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EXPRESSION OF HEAT SHOCK PROTEINS HSP70 IN PIGS WITH CHRONIC AFFLICTION OF LUNGS AND THEIR DETECTION BY AN IMMUNOCYTOCHEMICAL METHOD*

VÝSKYT STRESOVÝCH PROTEINŮ HSP70 U PRASAT S CHRONICKÝM POSTIŽENÍM PLIC A JEJICH DETEKCE IMUNOCYTOCHEMICKOU METODOU

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ABSTRACT: The presence of heat shock proteins (HSP70) in pig pulmonary tissue was detected using an immunocytochemical method. The pigs underwent a respiratory disease leading to coalescenses of visceral and parietal pleurae and fibrous reparation in lungs in the past. During the pre-slaughter handling, acute processes as congestion, edema, and cracking of alveolar sides occurred in animals with chronic affections of lungs. These pathological changes were accompanied by stress response in single alveolar cells, especially granulated pneumocytes (type II cells). An increased expression of HSP70 was found in pulmonary macrophages as well, their number in alveolar lumen markedly increased. HSP70 positive response in vessels was abnormally increased in fibrous coalescenses, while other fibrous tissue was quite negative.

immunocytochemical reaction; HSP70; lung; pulmonary cells; pre-slaughter stress; fibrous coalescenses

ABSTRAKT: Imunocytochemicky byla detekována přítomnost HSP70 v plicní tkáni prasat. Tato prasata v minulosti prodělala respiratorní onemocnění, vedoucí ke srůstům poplicnice a pohrudnice a vazivové reparaci plic. V průběhu předporážkové manipulace došlo u zvířat s chronickým postižením plic k akutním změnám, jakými bylo překrvení, edém až popraskání stěn alveolů. Tyto patologické změny byly provázeny stresovou reakcí na úrovni jednotlivých alveolárních buněk, zvláště granulovaných pneumocytů (buňky II. typu). V plicních makrofázích došlo rovněž k nárůstu exprese HSP70, jejich počet v luminu alveolů zřetelně vzrostl. Pozitivní reakce cév na HSP70 byla abnormálně zvýšena ve vazivových srůstech, přitom ostatní vazivová tkáň byla zcela negativní.

imunocytochemická reakce; HSP70; plíce; plicní buňky; předporážkový stres; vazivové srůsty

INTRODUCTION

Mammalian cell defensive response to stress insults, which is induced by changes of the environment, is the synthesis of stress proteins named also „heat shock proteins" or HSPs. HSPs include several families of single proteins, where the numerical index identifies their molecular weight in kilodaltons (from 8 kDa to 110 kDa). Well-known and higly inducible is HSP70 that belongs among chaperones (Becker and Craig, 1994). The chaperones interact with nascent polypeptides already during their synthesis on ribosomes and participate in functional conformation of these proteins and their transport into organelles (Chaloupka, 1994).

HSP70 also represents a basic component of stress response on the cell level and participates significantly in reparative processes (Lindquist and Craig, 1988). The stress escalates the occurrence of denatured proteins in cell and increases the synthesis of this stress protein. Distinction and attachment of HSP70 to denatured proteins prevent their negative effect and accompany them until the hydrolysis by intracellular proteases. HSP70 is capable of interacting even with partially denatured proteins which have not lost their enzymatic activity (Souren et al.,1999). The wide protein spectrum allowing their gradual compensation during the recovery is thus protected (Welch, 1992; Chaloupka, 1994).

The rate of HSP70 production differs in single animal tissues. Beck et al. (1995) detected increased expression in pulmonary tissue and in skin already 1 hour after application of stress. HSP70 gene transcription decreases immediately after return to normal conditions and, more-

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over, the stability of mRNA HSP70 is reduced. It is probably a mechanism limiting cytotoxic effect of these proteins (Theodorakis et al., 1999). That is why the highest levels of HSP70 occur at acute processes and do not occur at chronic affections.

The growth of HSP70 was demonstrated in pathological lesions which characterize numerous disorders, for example, atherosclerosis, oxidative stress in various organs, ischaemic diseases, liverishness, viral and autoimmune diseases (Lu et al., 1996). HSP70 belongs to the best characterized proteins in lung biology (Wu et al., 1999). The role of stress proteins in damaged human lungs was defined in studies concerning stress proteins in lungs of patients suffering from asthma, cancer and acute pneumonia (Wong and Wispé, 1997).

The aim of this work was to find out whether HSP70 takes part in defensive and reparative mechanisms after acute stress caused by pre-slaughter handling in pigs with respiratory syndrome.

MATERIAL AND METHODS

The biological material was obtained on abattoir lines in a slaughterhouse at the veterinary control of abattoir material. Lung affections were found in 12 hogs, which were therefore confiscated. Macroscopic changes were visible on tissues as a consequence of pleuropneumonia. They were manifested e.g. by discoloration of some organ parts, structural changes, presence of concretions in pleural sac, hemorrhagic sections of lungs and macroscopic perceptible fibrous degeneration.

Samples of pulmonary tissues from 6 slaughtered pigs, which were recognized as healthy by veterinary control, served as a control material.

Samples in form of blocks (1 x 1 cm) were taken from control and confiscated lungs. They were processed by modifed histological technique for immunocytological detection of HSP70. After a fixation in 4% buffered formaldehyde (1.5 h), the samples were thoroughly washed in running water (1 h), dehydrated in graded alcohols, cleared with xylene and embedded in paraffin. Histological sections (about 5 μm thick) were cut and stuck on slides.

One set of histological sections from both experimental and control groups was treated with routine hematoxylin-eosin staining. Another set of histological sections was subjected to imunocytochemical reaction including the negative control. Monoclonal antibody to HSP70 (SPA 810, StressGen, Biotechnologies Corp. Canada) was used for the detection of stress proteins.

The method of Milarski et al. (1989), with small modifications, was used in our study. The sections were deprived of paraffin, hydrated through a downward alcohol series and transferred into a buffered solution of Triton X100. To suppress endogenous peroxidase activities incubation in 0.3% hydrogen peroxide in methanol was used for 30 minutes. Non-specific bindings were blocked using a non-immune horse serum. Then the specific anti-HSP70 antibody was applied to the sections (dilution 1 : 200) at 4 °C overnight. The control sections on slides were incubated without the specific antibody.

To prove bindings of anti-HSP70 with antigen Vectastain ABC kit (Vector Laboratories, Canada), i.e. a biotinylated secondary antibody and avidin binding with horseradish peroxidase were used. Diaminobenzidin tetrahydrochloride (DAB; Vector Laboratories Canada) was used for the visualization of peroxidase reaction. DAB formed a brown precipitates in the sections.

The sections prepared in this way were no more stained. They were examined and photographed with JenaMed (Charlotte Zeiss Jena) optical microscope.

RESULTS

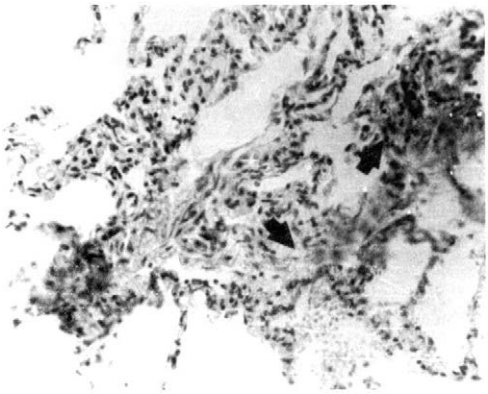
Histopatological changes in lung tissue after pleuropneumonia

Respiratory syndrome is the complex of aetiologically heterogeneous disorders, which are exhibited clinically as a sudden general setback with heavy pleuropneumonia. The permanent damage of pulmonary tissues lasted after recovery and it manifested itself as an expressive enhancement of collagen ligament in distal parts of respiratory apparatus – among pulmonary alveoli (Fig. 1). Visible concretions of visceral and parietal pleura arised after inflammation healing and exudate resorption in pleural sac. The damaged lungs had a limited ventilative ability, which probably led to evident aggravation of the chronic state at oppression and stress (pre-slaughter handling, pre-slaughter stress). This resulted in the exacerbation of inflammation, which was manifested by engorgement, edema, formation of inflammatory foci and hemorrhages (Fig. 2). An infiltration consisting of fluid and cellular elements cumulated in alveoli that stretched and cracked under the pressure. The interalveolar septa continuity was interrupted and this finally led to hemorrhage into alveoli and interstice (Fig. 3).

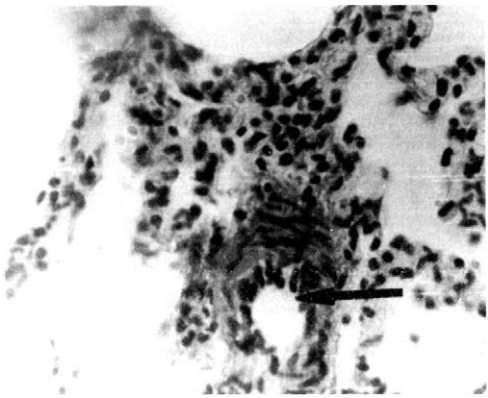
Histological examination of healthy animal pulmonary tissues showed no changes that could result from previous disorders or other kind of stress. The morphology of healthy pulmonary alveoli is shown in Fig. 4.

Results of immunocytochemical reaction to HSP70

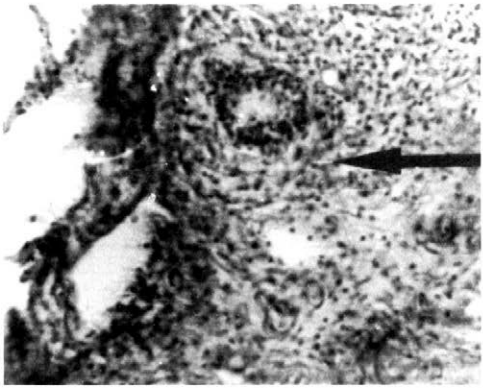
After the immunocytochemical reaction, a marked positive response was found in the cytoplasm of type II pulmonary cells (granulated pneumocytes), the large bodies of which protruded into the alveolar lumen on affected lung sections (Fig. 5). The type I pulmonary cells (membrane pneumocytes), which laid on the inner surface of alveolus like a very thin and continuous layer, also reflected an enhanced expression of HSP70. They looked like a dark continuous margin on the inner alveo-



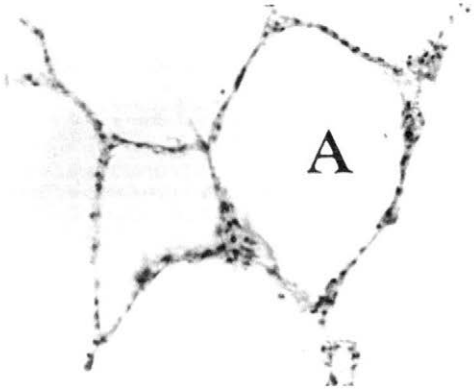
1. Respiratory syndrome, amplification of collagen ligaments (arrows); HE, 1 200×



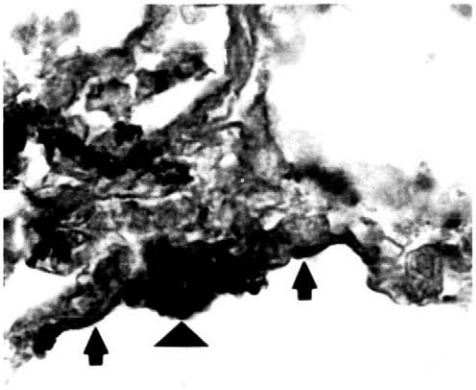
2. Respiratory syndrome, lymphocyte infiltration, cross-sectioned blood vessel – arrow; Weigert hematoxylin-eosin, 1 200×



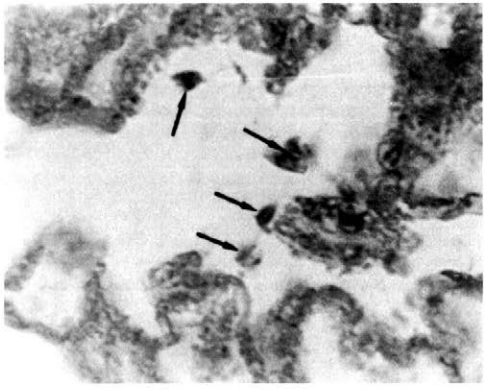
3. Respiratory syndrome, hemorrhage into the alveoli and interstice (arrow); Weigert hematoxylin-eosin, 1 200×



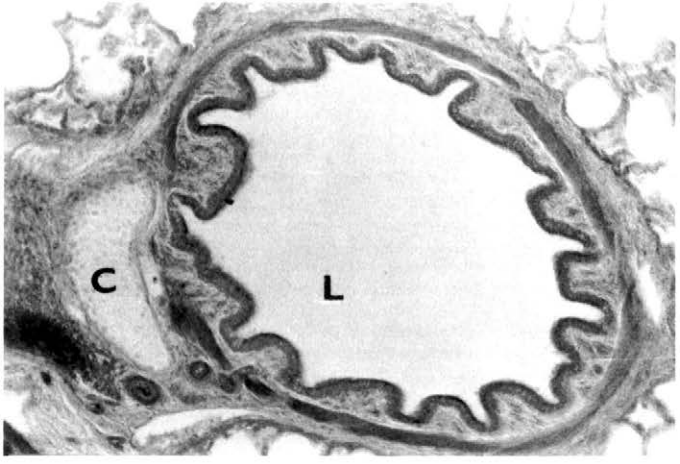
4. Control, healthy pulmonary alveoli (A); hematoxylin-eosin, 1 200×



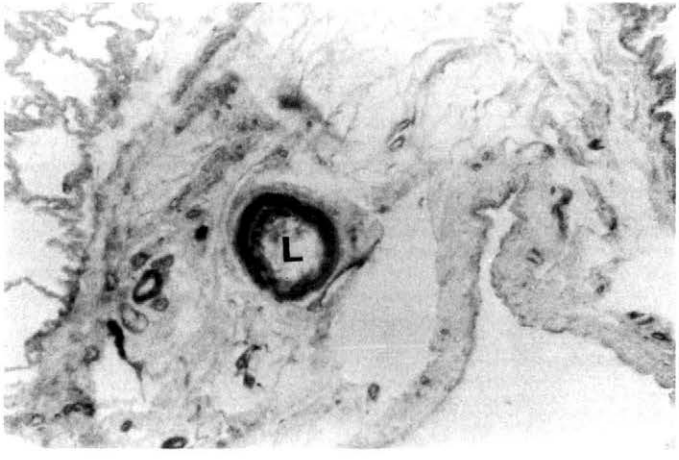
5. Respiratory syndrome, positive reaction in affected type II (arrow head) and type I (arrow) of pulmonary cels. Detection of HSP70; 1 200×



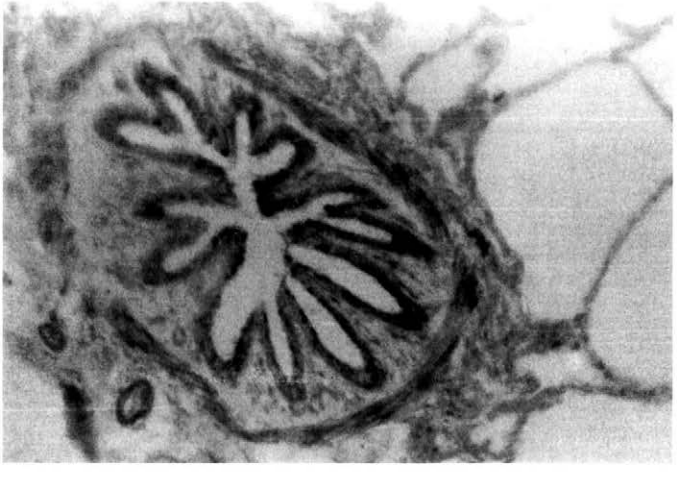
6. Respiratory syndrome, amplifited occurrence of macrophages (arrows) in alveoli. Detection of HSP70; 1 200×



7. Respiratory syndrome, transverse section through terminal bronchiole; L – bronchial lumen borded by ciliary epithelium, C – fragment of hyaline cartilage. Detection of HSP70; 1 200×



8. Respiratory syndrome, vessels in fibrous reparation; L – lumen of artery. Detection of HSP70; 1 200×



9. Control, healthy lung with respiratory bronchiole. Detection of HSP70; 1 200×

lus surface (Fig. 5). Alveolar macrophages were present individually or in clusters in alveolar lumen and their reaction was very positive too (Fig. 6). A distinct enhancement of these macrophages was observed in the lumen of alveoli.

Other structures, forming the pulmonary parenchyma, like bronchi, terminal and respiratory bronchioles, were markedly positive in some regions too. Fig. 7 clearly shows the bronchial epithelium, especially the ciliary margin was strongly positive in some places. The bronchi were clearly dilated according to decreasing and planishing of mucous plications. Blood vessels, especially small calibre arteries occurring inside pulmonary parenchyma, also positively responded to anti-HSP70 antibody, namely in the endothelium and media. Capillary endothelium was positive too, particularly in places of strong fibrous reparation (Fig. 8).

The reaction performed on lungs of control animals was much weaker in comparison with affected lungs. Only sporadic macrophages showed a high expression of HSP70. The sporadic reaction also occurred in the epithelium of bronchioles (Fig. 9). These were probably the secretory cells.

DISCUSSION

The respiratory syndrome affecting the respiratory tract of pigs still remains a relevant stress in Czech breedings and causes significant losses for the breeders. Although it occurs in lower-weight categories, its consequences appear with various intensities until slaughtering of animals.

The chronic affection of pig lungs was diagnosed on the basis of concretions in pleural sac and fibrous pulmonary reparation at slaughter and was also confirmed by the anamnesis. The stress preceding the slaughter (loading, transport, mixing of groups, etc.) led to a load of respiratory organs. The affected tissue could not respond adequately to this stress. This led to acute pathological changes manifested by a great tissue engorgement and edema, lymphocyte infiltration around bronchi, bronchioli and blood vessels and further by a gradual development of lesions in pulmonary tissue.

The occurrence of stress proteins in pulmonary tissue after stress was reported by many authors in various kinds of animals (both *in vivo* and *in vitro*). Cohen et al. (1991) described an increased expression of HSP70 in respiratory epithelium cells, pulmonary macrophages and endothelial cells of pulmonary arteries of guinea pig after application of sodium arsenite. The high expression of HSP70 at multifactorial disorders, as for example allergic asthma, results from the complex of interactions between environmental exposures and genetic background (Aron et al., 1999).

The present study demonstrates the expression of HSP70 in the lungs affected by a respiratory disorder. A strong positivity was found in both pulmonary cell types forming epithelium of alveoli and further in alveolar macrophages present freely in alveolar lumen.

Our results show that it was not only the chronic affection of lungs which led to the expression of stress proteins, but especially the sudden stress before the slaughter. In healthy animals the handling before slaughter did not mean such a serious stress that would lead to massive expression of HSP70. We suppose that the pre-slaughter stress can act cumulatively and can multiply the shock response (HSR) in animals with heavily damaged lungs. The relation of an acute infection to HSP70 expression is still not quite clear. For example, Chen et al. (1999) proved that HSP72 were not induced during an experimental sepsis.

The increased liability to stress is typical of pigs. At stress the temperature rises in tissues as well as in cells. The elevated temperature has an unfavourable effect on their metabolism. The cell transition to anaerobic metabolism can play a role in its survival at heat stress. Ischaemia and energy shortage are important stimuli of HSP70 accumulation in cells too (Kregel and Moseley, 1996). It is obvious that the afflicted lungs were exposed to a multiple stress which affected particulary the most sensitive cells.

The presence of positively responding single cells in samples from healthy animal lungs shows that HSP70 can be expressed even in these individuals. It can be due to a higher sensitivity of these cells, as implications of previous damage (of unknown origin), because the respiratory system is in a continual contact with the outer environment. It can also cohere with physiological phenomena, such as cell apoptosis. HSP70 not only markedly reduces the internucleosomal DNA fragmentation, but also enhances the cell viability (Ahn et al., 1999). Prevention of HSP induction results in sensitization and finally in apoptosis of cells. On the contrary, the sufficient expression of HSP70 has an antiapoptotic effect (Gorman et al.,1999).

When Su and Gordon (1997) used ozone to evoke stress, they proved the expression of HSP70 in alveolar macrophages although Wu et al. (1999) came to the opposite conclusion. In our material from lungs affected by respiratory disorder, the number of macrophages increased and the cells contained highly expressed HSP70.

It was found that HSP70 occur constitutively in both healthy and sick alveolar macrophages (Staton et al., 1995), but the expression is higher in the sick ones (Racine et al., 1999). In interstitial pneumonopathy cases of humans the levels of HSP70 were low, they were increased only in patients with leprosis and asbestosis. The expression of HSP70 is modulated by cytokinines, which activate monocytes. In addition, the alveolar macrophages are exposed to continuous stress caused by the environment or their own inflammatory products (Staton et al., 1995).

The pulmonary surfactant, the major physiological function of which is to confer mechanical stability to alveoli, also modulates the oxidative metabolism and

other pro-inflammatory functions of monocytes-macrophages, regulates the expression of HSP70 and protects the monocytes against damage, e.g. against vacuolization (Pinot et al., 1998). The changes in tissues, which are associated with the exacerbation of pleuropneumonia (edema up to seepage of fluid into alveoli), probably lead to spoiling of surfactant constitution and decreasing of its suppressive influence on expression of HSP70.

Leucocyte infiltration with icreased expression of HSP70 was registered in affected lungs. The induced transendothelial migration of polymorphonuclear leucocytes (PMN) resulted in an increased rate of apoptosis. PMN that migrate through the endothelium in response to a chemoattractant undergo activation as represented by increased phagocytosis and expression of adhesion receptors (Hennigan et al., 1999). The relation between apoptosis and stress reaction is known, so it is possible that similar processes proceed during the diapedesis of macrophages into lungs.

In our collected samples of confiscated lungs, a marked expression of HSP70 appeared in the IInd type of pulmonary cells. It can be explained by their high need of HSPs, because cells of intensive metabolism or secretory cells may be much more affected by the stress (Welch, 1992).

The increased expression of HSP70 is also induced by a sudden change of the environment. Brandes and Finkelstein (1989) succeeded in provoking the stress protein expression in both types of rabbit pulmonary cells only by their isolation and cultivation.

The positive reaction of endothelial cells and smooth-muscle cells of vessel media, which was manifested largely in the arterial segment of blood circulation in lungs, was probably caused by more factors. It has been proved that a high blood pressure increases the expression of stress proteins. Under such conditions, the arterial wall is stretched, which inflicts a dislocation of cytoskeleton and change of protein configuration. This is a signal for HSPs transcription (Xu and Wick, 1996). The incidence of toxins increases the synthesis of stress proteins in endothelial cells as well (Salminen et al., 1996, 1997), because the expression of HSP60 and HSP70 is a marker of induced endothelial cell activation at defence (Eberl et al., 1999).

Tanguay et al. (1993) arrived at the conclusion that HSP70 levels in livers, kidneys, stomach and colon may relate to exposure of these tissues to toxic metabolites or environmental substances. It is impossible to reduce their incidence by selection of conditions. Probably that is why the mild positivity occurs in the healthy animal endothelium. In addition, these animals were also exposed to a pre-slaughter stress. The maximum amount of HSP70 was in vessels of fibrous concretions. It may be connected with an extreme physical pain in these tissues at enhanced load of respiratory organs and a great alteration in blood pressure in them.

HSPs also play a major role in the pathophysiology of infection and inflammation. The induction of HSPs before onset of sepsis can reduce or prevent organ damage and death. Plasma glutamin is known to influence the expression of HSP70 in various cell types. In healthy donors, both granulocytes and lymphocytes showed a pronounced expression of HSP70, while a majority of polytraumatized patients with low glutamin levels showed no HSP70 expression in granulocytes. The expression of HSP70 in lymphocytes was similar to that of healthy volunteers (Weingartmann et al., 1999).

In our experiment, the tissue samples of animals with evident pulmonary damage exhibited a clear positive reaction in both types of alveolar cells and macrophages. We can conclude that the exacerbation of the chronic process by heavy physical stress can lead to an increased stress protein expression as a defensive response of the organism or of pulmonary tissue.

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EFFECT OF ANESTHESIA AND HYPOTHERMIA ON CHICKEN ERYTHROCYTE SUSCEPTIBILITY ON *IN VITRO* PEROXIDATION

VLIV ANESTEZIE A HYPOTERMIE NA CITLIVOST KUŘECÍCH ERYTROCYTŮ VŮČI PEROXIDACI *IN VITRO*

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ABSTRACT: Isoflurane is known as an inhalation anesthetic and hypothermia has the potential to cause oxidation stress in chicken erythrocytes. Anesthetized chickens were subjected to cooling by immersion in cold water (10–12 °C) from normal colonic temperature of 41 °C to 36 °C. Lipid peroxidation measured as the level of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) were determined in anesthetized chicken erythrocytes in hypothermia and posthypothermia before and after *in vitro* incubation with hydrogen peroxide. The level of TBARS before incubation increased in hypothermia ($P < 0.001$) although hypothermia without anesthesia might reduce free radical generation (Gradinski-Vrbanac et al., 1999). The concentration of GSH was not changed in hypothermia. The susceptibility of erythrocytes to *in vitro* peroxidation was increased in anesthetized ($P < 0.001$) and posthypotermic group ($P < 0.001$), but not in hypothermia. Erythrocytes from anesthetized and posthypothermic chickens seam to be the least resistant to peroxidative stress induced with hydrogen peroxide. These data show that increased lipid peroxidation in chicken erythrocytes is supposed to occur as a result of oxidative stress caused by anesthesia and hypothermia.

chickens; hypothermia; anesthesia; TBARS; GSH

ABSTRAKT: Isofluran je znám jako inhalační anestetikum a hypotermie může u kuřecích erytrocytů způsobit oxidační stres. Po anestezii byla kuřata vystavena ochlazení: byla ponořena do studené vody (10 až 12 °C) tak, že normální teplota v tračníku klesla z 41 °C na 36 °C. U anestetizovaných kuřecích erytrocytů jsme během hypotermie a posthypotermie stanovili před inkubací peroxidem vodíku *in vitro* a po ní peroxidaci lipidů zjišťovanou jako hladina látek reagujících na kyselinu thiobarbiturovou (TBARS), a dále redukci glutathionu (GSH). Hladina TBARS před inkubací se během hypotermie zvyšovala ($P < 0{,}001$), i když hypotermie bez anestezie by mohla tvorbu volných radikálů snižovat (Gradinski-Vrbanac et al., 1999). Koncentrace GSH se během hypotermie neměnila. Citlivost erytrocytů vůči peroxidaci *in vitro* se zvyšovala u anestetizované skupiny ($P < 0{,}001$) i u posthypotermické skupiny ($P < 0{,}001$), nikoliv však během hypotermie. Zdá se, že nejméně odolné vůči peroxidačnímu stresu navozenému peroxidem vodíku jsou erytrocyty anestetizovaných a posthypotermických kuřat. Výsledky naznačují, že ke zvýšené peroxidaci lipidů u kuřecích erytrocytů zřejmě dochází v důsledku oxidačního stresu způsobeného anestezií a hypotermií.

kuřata; hypotermie; anestezie; TBARS; GSH

INTRODUCTION

In physiological conditions, free radicals are continuously generated in erythrocytes (Giulivi et. al., 1994). However, these cells are furnished with very well developed antioxidant defence mechanisms (Edwards and Fuller, 1996). When the oxidant metabolism is disrupted, i.e. when the prooxidant-antioxidant system imbalance occurs, erythrocytes are exposed to a large quantity of reactive oxidant species. They attack unsaturated lipids in the membranes and cause the creation of a large quantity of lipid peroxides. These, in turn, are neutralised by means of glutathione peroxidase and glutathione.

It is well known that under hypothermic conditions metabolic processes slow down. Destructive processes, caused by the activity of free radicals, particularly those on biological membranes, are also slowed down in hypothermia. Such reduced generation of reactive oxidant species, which is manifested through a reduced TBARS level, was already observed in the plasma and hemolysate of chicken erythrocytes in hypothermia (Gradinski-Vrbanac et al., 1999). It is known that certain anesthetics affect the fluidity of the erythrocyte membrane (Yama-

guchi et al., 1998) with subsequent disruption of the phospholipid asymmetry (Roso et al., 1988), and at low levels these compounds even protect erythrocytes from the thermal lysis. (Jóźwiak and Watala, 1993). On the other hand, an intensified generation of free radicals may occur as a result of the inhalation of halothane (Monig and Asmus, 1984).

The objective of this experiment was to investigate the effects of anesthesia plus hypothermia on the GSH concentration and lipid peroxidation in chicken erythrocytes. In addition, we exposed the experimental erythrocytes to *in vitro* peroxidation in order to investigate the residual antioxidant status of these cells.

MATERIAL AND METHODS

The experiment was performed on 22 eight-week-old chickens of avian breed of both sexes weighing 2.5–3.8 kg. One week before and during the experiment the chickens were held in metal cages in a group of 7–8 birds at ambient temperature (20 °C) and 24-hours lighting. They had free access to feed (PPT-2-finisher for broilers, "POLJO-MIX" d.o.o. Bistra, Croatia) and drinking water.

When the experiment started chickens were divided into three groups: control (C, n = 8 birds), hypothermic (H, n = 7 birds) and posthypothermic (P, n = 7 birds). All groups were anesthetized by inhalation about 1–2 minutes with 3% isoflurane (FORENE: 1-chlor-2,2,2-trifluoroethyl-difluoromethylether; Deutsche Abbott GmbH, Wiesbaden) with an oxygen flow rate of 1.5 l/min.

Chickens in the experimental groups were immersed in cold water (10–12 °C) up to the neck until the colonic temperature fell from 41 °C to 36 °C. Hypothermia occurred after approximately 6–8 minutes. It takes much more time when cooling was performed without anesthetic (Alfaro and Palacios, 1993; Gradinski-Vrbanac et al., 1999). Chickens in posthypothermic groups were wiped by dry towels and reanimated by heating with electric heaters. After 3 hours their colonic temperature turned back to 39–40 °C and the reanimation was considered finished.

Blood samples were collected from the wing vein in heparinized tubes as follows: in controls immediately after anesthesia, in hypothermic group when colonic temperature fell to 36 °C and in posthypothermic group 24 hours after cooling. Red blood cells (RBC) were separated by centrifugation at 1 400 g for 10 minutes and washed three times with cold phosphate buffered saline (PBS; pH 7.4; osmolality 300 Osm/kg). Washed RBC's were then resuspended in PBS with a hematocrit value of 20%. The susceptibility of erythrocytes to *in vitro* peroxidation was examined by a modified method of Duthie et al. (1990). A cell suspension (2 ml) was added to 2 ml PBS containing 2mM sodium azide whereupon 0.44 ml of 0.05% hydrogen peroxide was added. Incubation was performed at 41 °C in a shaking water bath for 1 hour.

The TBARS and GSH levels in the cell suspension before and after the incubation were determined by applying the methods of Trotta et al. (1982) and Beutler et al. (1963) respectively. The molar extinction coefficient of 1.5×10^5 (Placer et al., 1966) was used to convert the absorbance reading (532 nm) into TBARS values. The hemoglobin level was determined spectrophotometrically using commercially available kits from Herbos d.d. (Sisak, Croatia, Cat. N° TR-1142).

The data are presented as mean ± S.E.M. Statistical significance was determined by Student's t-test. The difference was considered as significant when P was 0.05.

RESULTS

Table I shows that the TBARS level increased significantly in hypothermia ($P < 0.001$) and significantly decreased in posthypothermia ($P < 0.001$) in comparison with control group. The GSH level in chicken erythrocytes in the hypothermic group was higher by 16% than in control group, but it is not statistically significant. The lowest GSH level was recorded in the posthypothermic group and it is statistically significant in respect of the hypothermic group ($P < 0.001$).

I. Effect of hypothermia on anesthetized chicken erythrocyte TBARS and GSH levels

Groups	n	TBARS (nmol/g Hb)	GSH (μmol/g Hb)
Controls	8	39.18 ± 8.31	10.86 ± 2.10
Hypothermic	7	72.41 ± 11.36*	12.60 ± 2.75
Posthypothermic	7	13.78 ± 1.27*	9.39 ± 0.68

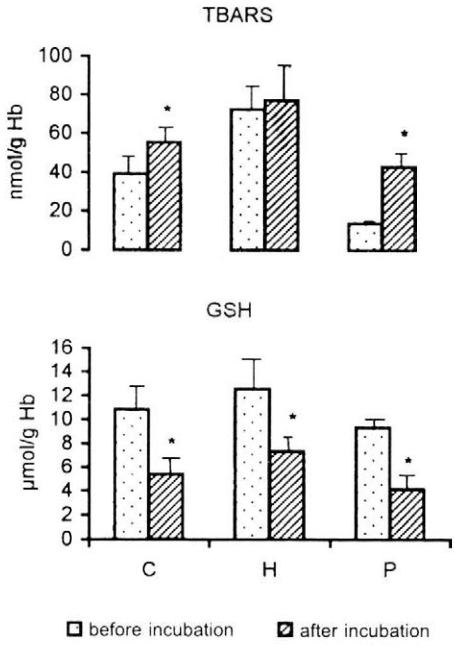
Significance of difference from control values: * $P < 0.001$

The erythrocyte susceptibility to *in vitro* peroxidation (Fig. 1) increased in control ($P < 0.001$) and posthypothermic group ($P < 0.001$), respectively. The erythrocytes GSH level after *in vitro* incubation significantly decreased in control ($P < 0.001$), hypothermic ($P < 0.001$) and in posthypothermic ($P < 0.001$) groups.

DISCUSSION

The mechanism of influence of certain anesthetics upon the erythrocyte membrane is not fully known. Yamaguchi et al. (1985) and Jóźwiak and Watala (1993) demonstrated that chlorpromazine at low levels first interacts with proteins of the erythrocyte membrane and then enters the lipid region. It is also known that an intensified generation of free radicals and consequently lipid peroxidation occur after the inhalation of halothane (Monig and Asmus, 1984; Akita et al., 1989).

The erythrocytes of fowls are nucleated and contain mitochondria. Their average lifespan (about 30 days) is much shorter than that of many larger mammals (Bell,



1. Effect of anesthesia and hypothermia on hydrogen peroxide-induced erythrocyte susceptibility to peroxidation (TBARS) and on erythrocyte GSH levels; significance of difference from the sample before incubation* $P < 0.001$

1971). The chickens have a more labile hematopoietic system than mammals and the regeneration in the chicken is very vigorous: it ends in a week, and in mammals about 3 weeks are necessary for the same result (Lucas and Jamroz, 1961). It is postulated that animals under relatively higher metabolic rates are also under a relatively higher level of oxidative stress because of elevated levels of reduced oxygen species.

In our experiment lipid peroxidation of erythrocytes in hypothermia significantly increased ($P < 0.001$) as shown in Tab. I. The results obtained in this experiment are contrary to those obtained in the previous experiment (Gradinski-Vrbanac et al., 1999). Precisely, the experimental protocols in both experiments were the same except that this time the anesthetic isoflurane was used before cooling. It is known (Nichelmann et al., 1986) that the lowering of the body temperature greatly affects the functioning of the organism and the heat generation capacity is seriously disturbed. In addition, in hypothermia the metabolic processes slow down with subsequent reduction of the creation of toxic metabolites (Prasad et al., 1992). During the anesthesia with isoflurane, oxygen was used as a carrier gas and increased oxygen consumption may result in increased generation of superoxide radicals, so that a significant increase of TBARS levels could be expected (Tab. I) besides of influence of hypothermia. We might say that significant lipid peroxidation occurred due to the isoflurane inhalation, which is in line with the data provided by Akita et al. (1989), who used halothane. We assume, on the basis of the literature (Slater, 1984), that the significant increase of the TBARS level in hypothermic group (Tab. I) was due to the fact that the peroxidative chain reactions, which had started during the anesthesia, continued in hypothermia even after the elimination of the initial oxygen trigger (anesthesia). We suppose that the kinetics of glutathione peroxidase was reduced in hypothermia and enables the accumulation of oxy-radicals, which resulted in a high lipid peroxidation.

The GSH levels did not change in hypothermia. This means that the interval of 6–8 minutes, i.e. the duration of cooling, was too short for GSH to oxidize, in spite of a very high lipid peroxidation.

In posthypothermia, the TBARS level decreased ($P < 0.001$) (Tab. I). We have assumed that these decreases appear within 24 hours, more exactly when the reanimation was finished. Further experiments are necessary to explore these possibilities. The lowest GSH value was recorded in posthypothermia (Tab. I). This decrease was statistically significant ($P < 0.001$) compared with the hypothermic group. One of the reasons for such a reduced GSH level might be its oxidation. Oxidized glutathione is readily transported through the erythrocyte membrane, but in the case of chicken erythrocyte, this transport is very slow (Srivastava and Beutler, 1969). In posthypothermia probably the -SH-rich -chicken hemoglobin readily interacts with oxidized glutathione and generates glutathionyl hemoglobin which is supposed to protect erythrocytes from the oxidant stress (Reishl and Dafré, 1992).

The susceptibility of erythrocytes to *in vitro* peroxidation was increased in control ($P < 0.001$) and posthypothermia ($P < 0.001$), but not in hypothermia (Fig. 1). This means that the erythrocyte resistance to oxidation in control and posthypothermia was reduced. In hypothermia an increase of TBARS levels after *in vitro* incubation was only 6.7%. Susceptibility of erythrocytes to *in vitro* peroxidation in posthypothermia was higher (211%). This means that the ability of the erythrocyte to resist oxidation is the biggest. We can assume that this was partly due to the increased vitamin E level (Duthie et al., 1990). If the vitamin E level in erythrocytes had been increased in posthypothermia, then the mobilization probably occurred from plasma, since it was determined that its level in plasma showed a continually growing trend after hatching (Mézes, 1997). Although our test did not include the vitamin E determination, its influence can not be neglected. Even more so because in the presence of hydroperoxide and free transition ions, vitamin E may act as a prooxidant (Yamamoto and Niki, 1988). GSH levels were decreased after *in vitro* peroxidation in all tested groups, but only in hypothermic group levels was 58% of those before incubation (Fig. 1).

The experimental results have shown that isoflurane anesthetic caused an increased lipid peroxidation in chicken erythrocytes in hypothermia, measured by TBARS

level. At the same time the GSH levels were not significantly changed in the course of experiment which indicates that the defense mechanism was rather stable. In posthypothermia the erythrocyte TBARS level turn back to the physiological level at 11.62 nmol/g Hb (Gradinski-Vrbanac – unpublished data).

The increased susceptibility to *in vitro* peroxidation, i.e. the reduced resistance of these cells to oxidation, was determined in the erythrocytes of control and posthypothermic chickens. No increased susceptibility was recorded in hypothermia. From this point of view one may speak about a protective role of hypothermia. The lipid peroxidation in chicken erythrocytes is supposed to result from the oxidant stress caused by anesthesia and hypothermia.

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RESISTANCE TO ANTIBIOTICS AND PRODUCTION OF β-LACTAMASE IN *PLESIOMONAS SHIGELLOIDES* STRAINS ISOLATED IN ANIMALS*

REZISTENCE K ANTIBIOTIKŮM A PRODUKCE β-LAKTAMÁZY U KMENŮ *PLESIOMONAS SHIGELLOIDES* IZOLOVANÝCH ZE ZVÍŘAT

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ABSTRACT: This research paper studies the resistance to antibiotics and the production of β-lactamase in *Plesiomonas shigelloides* strains isolated in animal population and environment in the Moravian region of the Czech Republic. At the National Veterinary Institute Olomouc in the period of 1994–1999, 6 370 samples of sectional, clinical material and environment have been examined for the presence of *Plesiomonas shigelloides*, out of which 75 strains have been isolated (1.2%). The isolated strains have been tested for β-lactamase production and sensitivity to 12 selected antibiotics. The production of β-lactamase was detected in 96% of the tested strains. 100% of the strains are resistant to penicillin and streptomycin. All strains were susceptible to aminopenicillins combined with β-lactamase inhibitors (ampicillin/sulbactam, amoxicillin/clavulanic acid), chloramphenicol, tetracycline, co-trimoxazole, ciprofloxacin, cefoperazone and norfloxacin.

production of β-lactamase; resistance to antibiotics; *Plesiomonas shigelloides*

ABSTRAKT: *Plesiomonas shigelloides* patří k bakteriálním druhům jejichž význam v patogenním uplatnění u lidí i zvířat není jednoznačně dořešen. Literární citace, věnující se problematice patogenity, rezistenci k antibiotikům a produkci β-laktamázy u infekcí zvířat vyvolaných *P. shigelloides* jsou řídké (Vladík and Vítovec, 1972, 1974; Bardoň, 1994,1999; Foster et al., 2000; Jagger et al., 2000; Avison et al., 2000). V letech 1994 až 1999 bylo na Státním veterinárním ústavu v Olomouci vyšetřeno 6 370 vzorků sekčního a klinického materiálu a vzorků prostředí, které pocházely z oblasti Moravy. Z tohoto souboru bylo izolováno 75 kmenů *P. shigelloides* (1,2 %). Testované kmeny byly rezistentní k penicilinu, streptomycinu a erytromycinu. Citlivost kmenů *P. shigelloides* byla prokázána na chloramfenikol, tetracyklin, aminopeniciliny s inhibitory β-laktamáz (ampicilin/sulbactam, amoxicilin/kyselina klavulanová), kotrimoxazol, ciprofloxacin, cefoperazon a norfloxacin. Acidometrickou metodou „betaLAKTAMtest" byla prokázána produkce β-laktamázy u 95 % testovaných kmenů. Použitím nitrocefinu byla produkce tohoto enzymu prokázána u 97 % testovaných kmenů. Výsledky naší práce, hodnotící rezistenci *P. shigelloides* k antibiotikům u kmenů izolovaných ze zvířat, korespondují s literárními výsledky, které vycházejí z větší části ze kmenů humánního klinického materiálu. Výjimku tvoří gentamicin, u kterého jsme prokázali 44% (disková difúzní metoda), respektive 39% (diluční mikrometoda), na rozdíl od jiných autorů, kteří popisují dobrou citlivost na gentamicin (Holmberg et al., 1986; Terpeluk et al., 1992; Hossain and Hossain, 1994; Rautelin et al., 1995; Marshall et al., 1996).

produkce β-laktamázy; rezistence k antibiotikům; *Plesiomonas shigelloides*

INTRODUCTION

Plesiomonas shigelloides as causitive agents in gastroenteritis in veterinary and human medicine is still inconclusive. Citations on the importance of *P. shigelloides* in the pathogenesis of infectious diseases in animals are relatively rare (Vladík and Vítovec, 1972;1974; Bardoň, 1994; 1999; Jagger et al., 2000; Foster et al., 2000). A detailed study of the antibiotic resistance of this species, including the β-lactamase production, or therapeutic

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practice in case of infections of animals with *P. shigelloides*, are sporadic. Avison et al. (2000) have examined the production of β-lactamases by 11 clinical and 9 environmental isolates of *P. shigelloides* from Czechoslovakia, the Czech Republic and Cuba.

In *P. shigelloides* strains isolated in a sepsis case from a lemur (*Varecia variegata*), sensitivity to cefoperazone, gentamicin, chloramphenicol, oxytetracycline, furadantin, and resistance to penicillin, bacitracin and polymyxin were determined (Bardoň, 1994). Other studies evaluating their sensitivity to antibiotics (ATB_S), including resistance to streptomycin mediated by a plasmid, isolated from blue crab (*Callinectes sapidus*), showed that these strains were susceptible to gentamicin, nalidixic acid, tetracycline and resistant to ampicillin, carbenicillin, kanamycin and streptomycin (Marshall et al., 1996).

In human medicine there exist a range of cases describing gastrointestinal infections, as well as systemic infections in humans (Korner et al., 1992; Gupta, 1995; Rautelin et al., 1995; Alcaniz et al., 1995; Delforge et al., 1995; Lee et al., 1996; Riley et al., 1996; Jonsson et al., 1997; Ahmad et al., 1998; Bravo et al., 1999). Opinions about the choice of antibiotic therapy are ambiguous. Hossain and Hossain (1994) evaluated 13 strains of *P. shigelloides* isolated from human clinical material in which all strains being susceptible to tetracycline, kanamycin, gentamicin, erythromycin a ciprofloxacin. In another study of *P. shigelloides* isolated from human ordure were susceptible to ciprofloxacin, doxycycline, trimethoprim/sulfamethoxazole (co-trimoxazole), gentamicin, cephalexin, cefuroxime, ceftriaxone and cefixime (Rautelin et al.,1995). An interesting case is a study of Thai children suffering from diarrhea in which the study presents 36 cases of Thai children suffering from diarrhea and finds that 19 children have been treated with ATB_S (norfloxacin, wintomylon, colistin, gentamicin, ceftriaxone, co-trimoxazole, ampicillin) and 17 children were not treated with antibiotics. In both groups the clinical course of the disease was monitored and the authors conclude that the antibiotic treatment had no influence on the length of the fever and/or diarrhea. 100% of the *P. shigelloides* strains in this study were susceptible to chinolons and cephalosporins and only 9% of the strains were susceptible to ampicillin (Visitsunthorn and Komolpis, 1995). As reported by Kain and Kelly (1989) the antimicrobial therapy considerably shortened the duration of the disease in patients with diarrhea caused by *P. shigelloides*. Holmberg et al. (1986) state that a subpopulation of people suffering from diarrhea caused by *P. shigelloides* improved their condition rapidly after treatment with trimethoprim/sulfamethoxazole or tetracycline. In the same study the authors describe a clinical manifestation of diarrhea caused by *P. shigelloides* after treatment with ampicillin prescribed for other reasons then the above mentioned disease. The *P. shigelloides* strains isolated afterwards from their stools were resistant to ampicillin. The study examined 30 isolates of *P. shigelloides* and all were susceptible to gentamicin, chloramphenicol, cephalothin, trimethoprim/sulfamethoxazole and trimethoprim. 27 of the strains were resistant to ampicillin and 28 of the strains to carbenicillin. One strain was resistant to sulfadiazine and two strains to tetracycline (Holmberg et al., 1986). Evaluation of the resistance to ATB_S in *P. shigelloides* strains isolated from human clinical material in association with antibiotic therapy is also presented by other authors (Olsvik et al., 1990; Kelly et al., 1991; Terpeluk et al., 1992; Jonsson et al., 1997; Bravo et al., 1998a, b). De Mondino et al. (1985) describe antibiotic susceptibility in 46 strains of *P. shigelloides* isolated from water in Rio de Janeiro. The isolated strains were susceptible to cephalosporins, penicillins with β-lactamase inhibitors, aminoglycosides, imipenem, norfloxacin, tetracycline, chloramphenicol and trimethoprim/sulfamethoxazole. All isolated samples were penicillin resistant (de Mondino et al., 1995).

The ability of the bacterial strains to produce β-lactamase correnates with some of their resistance patterns to ATB_S. The effort to find detailed literary reference of the β-lactamase production in strains with clinical significance in animals was not successful. The production of this enzyme in strain of *P. shigelloides* which caused a fatal sepsis with meningoencephalitis in a child is supposed by Terpeluk et al. (1992). Bravo et al. (1998b) proved the production of β-lactamase in 29 strains of *P. shigelloides* isolated from children suffering from diarrhea. Avison et al. (2000) found evidence of expression of a single β-lactamase in only 10 of the 20 isolates (50%).

Considering the fact that *P. shigelloides* is capable of causing clinically manifested infections in humans and animals, further studies on antibiotic resistance is advisable.

MATERIAL AND METHODS

6 370 samples from clinical, sectional and environment sources were inspected in the period of 1994–1999 at the National Veterinary Institute in Olomouc. The samples come from the area of Moravia. From this set of samples, 75 *P. shigelloides* were isolated. Number of examinations per type of material are shown in Tab. I.. Strains were examined for their resistance patterns to selected antibiotics as well as production of β-lactamases. The resistance to antimicrobials were tested at the National Veterinary Institute in Olomouc (NVI) by standard disc diffusion (75 strains) according to the methodology of the National Referential Laboratory for Antibiotics (Urbášková, 1998) and at the Department of Microbiology of the Medical Faculty in Olomouc (MF) by the standard dilution micromethod (74 strains). For the examination by disc diffusion a standard Mueller Hinton agar (Hi Media) was used with various antibiotic discs (Oxoid). The examination by standard dilution micromethod (MIC) was carried out in correspondence with the guidance of NCCLS (National Committee for Clinical Laboratory Standards, 1995). Breakpoints (criterion of susceptibility/resistance) for the antibiotics examined

I. Number of examinations per type of material and number of positive findings

Year	Material			Total	Number of positive findings	Percentage of positive findings
	sectional	clinical	environment			
1994	898	538	12	1 448	13	0.9
1995	724	521	10	1 255	19	1.51
1996	508	613	18	1 139	11	0.97
1997	376	320	14	710	12	1.69
1998	452	385	8	845	7	0.83
1999	523	438	12	973	13	1.34
Total	3 481	2 815	74	6 370	75	1.18
Number of positive findings	45	28	2	75		
Percentage of positive findings	1.30	0.99	x	1.18		

x – percentage is not stated

were: 0.125 mg/l for penicillin, 0.5 mg/l for erythromycin, 1 mg/l for ciprofloxacin, 2 mg/l for tetracycline, 4 mg/l for chloramphenicol, gentamicin, streptomycin, 8 mg/l for ampicillin/sulbactam and 32 mg/l for co-trimoxazole.

Tab. II shows the list of examined antibiotics. For examination by the disc diffusion methods, antibiotic groups currently employed in veterinary medicine were used. For the dilution micromethod, antibiotics used in human clinical medicine were used.

II. Resistance to ATB_S in *Plesiomonas shigelloides* strains by means of disc diffusion method and dilution micromethod

Tested antibiotic	Determination of resistance of tested strains of *P. shigelloides* (%)	
	disc diffusion method	dilution micromethod
Penicillin	100	100
Ampicillin/sulbactam	n	0
Amoxicillin/clavulanic acid	0	n
Chloramphenicol	0	0
Tetracycline	0	0
Co-trimoxazole	n	0
Erythromycin	97	100
Ciprophloxacin	n	0
Gentamicin	44	39
Streptomycin	100	n
Cefoperazone	0	n
Norfloxacin	0	n
Number of tested strains	75	74

n – not tested

β-Lactamase production was detected in 75 isolated strains of *P. shigelloides* by a commercial acidometer set (betaLACTAMtest, Lachema, Czech Republic). β-Lactamase production in 74 strains was also detected by hydrolysis of the chromogenic cephalosporin, NITROCEFIN (Oxoid).

RESULTS

During the monitored period 75 *P. shigelloides* strains (1.2%) out of 6 370 tested samples were isolated. Incidence per type of material is shown in Tab. I. Tab. II evaluates the results of *P. shigelloides* resistance to selected ATB_S obtained by disc diffuse method and dilution micromethod. The table shows 100% resistance to penicillin by *P. shigelloides* by both methods. One method reveals 100% resistance to streptomycin (disc diffusion method) and erythromycin (dilution micromethod). Both methods confirmed the susceptibility of all strains to chloramphenicol and tetracycline. One of the methods showed all strains were susceptible to ampicillin/sulbactam, amoxycillin/clavulanic acid, co-trimoxazole, ciprofloxacin, cefoperazone and norfloxacin. Comparison of both methods shows minor differences in erythromycin (3%) and gentamicin (5%) (Tab. II).

Detection of β-lactamase production by *P. shigelloides* strains is shown in Tab. III. By application of the betaLAKTAMtest, the production of β-lactamase has been shown in 95% of the 75 examined strains (4 strains did not produced β-lactamase). Using nitrocefin, the β-lactamase production was shown to be 97% (2 strains were negative). Both strains of *P. shigelloides* in which the β-lactamase production was negative by nitrocefin hydrolysis are the same strains which were negative by the betaLAKTAMtest. In spite of the negative result of β-lactamase production, the strains are resistant to penicillin. Correnating the pattern of resistance in particular strains from various origins, no connection could not be proved.

III. β-lactamase production in *Plesiomonas shigelloides* strains

Method applied	Number of tested strains	Percentage of strains producing β-lactamase
BetaLACTAMtest	75	95
Nitrocefin	74	97

DISCUSSION

Extensive information about *P. shigelloides* resistance to ATB_S, information about the β-lactamase production and an in-depth analysis of veterinary therapeutic practices with regards to *P. shigelloides* are rare in scientific literature. The probable reason for this is that *P. shigelloides* is not commonly diagnosed or directly associated with infectious diseases in animals.

The results of *P. shigelloides* resistance to ATB_S correspond in most cases with previously reported data. An exception is the susceptibility to gentamicin. In *P. shigelloides* strains isolated from a case of sepsis of half-ape lemur (*Varecia variegata*), susceptibility to cefoperazone, gentamicin, chloramphenicol, oxytetracycline, furadantin, and resistance to penicillin, bacitracin and polymyxin by disc diffuse method has been detected (Bardoň, 1994). These results correspond with the results of our research except for gentamicin, where resistance, 44% and 39%, by disc diffusion and dilution micromethod, respectively, has been shown. Animal strains of *P. shigelloides* isolated from blue crab (*Callinectes sapidus*), were susceptible to gentamicin, nalidixic acid, tetracycline and resistant to ampicillin, carbenicillin, kanamycin and streptomycin (Marshall et al., 1996). Again, our results correspond with this study except results for gentamicin. Kelly and Kain (1991) show most strains are resistant to ampicillin and other penicillins as well as to tetracycline, erythromycin and aminoglycosides. In our study, all the strains were resistant to penicillin, contrary to 100% susceptibility detected for tetracycline. 100% of strains were resistant to streptomycin, and 44% and 39% to gentamicin according to the results obtained by disc diffusion and dilution micromethod, respectively. Good *in vitro* susceptibility to tetracyclines (tetracycline, doxycycline) proved Olsvik et al. (1990) in 11 tested strains of *P. shigelloides* isolated from Peruvian children suffering from diarrheal disease. Also results of the tests with *P. shigelloides* obtained from human clinical material reported by other authors (Hossain and Hossain, 1994; Rautelin et al., 1995) correspond with the outcome of our study except for gentamicin. Also Holmberg et al. (1986) indicate in all 30 tested strains the susceptibility to gentamicin. A strain isolated from a case of child's meningoencephalitis, susceptible to gentamicin is described in a study by Terpeluk et al. (1992), the resistance to ampicillin was ascribed to the presumptive production of β-lactamase (Terpeluk et al., 1992). The production of β-lactamase has also been determined in all 29 tested strains of *P. shigelloides* isolated from clinical material of children (Bravo et al., 1998b). In our study, production of β-lactamase was demonstrated in 95% and 97% of strains by betaLACTAMtest and Nitrocefin, respectively.

Selected ATB_S for the disc diffuse method in this study are primarily representatives of the groups of ATBs used by veterinary clinicians. Both methods employed gave practically identical results and the differences did not exceeded 5%, which is an acceptable error. A similar situation occurred in the case when testing for the production of β-lactamase. Despite different method of detection of the β-lactamase activity the results of betaLAKTAMtest are comparable to examining nitrocefin hydrolysis.

Practical therapeutic experience with the application of ATB_S in infections with *P. shigelloides* is not recommended. The rule remains that in each strain isolated from clinical material, the resistance to ATB_S should be verified, because of the possibility of acquired resistance mediated by plasmids (transpozons, integrons) (Kelly and Kain, 1991), which has been described e. g. in case of streptomycin by Marshall et al. (1996).

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PLESIOMONAS SHIGELLOIDES SEROVARS IN ANIMALS

SÉROVARY *PLESIOMONAS SHIGELLOIDES* U ZVÍŘAT

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ABSTRACT: Thirty-one O serogroups of the international antigenic scheme and ten O serogroups of the provisional "Schubert" antigenic scheme were found in *Plesiomonas shigelloides* strains of animal origin. Serovar O18:H2 and serovar O22:H3 are probably exclusively feline serovars. Fish, water birds and reptiles carry "Schubert" serovars (i.e. serovars originating from water and water insects).

Plesiomonas shigelloides; serotyping; animals

ABSTRAKT: U kmenů *Plesiomonas shigelloides* zvířecího původu bylo objeveno 31 O séroskupin mezinárodního antigenního schematu a 10 O séroskupin provizorního antigenního schematu, zatím označeného „Schubert", jehož referenční kmeny pocházejí výhradně z vody a z vodního hmyzu. Sérovar O18:H2 a sérovar O22:H3 jsou pravděpodobně typickými sérovary kočkovitých. Naproti tomu sérovary „Schubert" byly nalézány u ryb, vodních ptáků a plazů.

Plesiomonas shigelloides; sérotypizace; zvířata

INTRODUCTION

The first strain of *Plesiomonas shigelloides* under the name "*Paracolon C27*" was isolated in Michigan from human stool (Ferguson and Henderson, 1947). But already in 1954, *Paracolon* strains were isolated from sheep, goat, polecat and cow hosts in Ceylon (Schmidt et al., 1954) and Bader published its *Pseudomonas shigelloides* from the intestinal content of a dog with enteritis (Bader, 1954). Bader´s strain became the type strain for *Plesiomonas shigelloides*. All these animal strains, as well as the first human strain, displayed an O antigen later labelled as "*O17*", identical with *Shigella sonnei* phase I. Very soon attempts at serotyping further *P. shigelloides* isolates were made (Sakazaki et al., 1959; Ewing et al., 1961; Quincke, 1967). As *P. shigelloides* strains display almost the same biochemical characters the only tool for distinguishing them has been, so far, serotyping. At present, two antigenic schemes are known, the international one with 102 O and 52 H antigens (Shimada and Sakazaki, 1978, 1985; Aldová, 1992, 1997; Shimada et al., 1994; Aldová et al.,1992, 1994) and a provisional one, called "Schubert" with 32 O and 7 H antigens, comprising environmental reference strains (Aldová and Schubert, 1996).

During our study, since 1964, we have collected strains from man, various animals, and water. This paper presents our animal isolates.

MATERIAL AND METHODS

Strains were isolated by the authors or offered to the laboratory of the senior author. All strains presented in this paper are harboured at the Czech National Collection of Type Cultures, National Institute of Public Health, Prague. For isolation routine methods or the isolation technique for *Vibrio cholerae* were used.

Methods of serotyping were described in previous papers mentioned in the introduction.

RESULTS AND DISCUSSION

Our animal strains belong to 31 O serogroups (Tab. I). Only some of them carry more than one H antigen, namely O2:H1a1c and O2:H2, O3:H2 and O3:H3, O38:H3 and O38:HUK (unknown H antigen), O60:H5 and O60:H22, O66:H1a1b and O66:H30, O79:H22, O79:H43 and O79:H3. The other ones occur as a single combination of one O and H antigen.

Various animal species were examined: domestic animals, pet dogs and cats (sometimes feral cats), as well as animals from zoological gardens (mammals, birds, eventually reptiles) and surface water and aquarium fish. Pets and animals from zoological gardens were mostly examined for illness or deaths and so the table contains strains isolated from rectal swabs as well as from internal organs.

1. *P. shigelloides* serovars of the international antigenic scheme

O	H	Animal, locality, year of isolation	Total	
			OH	O
2	1a1c	dog, Bratislava, 1970		
		gull *Larus ridibundus*, Blatná, 1973		
		fish *Abramis brama*, Prague, 1997		
		fish *Carassius carassius*, Prague, 1997	4	
	2	cat, dog, Prague, 1970	2	6
3	2	cat, Prague, 1970, 1995	2	
	3	cat, Prague, 1990	1	3
5	4	sewer rat *Rattus norvegicus*, Mělník, 1977	1	1
15	17	dog, Prague, 1969	1	1
17	2	dog, Prague, 1969	1	
		pig, Prague, 1978	2	3
18	2	cat, Prague, 1969, 1970, 1988, 1989, 1990, 1994	9	
		cat, Zeleneč, Prague-East, 1967	1	
		cat, České Budějovice, 1986, 1987	3	
		cat, Liberec, 1991	2	
		lynx caracal, Liberec, 1990	1	
19	2	penguin, Prague, 1999	1	1
20	2	duck, Prague, 1981	1	1
22	3	cat, Prague, 1994	1	1
26	1a1c	dog, Prague, 1990	1	1
30	1a1c	cat, Prague, 1969	1	1
31	Schubert H4	fish *Abramis brama*, Prague, 1997	1	1
32	4	pig, Prague, 1978	1	
	NM	dog, Prague, 1969	1	2
33	3	cat, Prague, 1969	1	1
35	1a1b	aquarium fish *Symphysodon discus*, Opava, 1998	1	1
38	3	cat, Radotín, Prague-West, 1968	1	
	HUK	pike, Leon (Spain), 1995	1	2
41	1a1b	cat, Beroun, 1969	1	
		cat, Prague, 1994, 1996	3	4
60	22	duck, Prague, 1969	1	
	5	fish *Abramis brama*, Prague, 1996	1	2
61	2	seal *Otaria byronia*, Liberec, 1991	1	1
63	22	chough *Pyrrhocorax pyrrhocorax*, Prague, 1998	1	1
66	1a1b	cat, Prague, 1994	1	
	30	grey heron *Ardea cinerea*, Hannover, 1991	1	2
68	2	seal *Eumetopias* sp., Liberec, 1998	1	1
74	22	ibis, Chomutov, 1998	1	1
75	34	seal *Otaria byronia*, Ústí nad Labem, 1998	1	
		little bittern *Nycticorax nycticorax*, Chomutov, 1999	1	2
76	39	pig, Prague, 1978	1	1
78	2	antelope *Tragelaphus spekei*, Prague, 1989	1	
		starling *Creatophora cinerea*, Prague, 1989	1	2
79	22	turtle *Mauremys caspica*, Prague, 1996	2	
	43	cat, Prague, 1994	1	
	3	cat, Prague, 1996	1	4
81	22	seal *Eumethopias* sp., Liberec, 1991	1	
		penguin, Prague, 1997	1	2
84	48	fish *Abramis brama*, Prague, 1997	1	1
88	2	gull *Larus ridibundus*, Blatná, 1973	1	
		fish *Abramis brama*, Prague, 1996	1	2
89	2	fish *Abramis brama*, Prague, 1996	1	1

NM = non-motile strain
HUK = H antigen unknown

II. Occurrence of *P. shigelloides* serovars from Tab. I in other materials

Animal strains	Strains from other sources (authors, collection)	Countries
O2:H1a1c O2:H2	man, trout (Dr. Bardoň, Olomouc)	CZ, SK, C, CDN, J(RS)
O3:H2	man, surface water, fish (Olomouc)	CZ, SK, S, US, J (RS)
O5:H4	man, sewage	CZ, SK, S, CDN, UK, J (RS)
O15:H17	man	CZ, J (RS)
O17:H2	man, sewage	CZ, SK, S, D, NL, HR, US, CDN
018:H2	two strains water D	CZ, J(RS-source unknown)
O19:H2	man, water, sewage	CZ, SK, HR, C, J (RS)
O20:H2	man	CZ, US
O22:H3	0	J (RS-source unknown)
O26:H1a1c	man	CZ, C, J (RS)
O30:H1a1c	0	J (RS-source unknown)
O31:Schubert H4	O31:H3 man	CDN, J (RS)
O32:H4 O32:NM	man, sewage	CZ, SK, S, C (RS-O32:Hl4)
O33:H3	man	C (RS)
035:H1a1b	man	C
O38:H3 38:HUK	man	IRQ, CZ, SK, C
O41:Hlalb	man, fowl (Olomouc)	CZ, IRQ
O60:H5 O60:H22	other H antigens : water fish (Olomouc)	O60:H21 THA(RS bird)
O61:H2	man	CZ, C, J (RS)
O63:H22	man	CDN, THA (RS cat)
O66:Hlalb O66:H30	man	NL
O68:H2	man	CZ
O74:H22	other H antigens : man	CZ, US
O75:H34	man	CZ, SK, CDN, HR
O76:H39	0	CZ (RS, the same strain)
O78:H2	sewage	SK
O79:H22 O79:43 O79:H3	man	CZ
O81:H22	0	CZ (RS, the same strain)
O84:H48	man, surface water	CZ, D
O88:H2	aquarium water	CZ
O89:H2	surface water	CZ

Countries: CZ = Czech Republic
SK = Slovak Republic
C = Cuba
CDN = Canada
D = Germany
GB = Great Britain
HR = Croatia
IRQ = Iraq
J = Japan
NL = Netherlands
S = Sweden
THA = Thailand
US = USA

RS = reference strain

III. *P. shigelloides* strains with unknown O antigen

O	H	Animal isolate	Total	
			OH	O
OUK	2	itone cuclew *Burhinus oedicnemus*, Prague, 1998	1	
		crane *Grus* sp., Prague, 1998	1	
		aquarium fish, Beroun, 1998	1	3
OUK	3	otter *Lutra lutra*, Prague, 1998	1	
		cherrug *Hierofalco* sp., Prague, 199	1	2
OUK	16	fish *Abramis brama*, Prague, 1997	1	1
OUK	HUK	aquarium fish, Prague, 1998	1	1
R	2	duck, Prague, 1981	1	1

OUK = O antigen unknown
HUK = H antigen unknown
R = autoagglutination of boiled culture

IV. *P. shigelloides* serovars of the provisional "Schubert" antigenic scheme

O	H	Animal isolate	OH	O
Schubert O2	H2	fish *Carassius carassius*, Prague, 1996	1	1
Schubert O4	H27	fish *Scardinius erythrophthalmus*, Prague, 1997	1	1
Schubert O5	H2	viper *Vipera berus*, Prague, 1998	1	
		fish *Abramis brama*, Prague, 1998	2	3
Schubert O9	H2	fish *Symphysodon discus*, Opava, 1999	2	2
Schubert O11	HUK	cayman *Caiman* sp., Nymburk, 1999	1	1
Schubert O18	H2	aquarium fish, Prague, 1999	1	1
Schubert O21	HUK	pelican, Prague, 1999	1	1
Schubert O25	Schubert H6	fish *Abramis brama*, Prague, 1996	2	
		fish *Carassius carassius*, Prague, 1996	2	
		aquarium fish, Prague, 1997	1	5
Schubert O27	H2	condor *Vultur gryphus*, Prague, 1998	1	
	H3	aquarium fish, Prague, 1999	1	
	Schubert H7	penguin, Prague, 1999	1	3
Schubert O28	H3	swan *Cygnus* sp., Prague, 1999	1	
		aquarium fish, Prague, 1998	1	2

HUK = H antigen unknown

Interesting is serovar O18:H2. This serovar has been, so far, isolated only from cats and from a lynx caracal and only once from surface water of a small pond in Frankfurt/M. This serovar, known to us since 1967 (the first strain 12129 was found in a cat in Zeleneč, Prague-East district) has never been isolated from human material. Unfortunately, the source of the Japanese reference strain was not given (Tab. II).

Tab. II is a supplement of Tab. I giving a survey on sources of particular serovars. The number of countries in which they were found lends fond for our imagination how frequent they are. Except for serovar O18:H2, which could be considered as a typical serovar of Felinae because it was isolated not only from sick cats but also from the intestinal content of healthy cats, and the single isolation of this serovar in Frankfurt's pond does not exclude the possible presence of a cat. Also O22:H3 and O30:H1a1c originate, in our table, from cats. Serovar O22:H3 was also isolated by Dr. Jan Bardoň from five cats in Olomouc (will be published elsewhere). Unfortunately, like in serovar O18:H2 the origin of the Japanese reference strain O22:H3 is unknown. Our feline strain O30:H1a1c has been, so far, our single isolate of this serovar, the source of the Japanese reference strain is not known. Two other strains, O76:H39 and O81:H22, represent only animal isolates, both also being reference strains for these O antigens.

Tab. III shows unknown O antigens (OUK) in combination with known H antigens and once in combination with an unknown H antigen (HUK). In such a heterogenous material, new unknown O or H antigens emerge each year.

"Schubert" serovars are presented in Tab. IV. These serovars as "environmental" ones originating from water and water insects found by Professor R. H. W. Schubert in Frankfurt/M. ponds, occurred also in our material exclusively in fish, water birds and in one cayman. However, the condor (Schubert O7:H2) and viper (Schubert O5:H2) are not aquatic animals.

One "Schubert" H antigen, Schubert H4, was introduced in Tab. I and was determined in a strain from surface water fish (O31:Schubert H4).

CONCLUSION

P. shigelloides strains presented in this paper do not represent material collected intentionally, they are mostly the result of random findings in routine examination. Nevertheless, most serovars of *P. shigelloides* found in animals show a connection with the same serovars from other sources.

Remarkable is the fact that the most frequently occurring serovar in humans, serovar O17, with H antigens H2, H11 or H34, was found in our animal strains only three times, in synanthropic animals, namely a dog and two pigs. On the contrary, among strains of Dr. Bardoň (not published here) this serovar was isolated from a bitunrong kept in the zoologic garden in Olomouc.

An exception is O18:H2, which is probably a typical feline serovar, and maybe also O22:H3.

Animals the habitat of which is water had strains in their intestinal content or organs carrying besides antigens of the international antigenic scheme also "Schubert" antigens.

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A CONTRIBUTION TO THE HISTORY OF GLANDERS IN THE CZECH REPUBLIC*

K HISTORII VOZHŘIVKY V ČESKÉ REPUBLICE

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ABSTRACT: The first and the only victim of glanders at the University School of Veterinary Medicine was MVDr. Miroslav Derbek (born 3 Aug. 1894). Derbek was a lecturer at the Institute of Pathological Anatomy headed by Professor MUDr. Jan Lukeš, who was the first to identify and describe *Leptospira canicola*. Derbek's hopeful educational and scientific career was dramatically interrupted at the age of 29 by what is known as the "Věrovany case". M.V., a farmer in Věrovany in the district of Přerov, owned a mare and gelding purchased from the army. The gelding suffered from a chronic fistular lesion in the withers region. The first to die under unclear circumstances was a stable boy (in 1921). The farmer's daughter, who cared for the horse, developed signs of a serious disease on 6 March 1923 and died 10 days thereafter. The farmer's stepson died on 30 March 1923 showing the same clinical signs. After his death, the horses were killed and autopsied by Dr. Derbek. During the autopsy, Dr. Derbek's face and mouth were accidentally sprinkled with infectious material. In spite of immediate desinfection by all means then available, Dr. Derbek contracted the infection and died on 30 Sept. 1923 in Prague hospital. His corpse was autopsied at the Professor Hlava's Institute of Pathological Anatomy by the lecturer MUDr. Jiří Šolc who also became infected and died of glanders several days later. Dr. Derbek's wife died on 9 Oct. 1923, the fiancée of Dr. Šolc also contracted glanders and died Professor Hlava forbade any further autopsies. Dr. Derbek and Dr. Šolc were not the last victims of occupational glanders in Czechoslavakia. In the same year Dr. Josef Pulkrábek, the vice-director of the Bioveta Institute contracted the infection and died on 4th Jan. 1924.

glanders; history; Czech Republic

ABSTRAKT: První a jedinou obětí na Vysoké škole veterinární v Brně (nynější Veterinární a farmaceutická univerzita) byl MVDr. Miroslav Derbek (nar. 3. srpna 1894). Derbek byl asistentem Ústavu patologické anatomie, jehož přednostou byl prof. MUDr. Jan Lukeš, objevitel *Leptospira canicola*. Derbekova nadějná pedagogická a vědecká kariéra byla dramaticky ukončena v důsledku tzv. Věrovanských událostí. M.V., rolník ve Věrovanech v okrese Přerov měl dva koně, valacha a klisnu, vyřazené z armády. Valach měl chronickou fistulární lézi v kohoutku. V roce 1921 zemřel za nejasných okolností čeledín. Rolníkova dcera, která pečovala o koně, onemocněla vážně 6. března 1923 a po 10 dnech zemřela. Nevlastní rolníkův syn zemřel 30. března 1923 za stejných příznaků onemocnění. Po smrti těchto dvou obětí byly koně utraceni a pitváni. Pitvu prováděl Dr. Derbek. Během pitvy byl jeho obličej potřísněn infekčním materiálem. Navzdory bezprostřední dezinfekci Dr. Derbek onemocněl a zemřel na vozhřivku 30. září 1923 v Praze. Při pitvě zesnulého se infikoval Dr. Jiří Šolc, asistent patologického ústavu prof. Hlavy. Zemřel za několik dnů a prof. Hlava další pitvu zakázal. Vdova po Dr. Derbekovi ze strachu před infekcí spáchala sebevraždu 9. října 1923, snoubenka Dr. Šolce onemocněla a zakrátko umřela. V témže roce (1923) se vozhřivkou nakazil Dr. Josef Pulkrábek náměstek ředitele z ivanovické Biovety. Zemřel 4. ledna 1924.

vozhřivka; historie; Česká republika

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Glanders is a highly contagious and fatal disease of horses, donkeys, and mules caused by the bacterium *Burgholderia mallei* (previously classified generically with *Pfeiferella, Loefflerella, Malleomyces, Actinobacillus* and *Pseudomonas*. Recently, the causative agent has been renamed to *Burgholderia mallei*, but the name *Pseudomonas mallei* is still in general use. Infected animals develop nodules and ulcers in the upper airways and lungs. A skin form, known as "farcy", has also been described. The control of glanders requires testing of suspect clinical cases, screening of normal equids, and prompt and safe disposal of positive reactors.

The infection is easily transmitted to humans. Carnivores can also become infected by eating infected meat, but cattle, sheep, and swine are fully resistant. Acute glanders, killing the patient within a few days, affects most frequently donkeys and mules, while in horses the infection generally runs a more chronic course and patients can survive up to several years.

The incubation period is from a few days to several months.

Glanders can be transmitted to humans by direct contact with an infected animal, its tissues, or secretions. The mortality of untreated cases of an acute infection is 95%; the patients die within 3 weeks. Chronic cases, characterised by abscessation, have also been described.

Ossiander was probably the first to describe human glanders as early as in 1783. However, a reliable report came from Schilling only in 1821. The causative agent – *Bacillus mallei* (now *Burgholderia mallei*) – was identified independently by Loeffler & Schütz and Bonchard with coworkers in 1882 (Kabelík, 1975).

According to the International Animal Health Code (1999 Edition, Chapter 3.4.8., Glanders, art. 3.4.8.2.) a glanders-free country is defined as follows:

"A country may be considered free from glanders when:

1. glanders is notifiable in the country and
2. no case of glanders has been confirmed for at least the last two years."

To this effect, the Czech Republic is free from glanders now. In the past, there were two documented periods of glanders in the former Czechoslovakia. During the first period, covering the years 1920 to 1934, 894 cases in horses were recorded and several people contracted the infection. No cases of glanders were reported from this territory from 1934 to 1944. Another outbreak of glanders, involving 178 horses on 107 farms in 82 villages, 52 districts, and 15 counties, was recorded after the Second World War. No cases of human glanders were reported during the second outbreak, which was successfully controlled in 1947. It is evident that in this country glanders was a typical war and post-war infection which could be readily combated under normal conditions (Nižnánsky, 1951).

Glanders at the University of Veterinary and Pharmaceutical sciences, Brno (formerly University School of Veterinary Medicine), is associated with a unique event. In 1918, the epizootiological situation in the former Czechoslovakia was very serious and glanders ranked among the priority problems the teachers of the then newly established University School of Veterinary Medicine had to cope with. Thus, already in 1920, the founder and the first head of the Institute of Microbiology Dr. Ševčík (1986–1930) defended his second doctorate thesis "A Contribution to Serological Diagnostics of Glanders", which became the starting point for his further principal paper ("Diagnostics and Control of Glanders in the Armed Forces" published in Zvěrolékařský obzor) and theses of undergraduate students working under his supervision.

The first and the only victim of glanders at the University School of Veterinary Medicine, Brno, was MVDr. Miroslav Derbek (born 3rd Aug. 1894). Derbek was a lecturer at the Institute of Pathological Anatomy headed by Professor MUDr. Jan Lukeš, who was the first to identify and describe *Leptospira canicola*. Derbek's hopeful educational and scientific career was dramatically interrupted at the age of 29 by what is known as the "Věrovany Cause".

Věrovany is a village in the district of Přerov where three people died under rather suspicious circumstances in the early twenties. Tentative diagnosis was glanders caused by a highly virulent strain, because the infection was contracted also by goats sharing their premises with horses.

M.V., a farmer, owned a mare and a gelding purchased from the army. Many horses of disbanded military formations were sold to farmers after the First World War. Some of such animals suffered from latent or non-typical glanders. One of them was also a gelding which reached, along with a mare, the farm of J. V. as a "revertible horse". A small abrasive lesion with loss of hair, found at take-over already, was treated by a local veterinarian.

The animals were used as work horses on the farm and were attended to by the farmer's daughter Anna and son Josef. The first to die under unclear circumstances was a stable boy (in 1921). The next to become ill was Anna who developed ocular lesions. She was treated by a local physician and later in the Olomouc hospital. After an unsuccessful surgical treatment, the disease proceeded rapidly and the patient died on 6th March 1923, i.e. ten days after the first signs had developed.

Her older brother Josef suffered initially from the same clinical signs and from various joint ailments and was treated by the local physician. The disease proceeded rapidly and the patient died on 30th March 1923.

The deaths of the two young people under unclear circumstances prompted intensive investigations aimed at the identification of the causative agent.

Soon after the death of the siblings, the local veterinarian diagnosed glanders in the gelding. He carried out the skin test for glanders. A painful oedema with a size of a man's palm and a height of 2 cm developed at the

site of injection after approximately one hour. The veterinarian prepared a record and reported the result to the official veterinarian who immediately ordered killing of the horse.

The animal was shot on the spot and transported to the district town for necropsy.

The official necropsy, carried out by the lecturer of the University School of Veterinary Medicine, MVDr. Miroslav Derbek, was assisted by the official district and municipal veterinarians.

The post mortem gross finding was indicative of glanders. Necropsy findings of the gelding: Nasal septum seriously damaged, practically absent. Lesions indicative of glanders in the lung, heart and liver.

The remaining two horses of the farmer, a mare and a foal, were destroyed and necropsied in the district town as well.

During the autopsy, Derbek's face and mouth were accidentally sprinkled with infectious material. Prof. MUDr. Jan Lukeš, the head of the Institute of Pathological Anatomy of the Veterinary high School issued the following statement on the death of Dr. Derbek:

The lecturer MVDr. Miroslav Derbek contracted a fatal disease during an official necropsy in Přerov. Due to careless behaviour of an auxiliary, a drop of blood of the necropsied animal penetrated into his mouth (document No. 1495 of 3rd Nov. 1923).

In spite of immediate disinfection by all means then available, Derbek contracted the infection and died on 30th Sept. 1923 in a Prague hospital. The physicians treating Dr. Derbek were puzzled by the rather unspecific signs developing in the patient and considered not only glanders, but also typhus and tuberculosis. The dominating manifestations included fatigue and increase in body temperature, in particular in the evening.

Dr. Derbek was first hospitalised in the Provincial Hospital, Brno (from 28th April to 31st May 1923) and later in the hospital of his birthplace Jičín (where he was visited by the most prominent internists of that time Professor Prusík and Professor Pelnář). The condition deteriorated rapidly. The patient was transported to the Prague clinic of Professor Thomayer on 26th September and died there on 30th September 1923. Pneumonia and sepsis were denoted as the immediate causes of death. According to the official report, the patient suffered from chronic glanders confirmed by bacteriological examination. A culture of the causative agent was isolated from a lesion found in the frontal sinus. Necropsy further revealed granulomatous infiltrations in the stomach in which Šikl and Jedlička, who were later appointed professors, identified the causative agent of glanders (then *Mycobacterium mallei*) upon histological examination. It is probable that the site of entry of the infection was the mouth cavity.

The post mortem finding was reportedly non-typical for glanders; the most serious lesions were found in the stomach. Derbek's corpse was autopsied at the Institute of Forensic Medicine. Bacteriological examination was carried out by MUDr. Jiří Šolc, a lecturer of the Institute of Pathological Anatomy, who also contracted the infection and died of glanders several days later. Professor Jedlička expressed the following assumption on Dr. Šolc's accident: "Dr. Šolc suffered from epilepsy and, working with *Mycobacterium mallei,* crushed a test tube with the culture in his hand in an epileptic fit". Another explanation of the accident is that Dr. Šolc became infected during centrifugation of a sample of an infected body fluid collected during the necropsy of Dr. Derbek. Šolc developed high fever several days after the accident and died within a week. His body was not necropsied due to fear of further transmission of the infection.

Depression from her husband's death and fear of being affected by glanders led eventually Mrs. Derbek to suicide. She died on 9th Oct. 1923 and Professor Hlava, the head of the Institute of Pathological Anatomy, Prague, forbade any further autopsies. The fiancée of Dr. Šolc also contracted glanders and died.

Unfortunately, Dr. Derbek and Dr. Šolc were not the last victims of occupational glanders in Czechoslovakia. In the same year, Dr. Josef Pulkrábek, the deputy director of the Bioveta Institute and a renowned specialist, contracted the infection and died on 4th Jan. 1924. He became ill in the summer 1923 suffering from signs suggestive of joint rheumatism of the right arm. On 30th July, he developed fever and an abscess appeared on the dorsal side of his hand. Results of both bacteriological examination and the mallein skin test were negative. Further abscesses developed later and the mallein skin test became positive on 10th September. After a seeming initial success of the treatment with neosalvarsan, the infection broke out with full strength around Christmas 1923 and Dr. Pulkrábek died at the beginning of January 1924.

The Věrovany tragedy is a significant record in the history of veterinary and human medicine and the dangerous contagious disease glanders.

However this story of deaths of young, hopeful people, which resembles an antic tragedy of fate ("*schicksalstragödie*") may seem incredible, it actually happened.

The whole story is very instructive because it shows the tricky character of glanders which:

1. is highly contagious,
2. can run a latent course and then exacerbate,
3. can run as an acute sepsis from the beginning,
4. can be associated with a high mortality.

World-wide eradication of glanders has not yet been accomplished as shown by reports from South America and several cases of occupational infections of laboratory staff in the USA from the last year.

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Změna publikačního jazyka ve vědeckých časopisech ČAZV

Předsednictvo České akademie zemědělských věd přijalo na zasedání dne 6. 4. 2000 usnesení, kde mj. doporučuje změnu publikačního jazyka ve vědeckých časopisech vydávaných pod gescí ČAZV. Předsednictvo navrhuje Vydavatelské radě ČAZV zavést angličtinu jako jediný jazyk ve všech vědeckých časopisech od 1. 1. 2001. Redakce časopisu Veterinární medicína přijímá od 1. 7. 2000 příspěvky psané pouze v angličtině.

A change of publication language in Scientific Journals of the Czech Academy of Agricultural Sciences

At its session on the 6th April 2000, the Presidium of the Czech Academy of Agricultural Sciences adopted a resolution recommending, among other things, to change the publication language in scientific journals published under the Academy patronage. The Presidium proposes to the Publishing Board of the Academy to introduce English as the only language in all scientific journals from the 1st January 2001. The papers written exclusively in English are accepted by the editor's office of the journal Veterinary Medicine – Czech from the 1st July 2000.

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