mast cell tumour. Sudden death was defined as being when the owner witnessed or reported unexplained sudden death, but it has to be noted that

Table 3: Survival days (mean and standard deviation) in different groups with aortic stenosis P value is calculated between individual groups

Group	No. dogs	Survival days	P value
1	35	2078±1158	0.287
3	13	1933±1299	
2	7	2148±1372	0.739
3	13	1933±1299	
1	35	2078±1158	0.271
2	7	2148±1372	

Legend: 1 – mild AS; 2 – moderate AS; 3 – severe AS

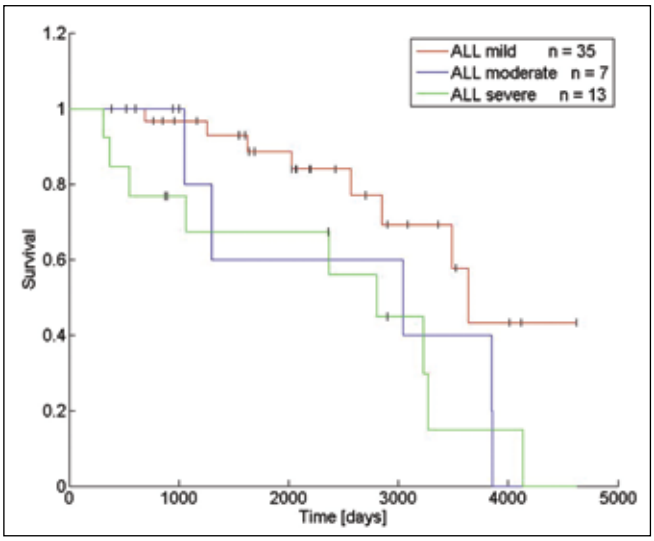


Figure 2: Comparison between survival of dogs with mild, moderate and severe aortic stenosis

it was not confirmed to be cardiac. Four dogs that were euthanized had concurrent non-cardiac disease (chronic renal failure, chronic hyperplastic gastritis, tetanus and neoplasia). Fifty-five dogs were included in graphical analysis of survival days. German Boxers, the most frequent breed (n=37) with AS, died on average earlier in the first 2.7 years than other breeds. Comparison between mild, moderate and severe AS showed surprisingly long survival of some dogs with moderate and severe disease, even 8 years or more. (Figure 2) Thirty-eight percent of dogs (n= 5) with severe AS lived as long as dogs from the other two groups.

Discussion

Aortic stenosis is the most common congenital heart defect in dogs in the last decade, both generally and in dogs in Slovenia (1,2,3,4,5). The popula-

tion of dogs included in our research showed similar numbers of cases as in other countries in the same period (1-3,5). The most affected breed in this study was German Boxer (n= 56), as in Italy and France (1,5). The reason for the high prevalence of AS in that breed in Slovenia could be its popularity in recent years and the facts that they are bred in a relatively small area and without obligatory screening for breeding. Golden Retrievers were the second most common breed (n=8) and are known to be highly predisposed (2, 6, 7). No gender predisposition was found in our study, as cited by some authors (2) but not others (1,8).

The most often heard murmurs were systolic crescendo-decrescendo grades 2/6 to 4/6. Murmur grade correlated weakly positively with AS and PS pressure gradient ($P<0.001$) in our study, while stronger positive correlations have been found by others (14).

Most dogs with mild AS are asymptomatic (8). Symptoms usually occur in dogs with moderate and severe AS (10). In our research 21.6% (11 dogs) with mild AS were symptomatic with one syncope or episodic weakness observed. 4 of those had syncope more than once. It has to be stressed that only in 5 dogs with mild AS did syncope occur before diagnosis and in 6 dogs syncope occurred later, according to the interview. That would indicate that these dogs might develop more severe disease while maturing. This is supported by findings from a British study that confirmed that AS is a developmental disease and can worsen by the time dogs mature. (15) Only one of the dogs in the mild AS group with syncope had concurrent tricuspid dysplasia and congestive heart failure. Others had concurrent mild PS or no cardiac/non-cardiac disease. Syncope is rare in dogs with mild stenosis, but it can occur also from non-cardiac causes, especially in brachicephalic breeds, so syncope in some mild AS dogs might be unrelated to AS. In the group with moderate AS, 2/7 dogs were symptomatic, with syncope or episodic weakness. In the severe group 7/19 (36%) were symptomatic: six dogs had syncope monthly with marked tiredness and extreme fatigue; 1 dog had syncope only once.

Congestive heart failure is a rare complication of AS (6, 7, 8, 10, 11). It is due mainly to coexisting mitral insufficiency or to concurrent cardiac defect. In our study 8 dogs (10%) had congestive heart failure (CHF). All our dogs with CHF had a concurrent problem such as mitral and tricuspid regurgitation, mitral and tricuspid dysplasia, ventricular septal defect, myxomatous mitral degeneration, pulmonic stenosis and/or myocardial failure. Mitral regurgitation, a consequence of ventricular remodelling and myocardial failure, can occur in late stages of moderate and severe SAS (6, 16) and was seen in 6 of our patients. Only 3 patients (3.7%) in CHF had no concurrent congenital heart defect, indicating that the true prevalence of heart failure in AS is low. In all other dogs, heart failure was more probably due to concurrent cardiac disorders.

The distribution of dogs according to the grade of AS was similar in our study to that reported (8), with 63% of dogs with mild AS, 12% with moderate AS and 23% with severe AS. Subvalvular AS (94%) was the most common form in our study, similar to that reported (1, 6, 7, 8, 10, 11). The valvular form is less common – in our dogs it occurred in 6 % of cases. It is characterised by a thickened valve with an abnormal motion of valve leaflets. Thickened aortic valve can also be seen in subaortic stenosis because of the damage caused by a high velocity jet across the orifice. (6, 15)

A positive correlation between murmur intensity and aortic velocity was found also by Linde et al et al. (14) A positive correlation between murmur intensity and deceleration time of the mitral valve can be explained by the slow closure of the early mitral valve opening that occurs in AS. Longer DT of the mitral E wave in Boxers with AS was also observed by Schober and Luis Fuentes (17). The negative correlation between murmur intensity and left ventricular diameter in systole can be explained by smaller LV diameter in AS, due to thickening of the LV walls. The negative correlation of pressure gradient with aortic diameter is understandable because of the smaller orifice in more severe cases. (18) Pressure gradient correlated positively with interventricular septum thickness in systole and diastole, and with left ventricular posterior wall diameter in systole and diastole, which is a logical result of left ventricular hypertrophy in dogs with AS. (6, 18) All correlations in our study were low and clinically significant inferences cannot be made.

Pulmonic stenosis was present in 35% of dogs with AS within this group. This proportion was 79% in German Boxers, the most common breed. A similar incidence was found in Italy, where 38% had concurrent PS (22). Much lower PS incidence in dogs with AS was found by Kienle et al (8), probably because of lower incidence of Boxers in that group.

Arrhythmias were diagnosed only in 17 dogs (21%), almost half of them in CHF. Ventricular pre-

mature complexes were present only in 5 dogs (6%). Detection of low proportions of arrhythmias were also found by Linde and Koch (14) and were observed only in the severe AS group. An event recorder would be more suitable to detect irregularities in heart rhythm.

The median age at diagnosis was 1.6 years, although more than half of the dogs were diagnosed before 2 years of age, usually at vaccination. Kienle et al. similarly found that 69 % of dogs were diagnosed before 1 year. (8) A wide age range at the time of diagnosis has been reported by other authors, possibly due to lack of symptoms (7, 8,10). The survival curve of dogs with severe AS is steeper in the first 2 years, when 50% of the dogs died. A similar finding was reported by Kienle et al. (8). Surprisingly, some of the dogs with moderate or severe AS lived 8 years or more. Individual older dogs in the severe AS group have also been reported by other authors (7, 8) Median survival for all our groups of dogs together was longer than in the study of Keinle et al. (72 vs. 40.5 months) (8). The reason could be that they were referred more severely affected dogs, that succumb sooner. Also some of our dogs in the severe AS group received beta blockers whereas their group contained only untreated dogs.

Conclusion

Aortic stenosis is common in German Boxers in Slovenia. Other breeds that are also affected are Golden Retriever (10%), Newfoundland (4%) and German shepherd (3%). Forty-six percent of patients were diagnosed at the age of 1.5 years or less. Pulmonic stenosis was the most common concurrent cardiac disease (35%). Fifty percent of dogs with severe AS died in the first three years after diagnosis, although some dogs with severe AS lived as long as dogs with mild AS.

A good breeding program is essential to lower the incidence of AS.

Limitations of the study

The first limitation of this study is its retrospective nature; most of the data were pooled from the records, in spite of the fact that all the owners were contacted when gathering the data for the study. In some records, the circumstances in which dogs had syncope or died suddenly were not described in detail. None of these dogs had a Holter monitor, so we cannot exclude some other cause for syncope such as right arrhythmogenic cardiomyopathy or dilated cardiomyopathy in boxers.

Acknowledgements

The authors thank Žiga Valentič for his help with calculating survival curves.

References

1. Bussadori C, Domenech O, Pradelli D. Canine subaortic stenosis -pathoanatomical observations in Italian Boxers. In: Proceedings of the 7th FECAVA Congress. Berlin, 2001: 16-8.

2. Tidholm A. Retrospective study of congenital heart defects in 151 dogs. J Small Anim Pract 1997; 38: 94-8.

3. Baumgartner C, Glaus TM. Congenital cardiac diseases in dogs: a retrospective analysis. Schweiz Arch Tierheilkd. 2003;145: 527-36.

4. Domanjko Petric A, Hozjan E. Epidemiological study of cardiovascular diseases in Slovenia. In: Proceedings of the 14th European College of Veterinary Internal Medicine. Barcelona, 2007: 212.

5. Le Bobinnec G. Canine Subaortic stenosis: epidemiology in France, ECG changes, antiarhytmic drug therapy. In: Proceedings of the 7th FECAVA Congress. Berlin, 2001: 12-5.

6. Kienle RD. Aortic stenosis. In: Kittleson MD, Kienle RD, eds. Small animal cardiovascular medicine. St Louis: Mosby, 1998: 260–72.

7. O'Grady MR, Holmberg DL, Miller CW, et al. Canine congenital aortic stenosis: a review of the literature and commentary. Can Vet J 1989; 30: 811.

8. Kienle RD, Thomas WP, Pion PD. The natural clinical history of canine congenital subaortic stenosis. J Vet Intern Med 1994; 8(6): 423–31.

9. Bussadori C. Amberger C, Le Bobinnec G, et al. Guidelines for the echocardiographic studies of suspected subaortic stenosis. J Vet Cardiol 2000; 2: 15–22.

10. Fuentes LV. Aortic stenosis in boxers. Vet Annu 1993; 33: 220–9.

11. Oyama MA, Sisson DD, Thomas WP, Bonagura JD. Congenital heart disease. In: Ettinger SJ, Feldman EC, eds. Textbook of small animal internal medicine. 6th ed. Philadelphia: WB Saunders, 2005: 972–1021.

12. Thomas WP, Kienle RD. Echocardiography. In: Nyland TG, Mattoon JS. Small animal diagnostic ultrasound. Philadelphia: W.B. Saunders company, 1995: 384–7.

13. Darke PGG, Bonagura JD, Miller M. Transducer orientation for Doppler echocardiography in dogs. J Small Anim Pract 1993; 34 :2 – 8

14. Linde A, Koch J. Screening for aortic stenosis in the Boxer: auscultatory, ECG, blood pressure and Doppler echocardiographic findings. J Vet Cardiol 2006; 8: 79–86.

15. French A, Fuentes VL, Dukes-McEwan J, et al. Progression of aortic stenosis in the Boxer. J Small Anim Pract 2000; 41: 451–6.

16. Opie LH, Commeford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. Lancet 2006; 367(9507): 356–67.

17. Schober KE, Fuentes VL. Doppler echocardiographic assessment of left ventricular diastolic function in 74 Boxer dogs with aortic stenosis. J Vet Cardiol 2002; 4: 7–16.

18. Oyama MA, Thomas WP. Two dimensional and M-mode echocardiographic predictors of disease severity in dogs with congenital subaortic stenosis. J Am Anim Hosp Assoc 2002; 38 (3): 209–15.

19. Bussadori C, Quintavalla C, Capelli A. Prevalence of congenital heart disease in Boxers in Italy. J Vet Cardiol 2001; 3: 7–11.

AORTNA STENOZA PRI PSIH

A. Domanjko Petrič, S. Cvetko

Povzetek: Med letoma 2002 in 2007 smo diagnosticirali 80 pacientov z aortno stenozo (AS) na Univerzitetni Kliniki za kirurgijo in male živali v Ljubljani. Zanimala nas je pasemska nagnjenost k AS, naravni potek bolezni in preživetje psov z različnimi stopnjami AS. Nemški bokserji so bili najpogosteje zastopana pasma, sledilo jim je 8 zlatih prinašalcev, 3 novofundlandci in 2 nemška ovčarja. 11 pasem pa je imelo le po enega predstavnika. Spola psov sta bila enakovredno zastopana. Sinkope so se pojavile pri 22 % psov z blago AS, 20 % psov s srednjo AS in 37 % psov s hudo AS. Pri psih v slednji skupini so se sinkope pojavljale pogosteje. Popuščanje srca je redek zaplet pri samo AS (4 %); večina psov s srčnim popuščanjem je imela poleg AS še eno srčno okvaro. Enainpetdeset psov je imelo blago, 10 srednjo in 19 hudo AS. Večina psov je imela subvalvularni tip AS (94 %). Stopnja šuma je značilno pozitivno korelirala s tlačnimi gradienti AS in pljučno stenozo (PS), medtem ko je značilno negativno korelirala s premerom levega prekata v sistoli in s preživetjem. Tlačni gradient AS pa je bil v negativni povezavi z premerom korena aorte. Pljučna stenoza je bila najpogostejša sočasna diagnoza z aortno stenozo (35 %). Psi z AS (n = 55) so živeli povprečno 2053 ± 1198 dni (razpon od 312 do 4619 dni). Med skupinami psov z AS ni bilo značilnih razlik glede preživetja. Do konca raziskave je poginilo 29 % psov, v skupini s hudo AS najpogosteje zaradi le-te, v drugih skupinah pa zaradi drugih srčnih ali nesrčnih bolezni. 38 % psov s hudo obliko AS je živelo tako dolgo kot psi iz ostalih dveh skupin.
Dober vzrejni program je nujen za zmanjšanje pojavnosti aortne stenoze.

Ključne besede: psi, bolezni – prirojene; srce, bolezni – prirojene; aortna stenoza, subvalvularna – ultrazvok; ehokardiografija; psi

Slov Vet Res 2009; 46 (4): 133-42
UDC 636.4.09:637.513.1:57.017:591.5:159.944.4

Original Research Paper

ASSOCIATION OF PLASMA STRESS MARKERS AT SLAUGHTER WITH CARCASS OR MEAT QUALITY IN PIGS

Martin Škrlep[1], Maja Prevolnik[1,2], Blaž Šegula[1], Marjeta Čandek-Potokar[1]*

[1]Agricultural Institute of Slovenia, Hacquetova 17, 1000 Ljubljana; [2]Faculty of Agriculture and Life Sciences, University of Maribor, Pivola 10, 2311 Hoče, Slovenia

*Corresponding author, E-mail: meta.candek-potokar@kis.si

Summary: The association between plasma level of three stress markers at slaughter (cortisol, neopterin, Hsp70) and carcass and meat quality traits was studied in pigs. The study comprised data from 51 pigs fattened on the same farm (one crossbreed, no *RYR1* mutation) and slaughtered in one abattoir in five batches. At slaughter blood was collected for the analysis of neopterin, cortisol and HSP70 level using commercial enzyme immunoassay kits. A day after the slaughter, carcass traits (fat and muscle thickness, lean meat percentage, *longissimus dorsi* (LD) muscle and fat area, belly leanness and ham weight) and meat quality traits (pH_1 at 1 h, pH_U at 24 h *post-mortem*, Minolta L*a*b* and drip loss) were assessed. No significant differences between slaughter batches were observed for stress markers and meat quality. However, individual animal variation of stress markers within slaughter batch was very high. Correlation analysis demonstrated no association between plasma neopterin and Hsp70 levels with any of carcass or meat quality properties. On the contrary, significant correlations of cortisol level were observed with some carcass or meat quality properties. Higher cortisol levels were associated with thicker subcutaneous fat (r=0.30 to 0.33) and lower carcass meat percentage (r=–0.37 to 0.41), which indicates that higher cortisol levels on the long term basis increase body fatness. Regarding meat quality, higher cortisol levels were associated with higher pH_1 (r=0.36 to 0.58) and pH_U (r=0.36 to 0.32), and consequently with lower LD Minolta L* (r=–0.44), b* (r=–0.38) and drip loss after 24 (r=–0.44) and 48 hours (r=–0.39) suggesting that cortisol level at slaughter reflects more than just acute preslaughter stress.

Key words: stress; pig; carcass quality, meat quality; cortisol; neopterin; Hsp70

Introduction

It has been recognized that stress in meat animals, and in particular in pigs, has a marked impact on meat quality. Prior to being slaughtered pigs endure food withdrawal, change of environment, loading, transport, mixing with unfamiliar animals, crowd, noise, spurring, very often cold or heat. It is difficult to measure animal's stress condition, therefore many markers (behavioural, physiological) have been used like body temperature, blood pressure, heart rate (1, 2), movement and jiggling (3), vocalisation (4, 5), different stress markers in body fluids and tissues such as cortisol (6, 7, 8, 9, 10, 11, 12, 13), neopterin (11), creatine phosphokinase (4, 9, 10, 13), heat shock proteins (14, 15, 16, 17, 18), lysine vasopressin (6, 8), lactate dehydrogenase (10), beta-endorphin (6, 7, 8), ACTH (9), adrenalin and noradrenalin (12, 19). Among many different biomarkers of stress we decided for plasma cortisol, neopterin and Hsp70. Cortisol is the main hormone of HPA (hypothalamic-pituitary-adrenocortical) axis released by the adrenal cortex in response to stress. It influences feeding behaviour, pancreatic hormone secretion, energy expenditure and protein/lipid balance (20). Although the role of neopterin (pyrazino-[2,3-D]-pyrimidin) is not fully understood, clinical studies in human suggest it is a good marker of cellular immunity activation since higher neopterin concen-

Received: 21 October 2009
Accepted for publication: 19 November 2009

trations were associated with infections, autoimmune diseases, tumours (11). At a cellular level, the stress response includes a synthesis of heat shock proteins, which are classified according to their molecular weight (21). One of the most abundant and best characterized is the 70-kDa family (Hsp70). Hsp70 (stress-limiting factor) during stress prevents inappropriate protein aggregation, mediates the transport of proteins for degradation, helps proteins to maintain their conformation and even assists in their repair (22, 23).

In pigs the quality of meat largely depends on the nature of post-mortem muscle pH decline, which is strongly influenced by stress prior to slaughter. An acute slaughter stress can induce PSE condition in pork due to a rapid pH decline (24), while fasting, transport (25, 26), or exercise (27) prior to slaughter can lead to glycogen depletion resulting in higher ultimate pH and better water holding capacity (28). Thus slaughter batch has been recognized as a major factor of variation in meat quality as demonstrated by various studies dealing with pork quality. The individual response of the animal to preslaughter stress will be determined by a complex interaction with genetics and previous experience (29). Relationship between stress status of individual pig and its meat quality has seldom been established. The objective of the present experiment was to analyze slaughter batch effect on the level of selected plasma stress markers and meat quality and to determine the association between stress status of individual pigs at slaughter with carcass or meat quality traits.

Material and methods

Animals

Current study was performed as a collateral study within another project (30). Our experiment comprised 51 pigs (20 females and 31 castrates) commercial four-breed crosses known to be free of *RYR1* mutation. Pigs were reared in the experimental station of Faculty of Agriculture and Life Sciences of Maribor University, and slaughtered at 85±14 kg live weight in a commercial abattoir (Košaki d.d., Maribor, Slovenia) in five batches. Slaughter batch stands for a group of pigs that were reared in the same pen and slaughtered in one batch (no mixing with unknown pigs). All batches were submitted to similar preslaughter treatment. Pigs were fasted 24 h prior to slaughter, they were loaded on the truck between 6 and 8 hour in the morning, and transported for 20 minutes to the abattoir where they were slaughtered between 8 and 11 a.m. according to the routine abattoir procedure. However, all other possible stress factors could not be controlled.

Carcass quality measurements

At the end of the slaughter line, pigs were classified according to SEUROP by official classification body, using a method known as ZP, which consists of taking two measurements at carcass split line; DM fat (minimal fat thickness over the *m. gluteus medius*) and DM muscle (shortest distance between cranial end of *m. gluteus medius* and dorsal edge of vertebral canal). Additionally, the measurements of muscle (HGP muscle) and fat thickness (HGP fat) were performed using a HGP4 Hennessy grading probe (Hennessy Grading Systems Ltd., Auckland, New Zealand) with a puncture between the 2nd and the 3rd last rib 7 cm laterally from the carcass split line. The carcass lean meat percentage (DM meat %, HGP meat %) was calculated according to the formulas approved for Slovenia (31). A day following slaughter, additional carcass traits were assessed. The hind leg (without shank) was cut off the carcass between 6th and 7th lumbar vertebra. It was weighed prior and after the removal of subcutaneous fat and the ratio between them was calculated. A digital image of carcass cross section (last rib) was taken with digital photo camera (Canon PowerShot G3, Canon Inc., Tokyo, Japan). Loin eye area (LD area), corresponding fat area (fat over LD), their ratio (LD meat:fat ratio) and belly leanness were determined on images with LUCIA.NET 1.16.5 software (Laboratory Imaging s.r.o, Prague, Czech Republic).

Meat quality measurements

Values of pH were taken with MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH; 8603 Schwarzenbach, Switzerland) fitted with a combined glass electrode (InLab427) one hour (pH_1) and 24 h post-mortem (pH_U). Duplicate measurements were taken in *longissimus dorsi* (LD) muscle at the level of last rib and in *semimembranosus* (SM) muscle app. 4 cm laterally to the *os pubis*. The measurements of colour (Minolta L*a*b*) were taken a day after the slaughter on a freshly cut surface of LD (level of last rib) using Minolta Chroma Meter CR-300 (Minolta

Co. Ltd, Osaka, Japan) with an 11 mm diameter aperture, D_{65} illuminant, calibrated against a white tile. Colour of LD was also assessed using 1-6 colour scale (32). A 2.5 cm thick slice of LD was taken from the loin at the level of the last rib for drip loss (EZ drip loss) determination according to Christensen (33). Drip loss was determined after 24 and 48 hours storage at 4°C and expressed as a percentage of the initial sample weight.

Blood sampling

At slaughter, blood samples (app. 4 ml) were taken into plastic tubes containing EDTA (against blood coagulation). After blood collection the tubes were immediately placed on ice. Within one hour blood samples were taken to the laboratory where blood was centrifuged at 1800 rpm for 15 minutes. Supernatants (plasma) were collected and stored at –20°C until further analyses.

Determination of plasma stress markers

The levels of plasma stress markers were determined using commercial kits based on the enzyme immunoassays for the in-vitro diagnostic quantitative determination of cortisol (34), neopterin (35) and Hsp70 (36) in plasma samples according to manufacturer instructions. The intensity of the colour was read at 450 nm using spectrophotometer Varioscan Flash and SkanIt Software Version 2.4.3. RE (Thermo Fisher Scientific Inc. Waltham, MA, USA). Plasma concentrations were expressed in μgdl^{-1}, $nmoll^{-1}$ and $ngml^{-1}$ for cortisol, neopterin and Hsp70, respectively.

Statistical analysis

Statistical analysis was performed using a statistical package SAS (37). Basic statistic parameters for all studied traits were calculated using the MEANS procedure. Relationships between plasma stress markers and carcass or meat quality traits were calculated using the CORR procedure on raw experimental data (phenotypic correlations) and after the adjustment for the effects in the model (residual correlations). To calculate residual correlation coefficients, a GLM procedure was used (model with the effect of sex, slaughter batch and their interaction and additionally carcass weight as covariate in case of carcass traits). Significant differences ($P<0.05$) in least square means (LS means) between slaughter batches were evaluated using the PDIFF option, adjust=*Tukey*.

Results

Carcass traits, plasma stress markers and meat quality traits

The effect of slaughter batch on carcass traits has no practical (biological) significance in the context of the present study (Table 1). In any case, well known effect of animal sex on carcass traits was confirmed, as well as a significant relationship between carcass weight and the majority of carcass traits. However, we do not present or discuss this data since they were not the objective of the present study. On the other hand, analysing slaughter batch effect for plasma level of stress markers as related to meat quality traits (Table 1) was the main point of the present study. Despite notable differences in the level of plasma stress markers at slaughter among slaughter batches (in particular HSP70 and neopterin) none of them was significant (Figure 1), which could be due to high within batch variability and low number of pigs per batch. A significant effect of slaughter batch was found for some of meat quality traits (LD ultimate pH, LD colour and Minolta L*), however with no consequences for main technological meat quality - drip loss (batch effect insignificant). Here again, high within batch variability and low number of pigs could be the reason for non significant differences between slaughter batches.

Relationship between plasma stress markers and carcass or meat quality traits

Phenotypic and residual (accounted for the effects in the model) correlation coefficients between the level of plasma stress markers and carcass quality traits (Table 2) or meat quality traits (Table 3) are presented. There were no significant phenotypic or residual correlations between different plasma stress markers ($r<|0.20|$; data not shown). No significant correlation between the level of neopterin or HSP70 with carcass traits was observed, indicating no relationship between them. On the other hand significantly higher cortisol levels were associated with more subcutaneous fat (HGP fat and DM fat) and consequently lower carcass meatiness (HGP meat %, DM meat %, LD meat:fat ratio, and ham meat %), meaning that fattier pigs exhibit higher cortisol

Table 1: Basic statistics for carcass, meat quality traits and plasma stress markers and results of analysis of variance

n=51	mean	sd	EFFECT				R^2	rmse
			sex	slaughter batch	slaughter batch × sex	[1]carcass weight		
CARCASS QUALITY TRAITS								
HGP fat, mm	13.2	2.2	***	NS	†	0.10***	0.65	1.5
HGP muscle, mm	45.5	8.3	NS	NS	NS	0.61***	0.73	4.8
HGP meat, %	60.4	1.8	***	NS	†	-0.04^{NS}	0.55	1.4
DM fat, mm	14.0	3.9	***	†	NS	0.20***	0.67	2.5
DM muscle, mm	62.1	6.0	*	NS	NS	0.43***	0.72	3.5
DM meat, %	58.1	2.7	***	*	NS	−0.09**	0.59	1.9
LD area, cm^2	37.6	7.8	**	***	NS	0.52***	0.87	3.1
Fat area over LD, cm^2	11.7	2.9	***	NS	NS	0.17***	0.86	1.2
LD meat:fat ratio	3.30	0.63	***	†	NS	-0.003^{NS}	0.69	0.39
Belly leanness, %	53.7	5.1	NS	*	NS	−0.21**	0.40	4.4
Ham (muscle+bone), kg	7.1	1.2	**	**	NS	0.10***	0.96	0.3
Ham, kg	8.5	1.6	NS	NS	NS	0.13***	0.93	0.5
Ham meat, %	83.7	4.9	*	NS	NS	-0.05^{NS}	0.20	4.9
LEVEL OF PLASMA STRESS MARKERS AT SLAUGHTER								
Cortisol, μgdl^{-1}	11.0	4.3	NS	NS	NS	/	0.17	4.4
Neopterin, $nmoll^{-1}$	3.1	1.9	NS	NS	NS	/	0.20	1.8
HSP70, $ngml^{-1}$	5.0	6.1	NS	NS	NS	/	0.22	5.9
MEAT QUALITY TRAITS								
SM pH_1	6.39	0.33	NS	NS	NS	/	0.21	0.33
SM pH_U	5.71	0.16	NS	†	NS	/	0.28	0.15
LD pH_1	6.19	0.46	NS	†	NS	/	0.22	0.44
LD pH_U	5.59	0.10	NS	***	NS	/	0.62	0.07
LD Minolta L*	53.8	2.9	NS	*	NS	/	0.31	2.6
LD Minolta a*	6.4	1.2	NS	NS	NS	/	0.22	1.1
LD Minolta b*	2.8	0.9	NS	NS	NS	/	0.23	0.8
LD colour (1-6)	3.6	0.5	NS	***	NS	/	0.49	0.4
LD drip loss 24h, %	5.0	2.3	NS	NS	NS	/	0.16	2.3
LD drip loss 48h, %	7.4	2.4	NS	NS	NS	/	0.22	2.3

LD – muscle *longissimus dorsi*; SM – muscle *semimembranosus*; pH_1 – pH measured one hour after slaughter; pH_U – pH measured 24 hours after slaughter; [1] coefficient of regression; *** P<0.001; ** P<0.01; * P<0.05; † P<0.10; ns - P>0.10.

levels. After the adjustment for the influence of the experimental factors, the obtained residual correlations were mainly similar. Additionally, significant correlation of cortisol level with fat over LD muscle (r=0.41), cortisol level with ham (muscle+bone) (r=−0.31) and neopterin level with LD muscle thickness (r=−0.33) was detected. Hsp70 showed no significant residual correlation with any of carcass quality traits. Cortisol level was also significantly correlated to all meat quality traits except Minolta a*. Higher cortisol levels were associated with higher pH_1 (r=0.36 and 0.58 in LD and SM muscle, respectively) and higher pH_U (r=0.36 and 0.32 in LD and SM muscle, respectively). Consequently higher cortisol levels were related to lower *LD* Minolta L* (r=−0.44), b* (r=−0.38) and lower drip loss (r=−0.44

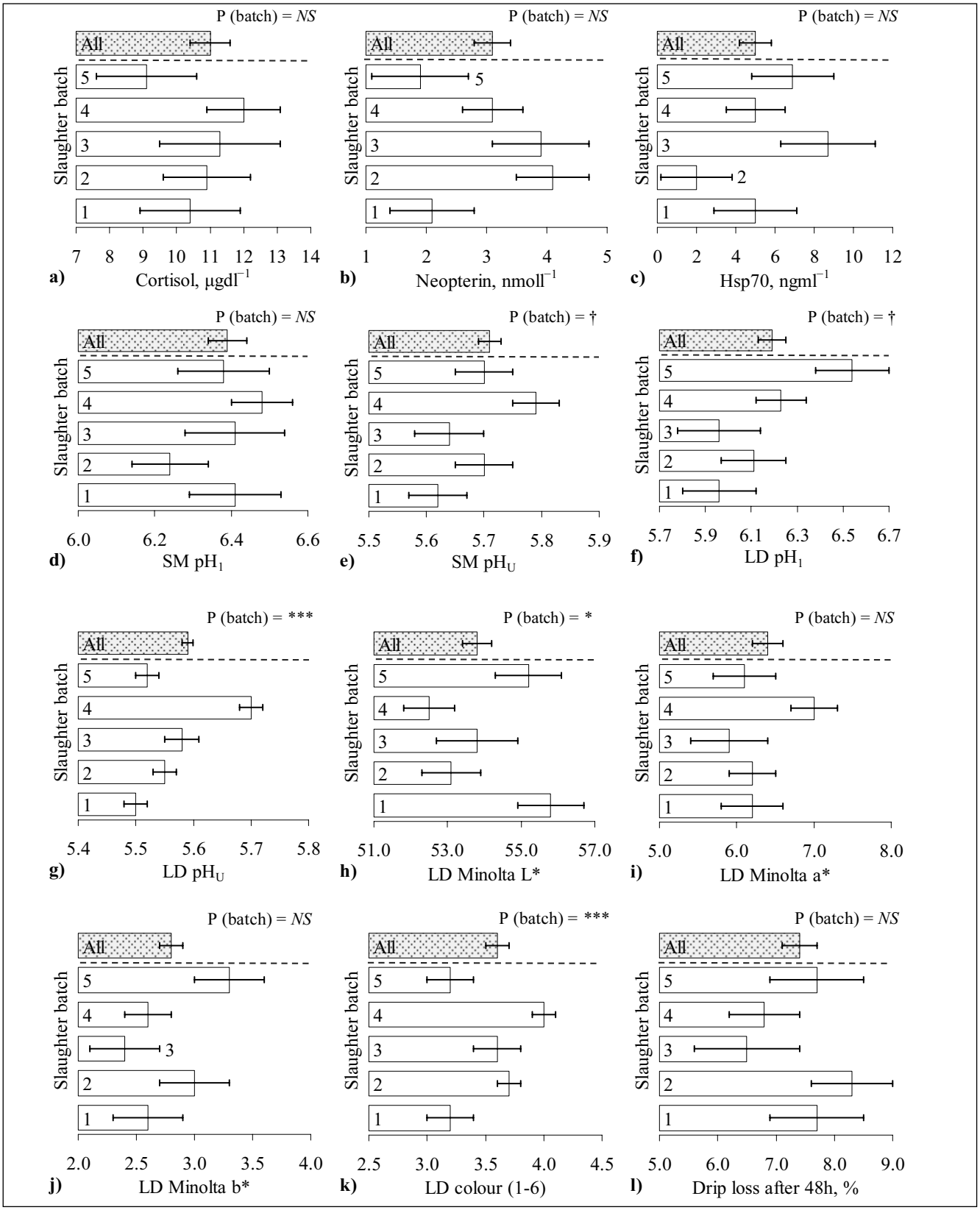


Figure 1: Effect of slaughter batch (LS means ± standard errors) on plasma stress markers and meat quality traits

LD – muscle *longissimus dorsi*; SM – muscle *semimembranosus*; pH$_1$ – pH measured one hour after slaughter; pH$_U$ – pH measured 24 hours after slaughter; *** - P<0.001; * - P<0.05; † - P<0.10; NS - P>0.10.

Table 2: Phenotypic and residual correlation coefficients between level of plasma cortisol and carcass quality traits[1]

N=51	Phenotypic correlations	Residual correlations
HGP fat, mm	**0.30**	**0.38**
HGP muscle, mm	–0.10	0.02
HGP meat, %	**–0.37**	**–0.36**
DM fat, mm	**0.33**	**0.53**
DM muscle, mm	–0.21	–0.15
DM meat, %	**–0.41**	**–0.54**
LD area, cm^2	–0.17	–0.15
Fat over LD, cm^2	0.14	**0.41**
LD meat:fat ratio	**–0.31**	**–0.32**
Belly leanness, %	–0.16	–0.16
Ham (muscle +bone), kg	–0.21	**–0.31**
Ham, kg	–0.09	0.07
Ham meat, %	**–0.32**	–0.26

LD – muscle *longissimus dorsi*;
DM – measurements of fat (minimal fat thickness over the *m. gluteus medius*) and muscle (the shortest distance between cranial end of *m. gluteus medius* and dorsal edge of vertebral canal) taken within 45 minutes *p.m.*;
HGP – measurements of fat and muscle taken within 45 minutes *p.m.* with HGP4 probe with puncture between 2nd and 3rd last rib;
[1]Correlation coefficients between carcass quality traits and neopterin or Hsp70 were statistically insignificant (p>0.05). Values in bold are statistically significant (p<0.05)

Table 3: Phenotypic and residual correlation coefficients among meat quality traits and level of plasma cortisol[1]

n=51	Phenotypic correlations	Residual correlations
SM pH_1	**0.58**	**0.53**
SM pH_U	**0.32**	**0.32**
LD pH_1	**0.36**	**0.49**
LD pH_U	**0.36**	**0.35**
LD Minolta L*	**–0.44**	**–0.39**
LD Minolta a*	–0.27	**–0.30**
LD Minolta b*	**–0.38**	**–0.30**
LD colour	**0.33**	0.17
LD drip loss 24	**–0.44**	**–0.39**
LD drip loss 48	**–0.39**	**–0.32**

LD – muscle *longissimus dorsi*; SM – muscle *semimembranosus*; pH_1 – pH measured one hour after slaughter; pH_U – pH measured 24 hours after slaughter;
[1]Correlation coefficients between meat quality traits and neopterin or Hsp70 were statistically insignificant (p>0.05). Values in bold are statistically significant (p<0.05)

and –0.39 for drip loss after 24 and 48 hours, respectively). After the adjustment for the influence of the experimental factors, the obtained residual correlations between plasma stress markers and meat quality traits remained similar, only correlation of cortisol level with Minolta a* became significant. No significant phenotypic or residual correlations were found for neopterin and Hsp70 with any of meat quality traits.

Discussion

Reported values for concentration of plasma stress markers in blood samples vary widely (Table 1, Figure 1). Despite notable differences in the average level of plasma stress markers (Figure 1) a lack of significance in the present study to our opinion is mainly due to high within batch variation and small number of pigs per batch. Stress endured by farm animals can be physical (hunger, thirst, fatigue, illness, injuries, exercise, thermal extremes), or psychological (fear related to restraint, handling, novelty), short term or long term, the later having much more complex impact (29). In fact, individual response of the animal to preslaughter stress is a result of a complex interaction with genetics and previous experience (29) and can explain why under similar stress conditions animals react differently. Regarding cortisol level, there are numerous experiments in pigs which demonstrate increased cortisol level due to preslaughter stress (38), slaughter day (19), exercise (39). An advantage of measuring cortisol is that the act of stunning and ticking has negligible effect on the level of plasma cortisol concentration (40), contrary to catecholamines. However, the interpretation of plasma cortisol levels remains difficult and has been a subject of debate (41). When considering plasma cortisol levels different factors (time elapsed between stress and sample taking, diurnal fluctuation, genetics, effect of chronic stress) must be taken into account. The concentration of cortisol increases rapidly (<30 minutes) after the

exposure to stress but then it diminishes or recovers in few hours (6). Another aspect to consider is the chronic stress. It is well known, that animal's social status within group can affect immune status (42), access to feed (43), and increase physiological stress (44). Low social status also increases reaction to acute stress (45). Circadian variation in cortisol level and genetic factors can be considered less influential in our study. Namely, blood samples were always collected between 9-10 a.m., and animals were free of stress susceptibility gene (RYR1), which moreover according to the literature, does not seem to increase cortisol level (11, 41). Our results demonstrate positive relationship between plasma cortisol levels with carcass fatness, in agreement with available studies (12, 19, 41) and general metabolic effect of cortisol favouring the accretion of fat at the expense of proteins (46, 47). In humans, many clinical studies suggest that chronic stress causes metabolic disorders like hyperglycemia, hypertension, hyperlipidemia, obesity (48, 49) with prolonged excessive cortisol elevation leading to symptoms resembling Cushing's syndrome. It could be speculated that the correlations observed in our study might have been higher if fat depot had been measured in different location (e.g. abdominal or neck area). Studies dealing with neopterin and HSP70 as related to preslaughter stress in pigs are rare (11, 14, 16). A significant increase of neopterin or cortisol plasma level was shown to be affected by 30 minutes transport prior to slaughter (11). In agreement with our results, no effect of preslaughter stress was also reported (14, 16). It is possible that experienced stress in our as well as in their studies was not intense enough, since elevation of HSP70 with exercise in rats has been shown to be intensity dependent (50). Correlation analysis demonstrated no association between plasma neopterin and Hsp70 levels with any of carcass or meat quality traits. To our knowledge few studies were made on relationship between meat quality in pigs and the level of heat shock proteins (14, 16, 51) and as in the present study, none of them showed any significant relationship.

Published studies show comparable results regarding the association between plasma cortisol and meat quality. From our results it would seem, that cortisol level is indicative of long term rather than acute stress associated with slaughter. Namely, we found positive correlation between plasma cortisol level and pH_1, an indicator of the intensity of post-mortem glycolysis leading to PSE meat, known to be enhanced by stress (24). Moreover, positive correlation of cortisol level with pH_U indicates that muscle glycogen stores were more depleted prior to slaughter in pigs with higher pH_U. Consequently higher plasma cortisol levels were associated with darker meat (higher values of Minolta L* and LD colour) and better water-holding capacity (lower drip). These findings are in accordance with other studies (41) which demonstrated elevated cortisol levels with occurrence of DFD meat, resulting from glycogen depletion due to long term stress (28). However, there are also studies that report no significant relationship between cortisol levels and meat quality (8, 19).

Conclusions

Among the studied stress markers, only cortisol levels were associated with carcass and meat quality traits. Higher cortisol levels were associated with higher carcass fatness, supporting the theory that higher cortisol levels on the long term basis increase body fatness. Higher cortisol level was associated with higher muscle pH (pH_1 and pH_U) and lower drip loss suggesting that cortisol level at slaughter reflects more than just acute preslaughter stress.

Acknowledgements

The authors acknowledge the financial support from the state budget by the Slovenian Research Agency (project J4-9532, program P4-0072) and Ministry of Agriculture Forestry and Food.

References

1. Marchant-Forde RM, Marlin DJ, Marchant-Forde JN. Validation of a cardiac monitor for measuring heart rate variability in adult female pigs: accuracy, artefacts and editing. Physiol Behav 2004; 80: 449–58.

2. Brown SN, Knowles TG, Wilkins LJ, Chadd SA, Warriss PD. The response of pigs to being loaded or unloaded onto commercial animal transporters using three systems. Vet J 2005; 170: 91–100.

3. Stooky JM, Nickel T, Hanson J, Vandenbosch S. A movement-measuring-device for objectively measuring temperament in beef cattle and for use in determining factors that influence handling. J Anim Sci 1994; 72: 207.

4. Warriss PD, Brown SN, Adams SJM, Corlett IK. Relationships between subjective and objective as-

sessments of stress at slaughter and meat quality in pigs. Meat Sci 1994; 38: 329–40.

5. Marchant JN, Whittaker X, Broom DM. Vocalisations of the adult female domestic pig during a standard human approach test and their relationships with behavioural and heart rate measures. Appl Anim Behav Sci 2001; 72: 23–39.

6. Bradshaw RH, Parrott RF, Forsling ML, et al. Behavioral and hormonal responses of pigs during transport: effect of mixing and duration of journey. Anim Sci 1996; 62: 547–54.

7. Bradshaw RH, Parrott RF, Forsling ML, et al. Stress and travel sickness in pigs: effects of road transport on plasma concentrations of cortisol, beta-endorphin and lysine vasopressin. Anim Sci 1996; 63: 507–16.

8. Bradshaw RH, Randall JM, Forsling ML, et al. Travel sickness and meat quality in pigs. Anim Welfare 1999; 8: 3–14.

9, Lebret B, Meunier-Salaün MC, Foury A, Morme`de P, Dransfield E, Dourmad JY. Influence of rearing conditions on performance, behavioral, and physiological responses of pigs to preslaughter handling, carcass traits, and meat quality. J Anim Sci 2006; 84: 2436–47.

10. Averos X, Herranz A, Sanchez R, Comella JX, Gosalvez LF. Serum stress parameters in pigs transported to slaughter under commercial conditions in different seasons. Vet Med-Czech 2007; 52: 333–42.

11. Breineková K, Svoboda M, Smutna M, Vorlova L. Markers of acute stress in pigs. Physiol Res 2007; 56: 323–9.

12. Foury A, Geverink NA, Gil M, et al. Stress neuroendocrine profiles in five pig breeding lines and the relationship with carcass composition. Animal 2007; 1: 973–82.

13. Gade PB, Barton P. Effect of rearing system and mixing at loading on transport and lairage behaviour and meat quality: comparison of outdoor and conventionally raised pigs. Animal 2008; 2: 902–11.

14. Van Laack RL, Faustman C, Sebranek JG. Pork quality and the expression of stress protein Hsp 70 in swine. J Anim Sci 1993; 71: 2958–64.

15. Khazzaka A, Figwer P, Poirel MT, Serrar M, Franck M. Hsp70 response in pigs is affected by their Halothane genotypes after heat stress. J Therm Biol 2006; 31: 605–10.

16. Young JF, Leoni F, Straadt IK, Williams JHH, Oksbjerg N. Heat shock proteins as markers for preslaughter stress and prediction of meat quality. In: 53rd International Congress of Meat Science and Technology, Beijing, China, 2007: 609-10.

17. Bao E, Sultan KR, Nowak B, Hartung J. Localization of heat shock proteins and histopathological changes in the kidneys of transported pigs. Livest Sci 2008; 118: 231-7.

18. Yu J, Tang S, Bao E, Zhang M, Hao Q, Yue Z. The effect of transportation on the expression of heat shock proteins and meat quality of m. *longissimus dorsi* in pigs. Meat Sci 2009; 83(3): 474–8.

19. Foury A, Devillers N, Sanchez MP, Griffon H, Le Roy P, Morme`de P. Stress hormones, carcass composition and meat quality in Large White × Duroc pigs. Meat Sci 2005; 69: 703–7.

20. Dallman MF, Strack AM, Akana SF, et al. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Front Neuroendocrin 1993; 14: 303–47.

21. Liu Y, Steinacker JM. Changes in skeletal muscle heat shock proteins: pathological significance. Front Biosci 2001; 6: 12–25.

22. Hendrick JP, Hartl FU. Molecular chaperone functions of heat-shock proteins. Annu Rev Biochem 1993; 62: 349–84.

23. Kiang JG, Tsokos GC. Heat shock srotein 70 kDa: molecular biology, biochemistry, and physiology. Pharmacol Ther 1998; 80: 183–201.

24. Briskey EJ. The etiological status and associated studies of pale, soft and exudative porcine musculature. Adv Food Res 1964; 13: 89–178.

25. Eikelenboom G, Bolink AH, Sybesma W. Effects of feed withdrawal before delivery on pork quality and carcass yield. Meat Sci 1991; 29: 25–30.

26. Leheska JM, Wulf DM, Maddock RJ. Effects of fasting and transportation on pork quality development and extent of postmortem metabolism. J Anim Sci 2002; 80: 3194–202.

27. Briskey EJ, Bray RW, Hoekstra WG, Phillips PH, Grummer RH. The effect of exhaustive exercise and high sucrose regimen on certain chemical and physical pork ham muscle characteristics. J Anim Sci 1959; 18: 173–7.

28. Fernandez X, Tornberg E. A review of the causes of variation in muscle glycogen content and ultimate pH in pigs. J Muscle Foods 1991; 2: 209–35.

29. Grandin T. Assessment of stress during handling and transport. J Anim Sci 1997; 75: 249–57.

30. Prevolnik M, Ocepek M, Čandek-Potokar M, Bavec M, Škorjanc D. The effect of housing (conventional vs. organic) on growth, carcass and meat quality in pigs. In: Research in pig breeding: international workshop. Kostelec nad Orlici, Czech Republic: Vyzkumny ustav živočišne vyroby, 2009: 15–6.

31. Commission decision of 8 December 2005 authorising methods for pig carcases in Slovenia. OJ EU L56/28, 29.2.2008.

32. Nakai H, Saito F, Ikeda T, Ando S, Komatsu A. Standard models of pork colour. Bull Natl Inst Anim Ind 1975; 29: 69-74.

33. Christensen LB. Drip loss sampling in porcine m. *longissimus dorsi*. Meat Sci 2003; 63: 469–77.

34. IBL International. Cortisol ELISA: enzyme immunoassay for the in-vitro-diagnostic quantitative determination of free cortisol in human saliva and of total cortisol in diluted serum. RE52611: instruction for use. Hamburg: IBL International GMBH, 2008: 7 p.

35. IBL International. Neopterin ELISA: enzyme immunoassay for the in-vitro-diagnostic quantitative determination of neopterin in human serum, plasma and urine. RE59321: isnstruction for use. Hamburg: IBL International GMBH, 2008: 6p.

36. Assays designs. Hsp70 high sensitivity EIA kit: enzyme immunoassay kit for quantitative determination of Hsp70 in human serum and plasma. EKS-715: instruction for use. Michigan: Assay designs, 2008: 16 p.

37. SAS Institute. SAS OnlineDoc®, SAS Version 8.2. Cary, NC: SAS Institute, 2002.

38. Hambrecht E, Eissen JJ, Nooijen RIJ, et al. Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants. J Anim Sci 2004; 82: 1401–9.

39. Heinze PH, Mitchell G. Stress resistant and stress susceptible landrace pigs: Comparison of blood variables after exposure to halothane or exercise on a treadmill. Vet Rec 1989; 124: 163–8.

40. Shaw FD, Tume RK. The assessment of preslaughter and slaughter treatments of livestock by measurement of plasma constituents: a review of recent work. Meat Sci 1992; 32: 311–29.

41. Shaw FD, Trout GR, McPhee CP. Plasma and muscle cortisol measurements as indicators of meat quality and stress in pigs. Meat Sci 1995; 39: 237–46.

42. McGlone JJ, Salak JL, Lumpkin EA, Nicholson RI, Gibson M, Norman RL. Shipping stress and social status effects on pig performance, plasma cortisol, natural killer cell activity, and leukocyte numbers. J Anim Sci 1993; 71: 888–96.

43. O'Connell NE, Beattie VE, Moss BW. Influence of social status on the welfare of pigs reared in barren and enriched environments. Anim Welfare 2004; 13: 425–31.

44. Ruis MAW, Brake JHA, Engel B, Buist WG, Blokhuis HJ, Koolhaas JM. Implications of coping characteristics and social status for welfare and production of paired growing gilts. Appl Anim Behav Sci 2002; 75: 207–31.

45. De Jong IC, Prelle IT, van de Burgwal JA, et al. Effects of rearing conditions on behavioural and physiological responses of pigs to pre-slaughter handling and mixing at transport. Can J Anim Sci 2000; 80: 451–58.

46. Devenport L, Knehans A, Sundstrom A, Thomas T. Corticosterone's dual metabolic actions. Life Sci 1989; 45: 1389–96.

47 Moss BW. Lean meat, animal welfare and meat quality. In: Johnston DE, eds. The chemistry of muscle-based foods. Cambridge: Royal Society of Chemistry, 1992: 62–76.

48 Andrews RC, Herlihy O, Livingstone DEW, et al. Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance. J Clin Endocrinol 2002; 87: 5587–93.

49 Mariemi J, Kronholm E, Aunola S, et al. Visceral fat and psychosocial stress in identical twins discordant for obesity. J Intern Med 2002; 251: 35–43.

50. Milne KJ, Noble EG. Exercise-induced elevation of HSP70 is intensity dependent. J Appl Physiol 2002; 93: 561–8.

51 Sepponen K, Pösö AR. The inducible form of heat shock protein 70 in the serum, colon and small intestine of the pig: comparison to conventional stress markers. Vet J 2006; 171: 519–24.

POVEZANOST MED VSEBNOSTJO STRESNIH OZNAČEVALCEV (KORTIZOL, NEOPTERIN, HSP70) V KRVNI PLAZMI OB ZAKOLU IN LASTNOSTMI KAKOVOSTI KLAVNEGA TRUPA OZ. MESA

M. Škrlep, M. Prevolnik, B. Šegula, M. Čandek-Potokar

Povzetek: Cilj raziskave je bil analiza povezanosti različnih stresnih označevalcev v krvni plazmi ob zakolu (kortizola, neopterina in Hsp70) s klavno kakovostjo in kakovostjo mesa pri prašičih. V raziskavo je bilo vključenih 51 prašičev (isto križanje, odsotnost mutacije RYR1), ki so bili vzrejeni pri istem rejcu in zaklani v isti klavnici v petih serijah zakola. Ob zakolu so bili odvzeti vzorci krvi za analizo stresnih označevalcev, ki smo jih določili s komercialnimi kiti. Dan po zakolu smo izmerili/ocenili lastnosti klavne kakovosti (debelina mišice in slanine, mesnatost trupa, površina mišice *longissimus dorsi* (LD) in maščobe nad mišico LD, mesnatost potrebušine, masa stegna) in lastnosti kakovosti mesa (pH vrednost eno (pH_1) in 24 ur (pH_U) po zakolu, barvni parametri Minolta L*a*b* in izceja vode). Med serijami zakola ni bilo značilnih razlik v vsebnosti stresnih označevalcev in kakovosti mesa, ugotovili pa smo veliko variabilnost znotraj serije zakola. Analiza korelacij je pokazala, da vsebnosti neopterina in Hsp70 v plazmi nista povezani z nobeno lastnostjo kakovosti klavnega trupa oz. mesa. Nasprotno pa smo ugotovili značilne korelacije med vsebnostjo kortizola v plazmi in nekaterimi lastnostmi kakovosti klavnega trupa oz. mesa. Višja vsebnost kortizola je bila povezana z debelejšo hrbtno slanino (r=0,30 do 0,33) in manjšim deležem mesa v trupu (r=–0,37 do –0,41), kar kaže na to, da višje vrednosti kortizola v daljšem obdobju povečujejo zamaščenost. Glede kakovosti mesa smo ugotovili, da so višje vsebnosti kortizola povezane z višjim pH_1 (r=0,36 do 0,58) in pH_U (r=0,32 do 0,36) ter posledično z nižjimi vrednostmi za Minolta L* (r=–0,44), b* (r=–0,38) in izcejenostjo vode po 24ih (r=–0,44) in 48ih urah (r=–0,39), kar kaže na to, da vsebnost kortizola v krvi ob zakolu izraža več kot samo akutni predklavni stres.

Ključne besede: stres; prašič; klavna kakovost, kakovost mesa; kortizol; neopterin; Hsp70

Slov Vet Res 2009; 46 (4): 143-7
UDC 636.3.09:618.14-002:612.621.5

Case Report

HYDROMETRA IN A SHEEP AFTER OESTRUS SYNCHRONIZATION AND INSEMINATION IN THE ANOESTRAL SEASON

Stanimir Yotov[1]*, Dimitar Dimitrov[2], Ivan Fasulkov[1]

[1]Department of Obstetrics, Reproduction and Reproductive Disorders, [2]Department of Anatomy, Hystology and Embryology, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

*Corresponding author, E-mail: stanrad@abv.bg

Summary: A case of hydrometra in a Blackhead Pleven sheep following oestrus synchronization and artificial insemination in the anoestral season is detected.

The causes of the disease are discussed, the clinical signs and the echographic findings are described. The haematological, blood biochemical parameters and progesterone concentration were assayed. The gross anatomical and histological changes in the genital tract are reported.

The presented case showed that in sheep hydrometra was not accompanied by deviations in the general health condition, haematological and blood biochemical profile. Progesterone level did not correspond to value indicating pregnancy. A strong thinning of the uterine wall, aseptic fluid in the uterine cavity and persistent corpus luteum in one ovary were found out. In the endometrium, there were extensive lesions and strands dilatation of superficially located uterine glands.

In order to diagnose hydrometra in sheep, we recommend two echographic examinations and visualization of intrauterine anechoic fluid, thinned uterine wall and lack of embryo and placentomas.

Key words: sheep; hydrometra

Introduction

Hydrometra or pseudo pregnancy is a common disease of the genital tract in goats, described by a number of investigators (1,2,3,4). It is characterized with accumulation of aseptic fluid into the uterus, presence of persistent corpus luteum in the ovaries, high blood progesterone concentration and is observed in goats with spontaneous ovulation or after oestrus synchronization (5,6,7).

The hydrometra in sheep is a genital pathological state leading to permanent infertility (8). It is rarely encountered and that is why is not studied in details.

In threefold echographic pregnancy examination of 1,411 sheep from the Rambouillet breed and its crosses, hydrometra was observed only in 2.9% of cases (9). After the second examination, the cases of hydrometra were found to increase and it is pointed out that the examination stress could be a cause for the onset of the diseases

In a study on genital tracts of 1,042 slaughtered Awassi sheep, hydrometra was registered in 0.3%(10). Another authors have investigated the genital tract of 9,970 culled and 23,536 non-inseminated sheep and detected hydrometra in 18 and 61 animals respectively (8).

Received: 23 March 2009
Accepted for publication: 15 October 2009

Case history

A case of hydrometra in a Blackhead Pleven sheep at the age of 4 years, weighing 62 kg, reared at a place situated at latitude of 42° 25' N.

The animal has given birth thrice, and the last parturition and the postparturient period were normal. In June 2008, echography of the genital tract of the sheep was performed. No pathologies have been observed.

The oestrus was synchronized by means of Chronogest® intravaginal sponges (30 mg Cronolone), that stayed into the vagina for 12 days and treatment with Folligon® (500 UI PMSG). Artificial insemination was done twice on hours 48 and 56 after sponge removal.

By the 40th and the 60th day after the insemination, the sheep was examined for pregnancy by transrectal and transabdominal echography with an echograph Aloka SSD 500 Micrus (Aloka Co. Ltd, Tokyo, Japan) supplied with a 5 MHz probe.

A physical examination was performed and blood samples for haematological, biochemical and hormonal analysis were obtained.

Haematological parameters were assayed on an automated haematological analyzer (Serono Plus, Germany), the biochemical profile on a biochemical analyzer BA 88 (Mindray, China), and progesterone level – by ELISA (Elisa kit for Progesterone, Human GmbH, Germany) with a sensitivity >0.03 ng/ml and a standard curve from 0 ng/ml to 40 ng/ml.

After the second echography, the sheep was slaughtered, a gross examination of the genital tract was performed and the volume of uterine fluid was determined.

Samples for histological examination were obtained from the uterine horns, fixed in 10% solution of neutral formalin and processed according to routine histological techniques (11). After dehydration, embedding in paraffin, preparation of histological cross sections on a microtome (Reichert–Jung, Austria) and staining with haematoxylin-eosin, they were examined under light microscope NU–2 (Carlzeiss, Jena, Germany) at magnifications from 1:50 to 1:250 (12).

Results

Physical examination: Rectal body temperature - 38.7^{0}C, heart rate - 52 beat per minute, respiratory rate - 18/ min, ruminal movements - 8/5min.

Hematology, blood biochemistry and hormonal assay: RBC - 9 T/L, Hb - 120 g/l, HCT – 0.32 l/l, WBC - 12.5 x 10^9/l, AST - 146, ALT - 78, Ca - 2.32 mmol/l, P - 1.46/mmol/l, Mg - 0.72 mmol/l, total protein – 55 g/l, cholesterol - 1.26 mmol/l, P_4 - 8.2 ng/ml.

Echography: Two echoic luteinized structures with a diameter of > 8 mm and four anechoic follicle of a diameter of 3–5 mm were observed in the left ovary (Fig.1a). In the right ovary, multiple follicles with diameters < 5 mm were present. In the uterus, several anechoic zones, located ventrocranially to the urinary bladder and thinned echoic uterine wall without visible embryo and clearly manifested placentomas were observed (Fig.1b).

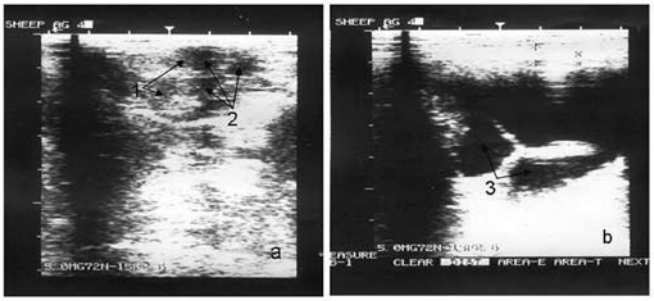


Figure 1: Echography of ovary (a) and uterus (b) in a sheep with hydrometra: 1 - corpora lutea; 2 - follicles; 3 - thinned uterine wall and anechoic uterine fluid

Gross anatomy and histological examinations: In the left ovary, two corpora lutea and several small follicles, and in the right ovary – multiple small and medium-sized follicles were observed. The left uterine horn was enlarged, with a strongly thinned and transparent uterine wall at the horn's tip (Fig. 2). The uterus was filled with 26.4 ml clear fluid without pus or blood, and on the surface of the endometrium in the left uterine horn, loci of darker colour and millet to lentils size were established.

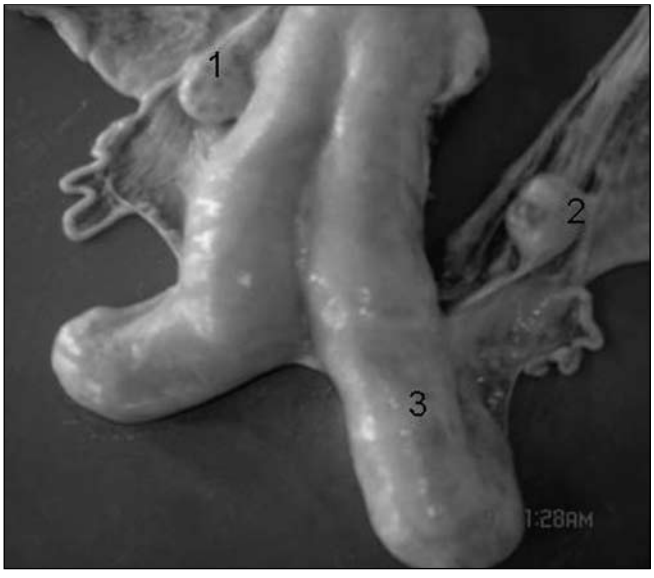


Figure 2: Uterus in a sheep with hydrometra: 1 - right ovary with small follicles; 2 - left ovary with corpora lutea; 3 - dilated uterine horn

The histological examination showed no alterations in the microstructure of the perimetrium and myometrium.

There was a thinning of the endometrium in the areas with hydrometra. Extensive lesions were present in lamina epithelialis mucosae.

The epithelial cells of the single-layered or double-layered prismatic epithelium that were in contact with altered areas, possessed microstructural changes. In these areas, Lamina epithelialis was built most frequently from one layer of cells, whose height was lower than that of cube-shaped cells.

In both types of lining epithelial cells – those with initial height reduction and those having completed this process, the cell cytoplasm was partially or totally vacuolated. Various amounts of diffusely located, basophilically stained granules were detected.

The most perceptible changes were observed in the uterine glands of the endometrium that was in contact with hydrometra. Significant differences in the number, density, and the extent of branching, lumen width and functional state were observed between uterine glands situated in the deep and the superficial endometrial zones (Fig. 3). Uterine glands were strongly dilated, with the width of their lumen exceeding many times that of deeply located ones.

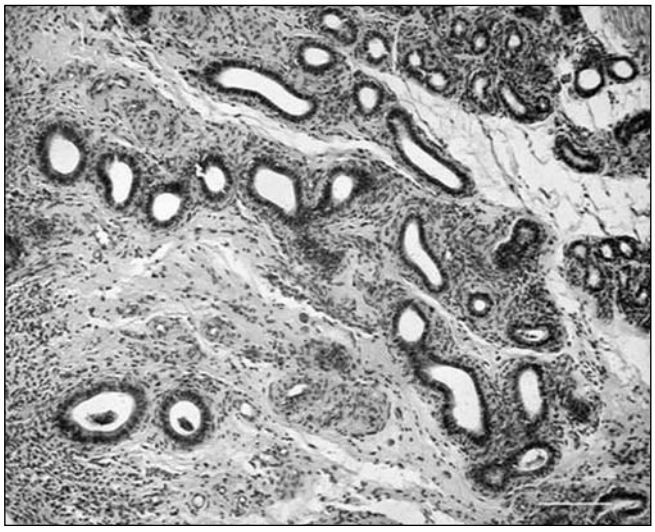


Figure 3: Endometrium with desquamated lining epithelium and structural changes in uterine glands in a sheep with hydrometra (H/E; 1:250, bar= 12.5 μm)

The glandular epithelium in some glands showed destructive changes and initial vacuolization. Single intraepithelial glands of a various size, at some places transformed into intraepithelial cysts, were observed.

Discussion

The presented case evidenced that hydrometra in sheep could be detected after oestrus synchronization and artificial inseminations in the anoestral season. In goats with this state was registered or without insemination (13), regardless of whether they were fertilized or not, whereas (14) found it after oestrus synchronization. These reports support the above mentioned statement.

The disease could pass without symptoms, without discharge from the external genitals and deviations in the haematological and blood biochemical indices. Only leukocytes were slightly elevated, but could not be a precise marker of inflammation of the uterus.

The blood serum progesterone concentration of 8.2 ng/ml showed the presence of corpus luteum in the ovaries, but was lower than the levels indicated a gestation of 40 days in this sheep breed as determined in previous studies of ours (15).

These results showed that the prolonged effect of progesterone is able to induce hydrometra in sheep too, not only in goats (3,16).

The echography of ovaries and the uterus is the most precise means to detect hydrometra in sheep *in vivo*. The visualization of corpus luteum in the ovaries, the thinned uterine wall, the lack of embryo and the clear visualization of placentomas are all proofs for the occurrence of hydrometra.

In agreement with the previous studies (9), we also believe that two echographies are needed for the correct diagnosis.

The diagnostic error is highly probably in case of a single echography, especially in the early gestational stages. Moreover, when non-fertilized or in cases of early embryonic death, sheep do not exhibit a regular sexual cycle, as they are not in the breeding season.

The gross examination of the genital tract did not show remnants of foetal membranes or an embryo, excluding early embryonic death as a cause. The nature of the fluid and the uterine changes suggest that this was not a case of pyometra and they were similar to those reported by previous authors (8).

In our view, the extensive lesions observed throughout the histological examination, could be probably due to the pressure exerted by the fluid, resulting in cell desquamation and lack of lining epithelium. The basophilic granulation and the total vacuolization of cellular cytoplasm in Lamina epithelialis provided evidence for destructive pro-

cesses in the cells. The increased incidence of cross sections of glands in areas of desquamated epithelium or reduction indicated that they were excessively coiled or branched. Similar changes in the endometrium are described in goats with hydrometra (13).

In conclusion, hydrometra in sheep could be detected after oestrus synchronization and artificial insemination in the anoestral season. There were not clear clinical manifestations of the disease, or any deviations in the haematological and blood biochemical parameters. The Histological changes of the endometrium consisted in vast lesions with destructive events and altered height of epithelial cells situated around them. The strong dilatation of the lumen of superficially located uterine glands is specific for the disease.

In order to diagnose hydrometra in sheep *in vivo,* two echographic examinations at 20-day interval are recommended, as well as visualization of persistent corpus luteum in the ovaries, anechoic fluid in the uterus, thinned hyperechoic uterine wall, lack of embryo and placentomas.

References

1. Hesselink WJ. Incidence of hydrometra in dairy goats. Vet Rec 1993; 132: 110–2.

2. Hesselink WJ. Hydrometra in dairy goats: reproductive performance after treatment with prostaglandins. Vet Rec 1993; 133: 186–7.

3. Kornalijnslijper JE, Bevers MM, Van Oord HA, Tavene MAM. Induction of hydrometra in goats by means of active immunization against prostaglandin $F2_{\alpha}$. Anim Reprod Sci 1997; 46: 109–22.

4. Moraes EPBX, Santos MHB, Arruda IJ, Bezerra FQG, Aguiar Filho CR, Neves JP, Lima PF et al. Hydrometra and mucometra in goats diagnosed by ultrasound and treated with PGF2α. Med Vet (Brasil) 2007; 1: 33–9.

5. Pieterse MC, Taverne MAM. Hydrometra in goats: diagnosis with real-time ultrasound and treatment with prostaglandins or oxytocin. Theriogenology 1986; 26: 813–21.

6. Kornalijnslijper JE, Kemp B, Bevers MM, van Oord HA, Taverne MAM. Plasma prolactin, growth hormone and progesterone concentrations in pseudopregnant, hysterectomized and pregnant goats. Anim Reprod Sci 1997; 49: 169–78.

7. Lopes-Júnior ES, Cruz JF, Teixeira DIA, Lima Verde JB, Paula NRO, Rondina D et al. Pseudopregnancy in Saanen goat (*Capra hircus*) raised in Northeast Brazil. Vet Res Commun 2004; 28: 119–25.

8. Smith KC, Long SE, Parkinson TJ. Abattoir survey of congenital reproductive abnormalities in ewes. Vet Rec 1998; 143: 679–85.

9. Bretzlaff KN. Development of hydrometra in a ewe flock after ultrasonography for determination of pregnancy. J Am Vet Med Assoc 1993; 203: 122–5.

10. Moghaddam A, Gooraninejad S. Abattoir survey of gross abnormalities of the ovine genital tracts in Iran. Small Ruminant Res 2007; 73: 259–61.

11. Vitanov S, Dimitrov D, Bochukov A. Manual of cytology and histology with histological techniques. Sofia: Zemizdat Press, 1995: 3–12.

12. Kiernan JA. Histological and histochemical methods: theory and practice. 4th ed. Bloxham: Scion Publishing, UK, 2008: 131–2.

13. Wittek T, Erices J, Elze K. Histology of the endometrium, clinical chemical parameters of the uterine fluid and blood plasma concentrations of progesterone, estradiol-17_{β} and prolactin during hydrometra in goats. Small Ruminant Res 1998; 30: 105–12.

14. Humbolt P, Brice G, Chemineau P, Broqua C. Embryo mortality in the dairy goat after oestrus synchronization and artificial insemination outside the normal breeding season. In: Les Colloques des 2emes Rencontres des Recherches sur Ruminants. Paris: Institut de l` Elevage, 1995: 387–9.

15. Yotov S. Determination of the number of fetuses in sheep by means of blood progesterone assay and ultrasonography. Bulg J Vet Med 2007; 10: 185–93.

16. Taverne MAM, Lavoir MC, Bevers MM, Pieters MC, Dieleman SJ. Peripheral plasma prolactin and progesterone levels in pseudopregnant goats during bromocriptine treatment. Theriogenology 1988; 30: 777–83.

HIDROMETRA PRI OVCAH PO SINHRONIZACIJI GONITVE IN UMETNI OSEMENTIVI V OBDOBJU NEGONITVE

S. Yotov, D. Dimitrov, I. Fasulkov

Povzetek: Pri plevenski črnoglavi ovci je po sinhronizaciji gonitve in umetni osemenitvi v obdobju negonitve prišlo do hidrometre. V članku so obravnavani vzroki bolezni, klinični znaki ter ugotovitve, pridobljene s pomočjo ultrazvoka. Analizirani so bili tudi hematološki in krvni biokemijski parametri ter koncentracija progesterona. V spolovilih ovce so bile opazne patološke in histološke spremembe

V opisanem primeru smo pokazali, da hidrometre pri ovci niso spremljali odkloni splošnega kliničnega zdravja ter hematološkega in krvnega biokemijskega profila. Raven progesterona ni ustrezala vrednosti, ki kaže na brejost. Maternična stena je bila močno stanjšana, v maternici je bila prisotna aseptična tekočina ter trajajoče rumeno telesce na enem jajčniku. V steni maternice so bile spremembe razširjene, opazno je bilo povečanje površinskih materničnih žlez.

Za diagnostiko hidrometre pri ovcah priporočamo dva pregleda z ultrazvokom, pri katerih je potrebno biti pozoren na prosto tekočino znotraj maternice, stanjšanje maternične stene, neopaznost zarodka in placentomov.

Ključne besede: ovca; hidrometra

Slov Vet Res 2009; 46 (4): 149-54
UDC 579.62:576.852.21:616-097.3

Case Report

HIGH PREVALENCE OF *MYCOBACTERIUM AVIUM* SUBSP. *HOMINISSUIS* IN A BATCH OF QUARANTINED PIGS IN CROATIA

Željko Cvetnić[1]*, Silvio Špičić[1], Sanja Duvnjak[1], Maja Zdelar-Tuk[1], Miroslav Benić[1], Mario Mitak[1], Mateja Pate[2], Brane Krt[2], Matjaž Ocepek[2]

[1]Croatian Veterinary Institute, Savska c. 143, 10000 Zagreb, Croatia, [2]University of Ljubljana, Veterinary Faculty, Gerbičeva 60, 1115 Ljubljana, Slovenia

*Corresponding author, E-mail: cvetnic@veinst.hr

Summary: Tuberculosis is a common occurrence in pigs, which are susceptible to infection with both *Mycobacterium (M.) avium subsp. avium* and *M. avium* subsp. *hominissuis*. The aim of this study was to present a case of tuberculosis in a batch of quarantined pigs, imported to Croatia from Austria. Diagnostic examination of 125 quarantined animals included a comparative tuberculin skin test with avian and bovine tuberculin. As 67.2% of pigs showed either positive or suspicious reaction to avian tuberculin, the animals were retested. The results of the second skin test revealed an even higher proportion of reactors (91.1%). Therefore, the State Veterinary Office ordered slaughtering of the animals, and post-mortem examination (gross examination and bacteriology) of the lymph nodes of 121 pigs was performed for diagnostic purposes. Gross tuberculous lesions were observed in 107 pigs (88.59%), mostly in mesenteric lymph nodes (52.1%). *M. avium* subsp. *hominissuis*, identified with molecular methods, was isolated from 113 pigs (93.4%). This report stresses the importance of effective preventive and control measures which are necessary on farms raising animals for breeding purposes.

Key words: swine; tuberculosis; lymph nodes; tuberculin skin test; PCR

Introduction

Swine tuberculosis (TBC) is a chronic infectious disease characterised by inflammatory changes prone to calcification in various body sites, mostly in the digestive system. There is no mycobacterial species specific to pigs, like *Mycobacterium (M.) tuberculosis*, to be found in humans, *M. bovis* in bovines or *M. avium* in poultry and birds. However, pigs are susceptible to infection caused by members of the *M. tuberculosis* and *M. avium* complex, and by opportunistic mycobacterial species, e.g. *M. fortuitum* and *M. chelonae* (1, 2).

Systematic control programmes for bovine TBC and reduced incidence of human TBC decreased the importance of *M. bovis* and *M. tuberculosis* infections in pigs (3). The possibility of infection caused by *M. caprae* exists in pigs fed with raw milk of infected cows (4). However, infections caused by *M. avium* complex are the most common in pigs and have been described worldwide (5-16). Environment (water, soil, sawdust, feedstuff, birds etc.) is a risk factor for human and animal infections caused by the *M. avium* complex, comprising *M. avium* and *M. intracellulare* (1, 17-20).

M. avium is currently divided into four subspecies, *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, *M. avium* subsp. *silvaticum* and *M. avium* subsp. *hominissuis* (21). *M. avium* subsp. *avium* is the causative agent of avian TBC. Primarily affecting birds, it may also infect other animal species. It comprises serotypes 1, 2 and 3; its genome contains mobile elements IS*901* and IS*1245*. *M. avium* subsp. *hominissuis* was proposed to distinguish organisms found in humans and pigs from those isolated from birds (22). Predominantly found in the environment, *M. avium* subsp. *hominissuis* isolates are weakly

Received: 3 July 2009
Accepted for publication: 28 September 2009

virulent for birds but are frequently encountered in tuberculous lesions in different animals, especially pigs (23). This subspecies includes serotypes 4-6, 8-11 and 21 and is of the IS*901*-, IS*1245*+ genotype. Pigs are susceptible to infection with both *M. avium* subsp. *avium* and *M. avium* subsp. *hominissuis*. The prevalence seems to be correlated with the presence of certain subspecies in the environment. However, *M. avium* subsp. *avium* is considered a much more serious pathogen for pigs than *M. avium* subsp. *hominissuis* (13).

The aim of this case report was to present the results of the skin tuberculin test, necropsy, isolation and identification of *M. avium* subsp. *hominissuis* performed as a part of quarantine procedures in breeding pigs imported from Austria.

Materials and methods

Anamnestic data

At the beginning of March 2006, 120 gilts and one boar of Edelschwein race from farm A, two boars of the same race from farm B and two boars of the same race from farm C were imported from Austria. The pigs intended for breeding were from six to nine months of age, weighing between 90 and 130 kg. The pigs were transported from Austria and placed in a quarantine unit of the farm near the town of Petrinja (County of Sisak-Moslavina). The quarantine conditions complied with the standards prescribed by the State Veterinary Office of the Republic of Croatia. Diagnostic examination of the quarantined animals was in accordance with the current legislation prescribed by the same government body.

Tuberculin skin test

All 125 pigs were tuberculin tested with 0.1 ml of avian PPD (20,000 IU) and bovine PPD (50,000 IU; VETERINA d.o.o., Kalinovica, Croatia) administered intracutaneously at auricular basis. The tuberculins were administered simultaneously: avian in the left and bovine in the right ear. Skin reactions were evaluated after 48 and 72 hours according to manufacturer's recommendation. Reaction was negative if no or only pea-sized oedema without redness appeared at the site of application. Reaction characterised by oedema of approx. 2 cm in diameter and visible redness not surrounded with a red circle was regarded as suspicious. Reaction was positive if the site of application was oedematous (2-5 cm in diameter), surrounded by a circular red zone with warmer, purple-red coloured centre and covered by scab. Since a high percentage of pigs were positive at the first skin tuberculin test carried out on 14 March 2006, the State Veterinary Office recommended a repeated skin test which was performed on 30 May 2006.

Gross examination

Due to the high percentage of pigs with positive and suspicious reactions in the second test, the State Veterinary Office ordered the slaughter of 121 pigs (118 gilts and three boars). During the quarantine period two gilts died. Two negative boars were not slaughtered, due to their high genetic value. Slaughtered pigs were checked for gross lesions, and lymph nodes (*ln. submandibularis, ln. mesenterialis, ln. inguinalis, ln. mediastinalis, ln. hepaticus, ln. ileocaecalis*) were collected for investigation.

Bacteriology

For microscopy, lymph node smears were Ziehl-Neelsen (ZN) stained and checked for the presence of acid-fast bacilli (AFB). Lymph nodes were then homogenised, concentrated, decontaminated with NALC-NaOH, inoculated on standard nutrient media (Löwenstein-Jensen with pyruvate, Löwenstein-Jensen with glycerine, Stonebrink and Middlebrook 7H10) and incubated at 37°C for two months. Media were checked for growth of mycobacteria at weekly intervals. The colonies were ZN stained; positive colonies were subcultured and identified by biochemical and molecular methods (25-27).

Identification of isolates by biochemical and molecular methods

ZN positive colonies were identified according to colony morphology, growth temperature and pigment production. Biochemical tests included the following enzymatic activities: nitrate reductase, catalase, Tween 80 hydrolysis, amidase, arylsulfatase, pyrazinamidase and urease (25).

The isolates were identified as members of the genus *Mycobacterium* also by amplification of DNA sequence containing the gene coding for 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 to amplify a 383 bp fragment (26). Subsequent-

ly, the isolates were subjected to identification with the commercially available identification kit GenoType *Mycobacterium* CM (Hain Lifescience, Nehren, Germany) which enables identification of mycobacteria commonly found in clinical samples. In order to differentiate *M. avium* subsp. *avium* and *M. avium* subsp. *hominissuis*, the isolates were tested by PCR using the primer set P1 FR300 (5'-CAG-CCA-GCC-GAA-TGT-CAT-CC-3') and P2 FR300 (5'-CAA-CTC-GCG-ACA-CGT-TCA-CC-3') described previously by Kunze et al. (27). Amplification product size depends upon the presence or absence of IS*901*. IS*901*+ positive isolates (*M. avium* subsp. *avium*) show an amplification product of 1700 bp, while IS*901*- isolates give amplicons of 300 bp (*M. avium* subsp. *hominissuis*).

PCR results were detected by electrophoresis in 2% agarose gels and visualised by UV transilluminator and camera (Bio-Capt, Vilbert Lourmat, France).

Results

Tuberculin skin test

Among 125 quarantined pigs tested in the first skin test, positive reactions to avian tuberculin were observed in 44 pigs (35.2%), while 40 pigs (32%) were regarded as suspicious reactors. In the second test, which included 123 pigs, the number of reactors and intensity of reactions increased in comparison to the first test (Table 1). All positive pigs originated from farm A. An example of positive reaction is shown in Figure 1.

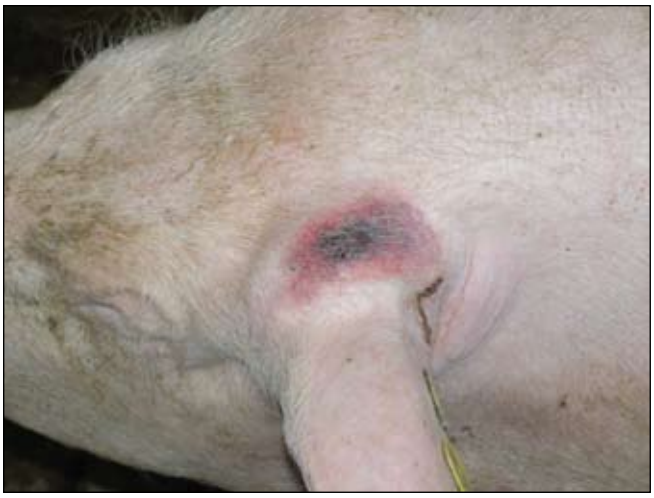


Figure 1: Positive reaction to avian tuberculin in a pig

Gross pathology examination

A total of 121 slaughtered pigs were inspected for gross lesions at the slaughterhouse. Visible granulomatous changes characteristic for TBC were found in 107 pigs (88.59%). Submandibular and mesenteric lymph nodes were moderately augmented with incorporated yellow foci of 3-10 mm in diameter (Figure 2). Calcified granules were visible in the caseous contents of dissected nodes. Mesenteric lymph nodes alone were visibly changed in 63 pigs (52.1%) and both mesenteric and submandibular nodes in 35 pigs (28.9%). Changes in other lymph nodes were rarely observed (Table 2).

Table 1: Results of tuberculin skin tests performed in quarantined pigs

Date of test	No. of tested pigs	Positive reaction		Suspicious reaction		Total reaction (+, +/-)		Negative reaction	
		No.	%	No.	%	No.	%	No.	%
16 March 2006	125	44	35.2	40	32	84	67.2	41	32.8
30 May 2006	123	95	77.2	17	13.8	112	91.1	11	8.9

Table 2: Localisation of necropsy changes found in investigated lymph nodes of 121 slaughtered pigs

No. of pigs	Location of changes				
	SLN	MLN	SLN + MLN	SLN + MLN + PLN	Total
121	7	63	35	2	107
100%	5.8	52.1	28.9	1.7	88.5

Legend: SLN – submandibular lymph node, MLN – mesenteric lymph node, PLN – portal lymph node

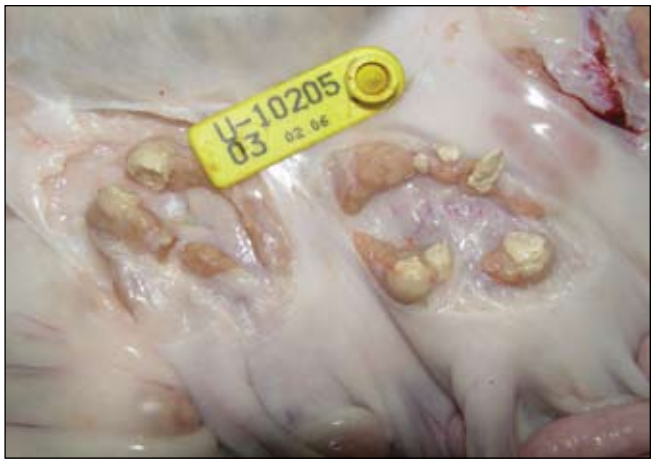


Figure 2: Tuberculous lesions in a mesenteric lymph node of a swine at slaughter

Bacteriology and identification of the isolates

Mycobacteria were isolated from the lymph nodes of 113 swine (93.4%). All isolates were identified as members of the genus *Mycobacterium*. Using the GenoType *Mycobacterium* CM kit, the isolates were identified as *M. avium*. PCR used for differentiation of *M. avium* subsp. *hominissuis* and *M. avium* subsp. *avium* resulted in amplicons specific for *M. avium* subsp. *hominissuis* only.

Discussion

Swine TBC can cause huge economical losses. Pavlik et al. (13) stated that direct losses due to TBC can reach 24% of the total swine price. In order to avoid them, effective diagnostic methods are needed to prevent the spread of the disease. The tuberculin skin test, recommended by the OIE and used worldwide, serves as an indirect diagnostic method for TBC since the infected animals develop hypersensitivity against the causative agent. Post-mortem examinations are used for definitive diagnostics of TBC. Standard identification methods of the causative agent, based on morphological and biochemical characteristics, made the differentiation within *M. avium* difficult. Development of molecular methods facilitated and improved the identification and contributed to the description of new subspecies.

In this study, a high proportion of pigs imported from Austria showed positive reactions to avian tuberculin. In the first tuberculin test, 67.2% of tested pigs were either positive or suspicious reactors, while in the repeated test the proportion increased to 91.1% of tested pigs. The reason for substantial increase of positive reactors (from 35.2% to 77.2%) most probably lies in the fact that the infection in young animals was acute and the organisms needed time to create an immunological response. Some pigs reacted also to bovine tuberculin but with weaker intensity. Subsequent bacteriological examination of specimens from these animals revealed only the presence of *M. avium* subsp. *hominissuis*.

In 121 carcasses of slaughtered pigs, tuberculous granuloma localised in submandibular and mesenteric lymph nodes were found in 107 pigs (88.5%). The changes were most frequently localised in mesenteric lymph nodes (52.1%), followed by both mesenteric and submadibular (28.9%) lymph nodes. Lesions in other lymph nodes were rarely observed. Pavlik et al. (13) reported similar results: 65.3% of granuloma in swine were detected in mesenteric lymph nodes, followed by submandibular (18.6%), both submandibular and mesenteric (15.9%) lymph nodes, while 0.1% of changes were observed in parenchymatous organs.

All isolates from this investigation were identified as *M. avium* subsp. *hominissuis*. This is a common finding in pigs (2, 18). Pate et al. (14) reported domination of *M. avium* subsp. *hominissuis* (60.9%) over *M. avium* subsp. *avium* (33.8%) among mycobacteria isolated from swine in Slovenia between 2000 and 2003. Pavlik et al. (13) described a reverse situation in a different time period (1990-1999), with *M. avium* subsp. *avium* being more prevalent (55.7%) than *M. avium* subsp. *hominissuis* (39.2%). However, in the second part of the 1990s the percentage of isolated *M. avium* subsp. *hominissuis* strains started to increase, which was probably connected to a change in the sources of infection for pigs. One of our previous studies (16) revealed that the majority of mycobacteria isolated from swine in Croatia belonged to *M. avium* complex (95.7%), while other identified species included *M. fortuitum* (3.3%), *M. chelonae* (0.5%) and *M. peregrinum* (0.5%). *M. avium* subsp. *hominissuis* was identified in larger proportion than *M. avium* subsp. *avium* (78.9% versus 21.1%).

Sawdust was used as bedding material on farm A (probably in the farrowing units) of this study. Therefore, the piglets were very likely exposed to high concentrations of *M. avium* subsp. *hominissuis* which is frequently found in the environment (12). Cvetnić et al. (16) found the highest proportions of tuberculin skin test reactors on farms where sawdust or wood shavings were used for bedding in farrowing and

boar units. In one case, the proportion of infected boars reached 70%. Isolation of *M. avium* subsp. *hominissuis* serotype 8 from the lymph nodes of pigs and from sawdust indicated the latter as the most probable source of infection. This has been suggested before (1, 28-30). The development of genotyping methods enabled an insight into molecular characteristics of investigated isolates and confirmation of previously suggested epidemiological links. Matlova et al. (2) managed to demonstrate identical genotypes of *M. avium* subsp. *hominissuis* from sawdust and from clinical samples of pigs, therefore confirming the hypotheses regarding the sawdust being a source of infection for pigs. The same situation was proven also in Croatia by Špičić et al. (31).

Knowledge about the ecology and epidemiology of mycobacteria is of great importance for successful prevention and control of the infections. Susceptibility of swine to various mycobacteria poses a constant risk for outbreaks of clinical disease in swine herds. Therefore, effective preventive and control measures are necessary on farms raising animals for breeding purposes.

Acknowledgements

The results presented herein were obtained within the research project Molecular epizootiology of the major bacterial zoonoses supported by the Ministry of Science, Education and Sport of the Republic of Croatia (Grant 048-0481153-1150).

References

1. Cvetnic Z, Kovacic H, Ocepek M. Mykobakterien in der Umwelt und im Futter von Schweinen in Kroatien (in German). Wien Tierärztl Monatscrift 1998; 85: 18–21.

2. Matlova L, Dvorska L, Palecek K, Maurenc L, Bartos M, Pavlik I. Impact of sawdust and wood shavings in bedding on pig tuberculous lesions in lymph nodes, and IS*1245* RFLP analysis of *Mycobacterium avium* subsp. *hominissuis* of serotypes 6 and 8 isolated from pig and environment. Vet Microbiol 2004; 102: 227–36.

3. Aleraj Z, Tunkl B, Karlovic M. Using of UV rays for fast TBC diagnosis and eradication from swine (in Croatian). Praxis Vet 1972; 3: 135–40.

4. Cvetnic Z, Spicic S, Katalinic-Jankovic V et al. *Mycobacterium caprae* infection in cattle and pigs on one family farm in Croatia: a case report. Vet Med – Czech 2006; 51: 523–31.

5. Zorawski C, Karpinski T, Skwarek P, Loda M. *M. intracellulare* serotip 8 (Davis), existing in sawdust as a cause of mass infection of pigs. Bull Vet Inst Pulawy 1983; 26: 1–5.

6. Karlović M, Lojkić M, Bilić V, Kovačić H. The health issues in swine farms (in Croatian). Vet Arh 1985; 55: S9.

7. Gradner IA, Hird DW. Environmental source of mycobacteriosis in a California swine herd. Can J Vet Res 1989; 53: 33–7.

8. Haugegaard J, Knudsen S, Bager, F. Tuberculous lymphadenitis caused by *Mycobacterium avium-intracellulare* in Danish swine herds. In: 12th International Pig Veterinary Society Congress Proceedings. Hague, 1992: 343.

9. Balian SC, Ribeiro P, Vasconcelos SA et al. Tuberculosis lymphadenitis in slaughtered swine from the State of Sao Paulo, Brasil: microscopic histopathology and demonstration of mycobacteria. Rev Saude Publica 1997; 31: 391.

10. Komijn RE, De Haas PE, Schneider MM et al. Prevalence of *Mycobacterium avium* in slaughter pig in the Netherlands and comparison of IS*1245* restriction fragment length polymorphism patterns of porcine and human isolates. J Clin Microbiol 1999; 37: 1254–59.

11. Offermann U, Bodmer T, Audige L, Jemmi T. The prevalence of salmonella, yersinia and mycobacteria in slaugtered pigs in Switzerland (in German). Schweiz Arch Tierheilkd 1999; 141: 509–15.

12. Matlova L, Dvorska L, Bartl J et al. Mycobacteria isolated from environment of pig farms in the Czech Republic during the years 1996 to 2002. Vet Med – Czech 2003; 48: 343–57.

13. Pavlik I, Matlova L, Dvorska L et al. Tuberculosis lesions in pigs in the Czech Republic during 1990–1999: occurrence, causal factors and economic losses. Vet Med – Czech 2003; 48: 113–25.

14. Pate M, Zdovc I, Pirš T, Krt B, Ocepek M. Isolation and characterisation of *Mycobacterium avium* and *Rhodococcus equi* from granulomatous lesions of swine lymph nodes in Slovenia. Acta Vet Hung 2004; 52: 143–50.

15. Pavlik I, Matlova L, Dvorska L, Shitaye JE, Parmova I. Mycobacterial infections in cattle and pigs caused by *Mycobacterium avium* complex members and atypical mycobacteria in the Czech Republic during 2000–2004. Vet Med – Czech 2005; 50: 281–90.

16. Cvetnić Ž, Špičić S, Benić M et al. *Mycobacterium* infections in pigs in Croatia. Acta Vet Hung 2007; 55: 1–9.

17. Martin G, Schimmel D. *Mycobacterium avium* infections in poultry – a risk for human health or not? Dtsch Tierarztl Wochenschr 2000; 107: 53–8.

18. Matlova L, Dvorska L, Ayele WY, Bartos M, Amemori T, Pavlik I. Distribution of *Mycobacterium avium* complex isolates in tissue samples of pigs fed peat naturally contaminated with mycobacteria as a supplement. J Clin Microbiol 2005; 43: 1261–8.

19. Reed C, Von Reyn CF, Chamble S et al. Environmental risk factor for infection with *Mycobacterium avium* complex. Am J Epidemiol 2006; 164: 32–40.

20. Fischer OA, Matlova L, Dvorska L et al. Various stages in the life cycles of syrphid flies (*Eristalis tenax*; Diptera: Syrphidae) as potential mechanical vectors of pathogens causing mycobacterial infections in pigs herds. Folia Microbiol (Praha) 2006; 51: 147–53.

21. Turenne CY, Wallace R Jr, Behr MA. *Mycobacterium avium* in the postgenomic area. Clin Microbiol Rev 2007; 20: 205–29.

22. Mijs W, de Haas P, Rossau R et al. 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 2002; 52: 1505–18.

23. Pavlik I, Svastova P, Bartl J, Dvorska L, Rychlik I. 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 2000; 7: 212–7.

24. Luna LG. Manual of histologic methods of the Armed Forces Institute of Pathology. New York: McGraw Hill, 1979.

25. Kent PT, Kubica GP. Public health mycobacteriology: a guide for level III laboratory. Atlanta: US Department of Health and Human Services, Centers for Disease Control, 1985.

26. Hance AJ, Grandchamp B, Levi-Frebauld V et al. Detection and identification of mycobacteria by amplification of mycobacterial DNA. Mol Microbiol 1989; 7: 843–9.

27. Kunze ZM, Portaels F, McFadden JJ. Biologically distinct subtypes of *Mycobacterium avium* differ in possession of insertion sequence IS*901*. J Clin Microbiol 1992; 30: 2366–72.

28. Reznikov M, Leggo JH, Taffley RE. Further investigation of an outbreak of mycobacterial lymphadenitis at a deep – litter piggery. Aust Vet J 1971; 47: 622–8.

29. Saitanu K, Holmgaard P. An epizootic of *M. intracellulare*, serotype 8 infection in swine. Nord Vet Med 1977; 29: 221–6.

30. Saxegaard F. Serological investigations of *Mycobacterium avium* and *M. avium* like bacteria isolated from domestic and wild animals. Acta Vet Scand 1981; 22: 153–61.

31. Spicic S, Pate M, Katalinic-Jankovic V et al. Molecular epizootiology of *Mycobacterium avium* subsp. *hominissuis* isolated from humans, animals and the environment in Croatia. J Comp Immunol Microbiol Infect Dis 2010 In press

MOČNA RAZŠIRJENOST *MYCOBACTERIUM AVIUM* SUBSP. *HOMINISSUIS* V SKUPINI PRAŠIČEV, KI SO BILI V KARANTENI NA HRVAŠKEM

Ž. Cvetnić, S. Špičić, S. Duvnjak, M. Zdelar-Tuk, M. Benić, M. Pate, B. Krt, M. Ocepek

Povzetek: Tuberkuloza je pogosti pojav pri prašičih, ki so dovzetni za okužbo z *Mycobacterium (M.) avium* subsp. *avium* kot tudi *M. avium* subsp. *Hominissuis*. Cilj pričujoče raziskave je opis primera tuberkuloze v skupini prašičev v karanteni, ki so bili v Hrvaško uvoženi iz Avstrije. Diagnostična preiskava 150 živali v karanteni je vključevala tuberkulinski kožni test s ptičjim in govejim tuberkulinom. Ker je bila pri 67,2 odstotkih prašičev opazna pozitivna ali sumljiva reakcija, so živali ponovno preiskali. Rezultati drugega kožnega testa so pokazali še večji delež živali, ki so reagirale na antigene (91,1 odstotka). Na podlagi rezultatov je Državna veterinarska uprava odredila zakol živali ter izvedbo posmrtnega pregleda (patologija, bakteriologija) bezgavk 121 prašičev iz diagnostične preiskave. Velike patološke tuberkulozne spremembe so bile opazne pri 107 prašičih (88,6 odstotka), in sicer večinoma v oporkovih bezgavkah (52,1 odstotka). *M. avium* subsp. *hominissuis*, dokazano z molekularnimi metodami, smo izolirali pri 113 prašičih (93,4 odstotka). To poročilo želi poudariti pomembnost učinkovite preventive in kontrolnih pregledov, ki so nujni pri gojenju živali za vzrejne namene.

Ključne besede: prašič; tuberkuloza; bezgavke; tuberkulinski kožni test; PCR

SUBJECT INDEX VOLUME 46, 2009

anaemia 19
anemija 28
animals, - feeding, - suckling 71
antihistamines 5
antihistaminiki 12
aortic stenosis, subvalvular 125
aortna stenoza, subvalvularna 131
atopija 12
atopy 5
bacterial DNA – 105
bakterijska DNK – 113
bezgavke 154
bolezni – prirojene 131
bolezni, - srce, - prirojene 131
brain 85
celokupna antioksidantna kapaciteta 103
composting 105
corticotrophinoma 115
cortisol 133
Cushing's disease 115
Cushingova bolezen 120
dermatitis, - atopic, - atopični 5, 12
diseases, – congenital, - heart 125
dogs 93, 125
echocardiography 125
ehokardiografija 131
experimental animals 47
feed, additives 71
feksofenadin hidroklorid 12
fexofenadine hydrochloride 5
flaxseed 13
fluorokinoloni 41
fluoroquinolones 29
glucocorticoids 5
glukokortikoidi 12
gnoj 113
hidrometra 147
hiperkortizolizem 120
hipofiza 120
histochemistry 61
histokemija 70
hrana, dodatki 79
Hsp70 133, 142
hydrometra 143
hypercortisolism 115
kakovost, - klavna, - mesa 142
kompostiranje 113
kortikotropni tumor 120
kortizol 142
kunci 79
laneno seme 18
legislation 47
lipid oxidation 13
lymph nodes 149
mammals 61
manure 105
meta-analiza 41
meta-analysis 29
methylprednisolone 5
metilprednizolon 12
microbial inactivation 105
mikrobna inaktivacija 113
miozinska veriga, težka 70
mišičnega vlakna, tip 70
možgani 91
muscle fiber type 61
Mycobacterium avium subsp. paratuberculosis 113, 105
myosin heavy chain 61
neopterin 133, 142
oksidacija maščob 18
oligosaharides 71
oligosaharidi 79
ovca 147, 113
PCR 149, 154
pig 29, 133
pituitary 115
porcine respiratory disease complex 29
pork 13
poročilo 60
poskusne živali 60
prašič 41, 142, 154
prašičji respiratorni bolezenski kompleks 41
psi 103, 131
Q_{10}, -coenzyme, - koencim 93, 103
quality, - carcass, - meat 133
rabbits 71
report 47

Salmonella enterica serovar Senftenberg 105, 113
sex chromosomes 85
sexual differentiation 85
sheep 143, 105
sialic acid 19
sialidase 19
sialidaza 28
sialna kislina 28
skeletal muscle 61
skeletna mišica 70
spolne razlike 91
spolni kromosomi 91
steroid hormones 85
steroidni hormoni 91
stress 133, 142
svinjina 18
swine 149
total antioxidant capacity 93
tripanosomiaza 28
Trypanosoma congolense 19, 28
trypanosomosis 19
tuberculin skin test 149
tuberculosis 149
tuberkulinski kožni test 154
tuberkuloza 154
ultrasonography 125
ultrazvok 131
zakonodaja 60
živali, - prehrana, - sesne 79

AUTHOR INDEX VOLUME 46, 2009

Adamu S, Useh NM, Ibrahim NDG, Nok AJ, Esievo KAN. Erythrocyte surface sialic acid depletion as predisposing factor to erythrocyte destruction in sheep experimental model of African *Trypanosomosis*: A preliminary report. . . 19

Benić M, see Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. 149

Blatter MFC, see Gallelli MF, Miceli DD, Blatter MFC, Brañas MM, Castillo VA.. 115

Brañas MM, see Gallelli MF, Miceli DD, Blatter MFC, Brañas MM, Castillo VA.. 115

Čadonič-Špelič V, Ornik D, Čadonič-Špelič V. . 47

Čandek-Potokar M, see Škrlep M, Prevolnik M, Šegula B, Čandek-Potokar M. 133

Castillo VA, see Gallelli MF, Miceli DD, Blatter MFC, Brañas MM, Castillo VA.. 115

Cvetko S, see Domanjko Petrič A, Cvetko S. . . 125

Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. High prevalence of *Mycobacterium avium* subsp. *hominissuis* in a batch of quarantined pigs in Croatia. 149

Dimitrov D, see Yotov S, Dimitrov D, Fasulkov I.. 143

Dobeic M, see Gobec I, Ocepek M, Pogačnik M, Dobeic M. 105

Domanjko Petrič A, Cvetko S. Aortic stenosis in dogs: Clinical characteristics and survival in 80 cases.. 125

Domanjko-Petrič A, see Plevnik A, Kobal S, Domanjko-Petrič A, Kotnik T. 5

Duvnjak S, see Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. 149

Esievo KAN, see Adamu S, Useh NM, Ibrahim NDG, Nok AJ, Esievo KAN.. 19

Fasulkov I, see Yotov S, Dimitrov D, Fasulkov I.. 143

Fazarinc G. Enzyme-immunohistochemical aspects of muscle fiber type classification in mammals.. 61

Gallelli MF, Miceli DD, Blatter MFC, Brañas MM, Castillo VA. The occurrence of corticotrophinoma in cross-breed and breed dogs. 115

Gancarčíková S, see Marcinčák S, Nemcová R, Sokol J, Popelka P, Gancarčíková S, Švedová M. 13

Gobec I, Ocepek M, Pogačnik M, Dobeic M. Inactivation of *Mycobacterium avium paratuberculosis* in sheep.. 105

Golinar-Oven I, see Ščuka L, Golinar-Oven I, Valenčak Z.. 29

Ibrahim NDG, see Adamu S, Useh NM, Ibrahim NDG, Nok AJ, Esievo KAN. 19

Jakovac-Strajn B, Pestevšek U, Knafelc T. Influence of gradual change in feed use ,of acidifier and prebiotic on rabbits in the period of weaning. 71

Knafelc T, see Jakovac-Strajn B, Pestevšek U, Knafelc T.. 71

Kobal S, see Plevnik A, Kobal S, Domanjko-Petrič A, Kotnik T. 5

Kotnik T, see Plevnik A, Kobal S, Domanjko-Petrič A, Kotnik T. 5

Krt B, see Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. 149

Lukanc B, see Tomsič K, Prošek M, Lukanc B, Seliškar A, Nemec SA.. 93

Majdič G. Is male brain different from female brain?. 85

Marcinčák S, Nemcová R, Sokol J, Popelka P, Gancarčíková S, Švedová M. Impact of feeding of flaxeed and probiotics on meat quality and lipid oxidation process in pork during storage. . . 13

Miceli DD, see Gallelli MF, Miceli DD, Blatter MFC, Brañas MM, Castillo VA. 115

Mitak M, see Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. 149

Nemcová R, see Marcinčák S, Nemcová R, Sokol J, Popelka P, Gancarčíková S, Švedová M. . 13

Nemec SA, see Tomsič K, Prošek M, Lukanc B, Seliškar A, Nemec SA.. 93

Nok AJ, see Adamu S, Useh NM, Ibrahim NDG, Nok AJ, Esievo KAN. 19

Ocepek M, see Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. 149
Ocepek M, see Gobec I, Ocepek M, Pogačnik M, Dobeic M. 105
Ornik D, Čadonič-Špelič V. Records on the use of animals in experiments in the Republic of Slovenia and in other EU member states within 15-years period. 47
Pate M, see Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. 149
Pestevšek U, see Jakovac-Strajn B, Pestevšek U, Knafelc T. 71
Plevnik A, Kobal S, Domanjko-Petrič A, Kotnik T. The efficacy of antihistamine fexofenadine versus methylprednisolone in the treatment of atopic dermatitis in dogs. 5
Pogačnik M, see Gobec I, Ocepek M, Pogačnik M, Dobeic M. 105
Popelka P, see Marcinčák S, Nemcová R, Sokol J, Popelka P, Gancarčíková S, Švedová M. . 13
Prevolnik M, see Škrlep M, Prevolnik M, Šegula B, Čandek-Potokar M. 133
Prošek M, see Tomsič K, Prošek M, Lukanc B, Seliškar A, Nemec SA. 93
Ščuka L, Golinar-Oven I, Valenčak Z. Porcine respiratory disease complex (PRDC) - A meta-analysis and systematic review of the efficacy of enrofloxacin. 29
Šegula B, see Škrlep M, Prevolnik M, Šegula B, Čandek-Potokar M. 133
Seliškar A, see Tomsič K, Prošek M, Lukanc B, Seliškar A, Nemec SA. 93
Škrlep M, Prevolnik M, Šegula B, Čandek-Potokar M. Association of plasma stress markers at slaughter with carcass or meat quality in pigs. 133
Sokol J, see Marcinčák S, Nemcová R, Sokol J, Popelka P, Gancarčíková S, Švedová M. 13
Špičić S, see Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. 149
Švedová M, see Marcinčák S, Nemcová R, Sokol J, Popelka P, Gancarčíková S, Švedová M. 13
Tomsič K, Prošek M, Lukanc B, Seliškar A, Nemec SA. 24-hour follow-up study of plas-ma coenzyme Q_{10}, total antioxidant capacity and selected blood parameters after a single oral dose of water-soluble coenzyme Q_{10} in healthy Beagle dogs. 93
Useh NM, see Adamu S, Useh NM, Ibrahim NDG, Nok AJ, Esievo KAN. 19
Valenčak Z, see Ščuka L, Golinar-Oven I, Valenčak Z. 29
Yotov S, Dimitrov D, Fasulkov I. Hydrometra in a sheep after oestrus synchronization and insemination in the anoestral season. 143
Zdelar-Tuk M, see Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. 149

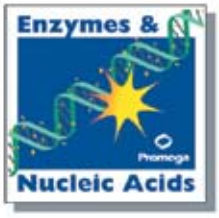
KEMOMED

PE: Stritarjeva 5, 4000 Kranj, Slovenija
tel.: (0)4/ 2015 050, fax: (0)4/ 2015 055
e-mail: info@kemomed.si
www.kemomed.si

Promega

SYNGENE

TPP



IZDELKI ZA MOLEKULARNO BIOLOGIJO

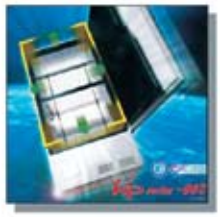
DOKUMENTACIJA IN ANALIZA GELOV

PLASTIKA ZA CELIČNE KULTURE

ELGA LABWATER

SANYO

Invitrogen life technologies



ČISTA VODA ZA LABORATORIJ

SKRINJE IN HLADILNIKI

CELIČNE KULTURE, GELI IN MOLEKULARNA BIOLOGIJA

BIOHIT

minerva biolabs

phenomenex

ELEKTRONSKE IN MEHANSKE AVTOMATSKE PIPETE

DIAGNOSTIKA MIKOPLAZEM IN LEGIONEL

HPLC in GC POTROŠNI MATERIAL

MD Svetovanje
Finančne storitve
www.vasefinance.si

Izterjava dolgov in upravljanje s terjatvami

Namen ustanovitve in delovanja podjetja MD svetovanje d.o.o. je pomagati podjetjem pri poslovanju z nudenjem produktov in storitev, ki ne spadajo v osnovno dejavnost podjetja. To dosegamo s celovito ponudbo predstavljenih produktov in storitev.

Zato smo naš moto Skupaj bomo uspešnejši! nadgradili še z motom in sloganom Vse za Vas na enem mestu!

Vizija

Postati vodilna neodvisna družba s celotno ponudbo za podjetja in posameznike na enem mestu in na ta način prihraniti podjetjem in posameznikom čas in denar.

Vse to nam bo uspelo s trdim delom in kakovostno izvedbo storitev in zaupanih nam nalog, predvsem če bomo sledili naslednjim načelom:

- zagotavljanje celovite ponudbe,
- vedno delo v dobro stranke,
- strokoven razvoj,
- organizacijsko izpopolnjevanje,
- zagotavljanje visoke stopnje kakovosti storitev z upoštevanjem predlogov naših strank,
- ustvarjanje novih delovnih mest,
- povečanje produktivnosti in dobičkonosnosti,
- visoko motiviran in usposobljen kader s primernim vodenjem, kar zagotavlja
- kakovost izvajanja storitev,
- postati vodilno podjetje, ki ponuja rešitve, ki podjetju omogočajo da si na enem
- mestu zagotovi vse dejavnosti, ki ne spadajo v njegovo osnovno dejavnost.

Prednosti poslovanja z nami:

- vse svoje potrebe in vizije uresničite s klicem na eno telefonsko številko,
- razbremenite se ukvarjanja z obrobnimi zadevami,
- posvetite se svojemu strokovnemu delu,
- informacijska tehnologija,
- prilagodljivost,
- zanesljivost,
- povečanje dobičkonosnosti,
- zmanjšanje stroškov dela,
- ...

MD svetovanje, poizvedbe in storitve d.o.o.
Dunajska cesta 421,
1231 Ljubljana – Črnuče

PE Ljubljana-Vič
Cesta dveh cesarjev 403,
1102 Ljubljana

01 / 620-47-01
01 / 620-47-04
041 / 614-090

www.mdsvetovanje.eu

mdinfo@mdsvetovanje.eu www.mdsvetovanje.eu

Zakaj MD Svetovanje d.o.o.

- visoka profesionalizacija,
- visoka strokovnost,
- visoka uspešnost,
- konkurenčne cene,
- vse na enem mestu.

MD MD MD MD

Do you need web site, web server and web application?You are in right place!

We offer you:

-web site consulting with unique, creative ideas;

-complex web application development with PHP and MySQL;

-web site design that is clean, professional and reflects who you are with 3 important elements :To make a first impression, Keep your visitor there, Help your visitor come back ;

-corporate identity:If you have a new business or would like to upgrade the look you have, our graphic designers can work with you to develop marketing identity that can take your business to the next level;

-ecommerce or online store that can be very successful on the web;

-content management with easy update tools which can be implemented on any new or restructured website. The staff logs in and has access to an interface that does not require HTML or XHTML knowledge. The web interface is as simple to use as typing in Word and attaching pictures as in any email program. Any page on the site can be selected, edited, and posted live to the site;

-application service provider;

roer informatika

Potrebujete spletno stran, spletne aplikacije ali spletni strežnik?

Pri nas vam nudimo:

- 'po meri' izdelani programi
- uporabniku prijazne aplikacije
- integracija v obstoječi sistem
- upravljanje s podatkovnimi bazami
- izdelava predstavitvene strani
- izdelava spletne trgovine
- zasnova in izdelava spletnega portala
- spletne aplikacije za delo s podatkovnimi bazami

Vas mučijo taki prikazi?

Mogoče pri konkurenci, pri nas jih poznamo samo iz zgodb :-)

Pri nas sodelujemo in ustvarjamo z naročnikom!

ROER informatika, Robert Resnik s.p., Puhova 3, PE Dunajska c. 421, 1000 Ljubljana , Slovenija
gsm: +386 41 427 494, e-mail: robert.resnik@roer.si, internet: www.roer.si

INSTRUCTIONS FOR AUTHORS

Slovenian Veterinary Research contains original articles which have not been published or considered for publication elsewhere. All statements in the articles are the responsibility of the authors. The editorial policy is to publish original research papers, review articles, case reports and abstracts of theses, as well as other items such as critical reviews of articles published in Slov Vet Res, shorter scientific contributions, letters to the editor, etc. Authors should send their contributions to the editorial board's address. All articles are subjected to both editorial review and review by an independent referees selected by the editorial board. The editorial board reserves the right to translate titles, summaries and keywords that have not been translated into Slovene by the authors.

Contributions should be written in English and should not exceed 12 pages (27 lines per page, approx. 75 characters per line). They should be submitted electronically (preferably to E-mail address, slovetres@vf.uni-lj.si), written in any word processor for Windows. Authors are requested to provide names of three potential reviewers. The text should be double spaced and the lines should be numbered on the left-hand side. The margin on the left-hand side of the page should be 4 cm.

The front page of a manuscript should start with the title, followed by the name and surname of the author(s). If there is more than one author, their names should be separated by commas. The next line ('Addresses of authors:') should contain the authors' full names and addresses (institution, street and number, postcode and place) after the colon. All the given data should be separated by commas. The name, address and E-mail and/or phone number of the corresponding author should be written in the next line.

The Summary of 200-300 words should follow on the next page.

Under 'Keywords:' (after the colon), keywords should be given. Individual words or word combinations should be separated by semicolons. Scientific papers and papers which present the author's research and findings should also include the following obligatory headings assigned by the author to appropriate parts of the text: Introduction, Materials and methods, Results, Discussion, and References. Review articles should consist of an introduction, sections logically titled according to the content, and references. Information on fund-providers and other matters important for the paper (e.g. technical assistance) should be supplied under 'Acknowledgements', which should be placed before the references. Figure legends should follow the references.

Tables, graphs and diagrams should be logically incorporated in the text file. Original photographs or drawings should be sent as separate files in bmp, jpg or tif format. They should be referred to by type and using Arabic numerals (e.g. Table 1:, Figure 1:, etc.). The colon should be followed by the text or title. All references cited in the text should appear in the References. They should be numbered in the text in the order in which they appear, marked with Arabic numerals placed in parenthesis. The first reference in the text should determine the number and order of the respective source in the References. If the author refers again to a source which has already been used in the text, he should cite the number the source had when it was referred to for the first time. Only works which have been published or are available to the public in any other way may be referred to. Unpublished data, unpublished lectures, personal communications and similar should be mentioned in the references or footnotes at the end of the page on which they appear. Sources in the References should be listed in the order in which they appear in the text. If the source referred to was written by six authors or less, all of them should be cited; in the case of seven or more authors, only the first three should be cited, followed by 'et al.'.

Any errata should be submitted to the editor-in-chief in good time after publication so that they may be published in the next issue.

Examples of references

Book: Hawkins JD. Gene structure and expression. Cambridge: University Press, 1991: 16.

Chapterorar ticle in a book: Baldessarini RJ. Dopamine receptors and clinical medicine. In: Neve KA, Neve RL, eds. The dopamine receptors. Totowa: Human Press, 1996: 475-98.

Article in a journal or newspaper: Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

Article in proceedings of a meeting or symposium: Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

NAVODILA AVTORJEM

Slovenski veterinarski zbornik (Slovenian Veterinary Research) objavlja izvirne prispevke, ki še niso bili objavljeni oz. poslani v objavo drugam. Za vse navedbe v prispevkih so odgovorni avtorji. Uredniška politika obsega publiciranje znanstvenih člankov, preglednih znanstvenih člankov, strokovnih člankov, povzetkov disertacij in drugih prispevkov, kot so kritične presoje o vsebini razprav, objavljenih v zborniku, kratke znanstvene prispevke, pisma uredniku in drugo. Avtorji pošljejo prispevke na naslov uredništva. Glavni urednik pregleda vse prispevke. Za vse članke je obvezna strokovna recenzija, za katero poskrbi uredništvo.

Prispevki naj bodo napisani v angleškem jeziku, z naslovom, povzetkom in ključnimi besedami tudi v slovenščini. Obsegajo naj največ 12 strani, kar pomeni 27 vrstic na stran s približno 75 znaki v vrstici. Prispevki naj bodo poslani v elektronski obliki v katerem koli urejevalniku besedil za okensko okolje. Zaželjena je uporaba elektronske pošte (slovetres@vf.uni-lj.si) in avtorji naj predlagajo tri možne recenzente. Besedilo naj ima dvojni razmik med vrsticami, pri čemer naj bodo vrstice na levi strani oštevilčene. Besedilo naj bo na levi strani od roba oddaljeno 4 cm.

Naslovna stran prispevkov se začne z naslovom, sledi ime in priimek avtorja. Kadar je avtorjev več, jih ločimo z vejicami. V naslednjih vrsticah je v rubriki Addresses of authors: za dvopičjem treba navesti polno ime in priimek ter naslov(e) avtorja(ev), tj. ustanovo, ulico s hišno številko, pošto in kraj. Vse navedene podatke ločujejo vejice. Sledi vrstica, kjer je treba navesti ime ter elektronski (E-mail:) in poštni naslov ter telefonsko številko (Phone:) odgovornega avtorja.

Sledi besedilo povzetka Summary v obsegu 200 do 300 besed. V naslednji rubriki Key words: se za dvopičjem navedejo ključne besede. Posamezne besede ali sklopi besed morajo biti ločeni s podpičjem.

Znanstveni članki in tisti, ki so prikaz lastnih raziskav in dognanj, morajo vsebovati še naslednje obvezne rubrike, s katerimi avtor sam naslovi ustrezne dele besedila v prispevku: Introduction, Material and methods, Results, Discussion in References. Pregledni članki naj vsebujejo uvod, poglavja, ki so glede na vsebino smiselno naslovljena, in literaturo. Podatke o financerjih ali drugih zadevah, pomembnih za prispevek, npr. o tehnični pomoči, avtorji navedejo v rubriki Acknowledgements, ki se uvrsti pred rubriko References. Za rubriko References sledijo spremna besedila k slikam.

Priloge, kot so tabele, grafikoni in diagrami naj bodo smiselno vključene v besedilo. Slikovni material naj bo poslan posebej v obliki bmp, jpg, ali tif.

Priloge in slike morajo biti poimenovane z besedami, ki jih opredeljujejo, in arabskimi številkami (npr. Table 1:, Figure 1: itn.). Za dvopičjem sledi besedilo oziroma naslov. Vsi navedki (reference), citirani v besedilu, se morajo nanašati na seznam literature. V besedilu jih je treba oštevilčiti po vrstnem redu, po katerem se pojavljajo, z arabskimi številkami v oklepaju. Prvi navedek v besedilu opredeli številko oziroma vrstni red ustreznega vira v seznamu literature. Če se avtor v besedilu ponovno sklicuje na že uporabljeni vir, navede tisto številko, ki jo je vir dobil pri prvem navedku. Citirana so lahko le dela, ki so tiskana ali kako drugače razmnožena in dostopna javnosti. Neobjavljeni podatki, neobjavljena predavanja, osebna sporočila in podobno naj bodo omenjeni v navedkih ali opombah na koncu tiste strani, kjer so navedeni. V seznamu literature so viri urejeni po vrstnem redu. Če je citirani vir napisalo šest ali manj avtorjev, je treba navesti vse; pri sedmih ali več avtorjih se navedejo prvi trije in doda et al.

Da bi se morebitni popravki lahko objavili v naslednji številki, jih morajo avtorji pravočasno sporočiti glavnemu uredniku.

Načini citiranja

Knjiga: Hawkins JD. Gene structure and expression. Cambridge: University Press, 1991: 16.

Poglavje ali prispevek v knjigi: Baldessarini RJ. Dopamine receptors and clinical medicine. In: Neve KA, Neve RL, eds. The dopamine receptors. Totowa: Human Press, 1996: 475-98.

Članek iz revije ali časopisa: Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

Članek iz zbornika referatov: Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

Slov Vet Res 2009; 46 (4)

Original Research Papers

Domanjko Petrič A, Cvetko S. Aortic stenosis in dogs: Clinical characteristics and survival in 80 cases. 125

Škrlep M, Prevolnik M, Šegula B, Čandek-Potokar M. Association of plasma stress markers at slaughter with carcass or meat quality in pigs 133

Case Reports

Yotov S, Dimitrov D, Fasulkov I. Hydrometra in a sheep after oestrus synchronization and insemination in the anoestral season. 143

Cvetnić Ž, Špičić S, Duvnjak S, Zdelar-Tuk M, Benić M, Mitak M, Pate M, Krt B, Ocepek M. High prevalence of *Mycobacterium avium* subsp. *hominissuis* in a batch of quarantined pigs in Croatia 149

Subject Index Volume 46, 2009. 155

Author Index Volume 46, 2009 157

Slov Vet Res 2009; 46 (4): 121-58