ÚZPI

ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

VETERINÁRNÍ MEDICÍNA

Veterinary Medicine – Czech

ČESKÁ AKADEMIE ZEMĚDĚLSKÝCH VĚD

10

VOLUME 42 (LXIX)
PRAHA
OCTOBER 1997
CS ISSN 0375-8427

VETERINÁRNÍ MEDICÍNA

Mezinárodní vědecký časopis vydávaný z pověření Ministerstva zemědělství České republiky a pod gescí České akademie zemědělských věd

VETERINARY MEDICINE – CZECH

An international journal published under the authorization by the Ministry of Agriculture and under the direction of the Czech Academy of Agricultural Sciences

Cíl a odborná náplň: Časopis Veterinární medicína uveřejňuje původní vědecké práce a studie typu review ze všech oblastí veterinární medicíny v češtině, slovenštině a angličtině.
Časopis je citován v bibliografickém časopise Current Contents – Agriculture, Biology and Environmental Sciences, a abstrakty z časopisu jsou zahrnuty v těchto databázích: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Toxline Plus, WLAS.

Periodicita: Časopis vychází měsíčně (12x ročně), ročník 42 vychází v roce 1997.

Přijímání rukopisů: Rukopisy ve dvou vyhotoveních je třeba zaslat na adresu redakce: Ing. Zdeňka Radošová, vedoucí redaktorka, Ústav zemědělských a potravinářských informací, Slezská 7, 120 56 Praha 2, tel.: 02/24 25 79 39, fax: 02/24 25 39 38, e-mail: fofo@uzpi.cz. Podrobné pokyny pro autory lze vyžádat v redakci.

Informace o předplatném: Objednávky na předplatné jsou přijímány pouze na celý rok (leden–prosinec) a zasílají se na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezská 7, 120 56 Praha 2. Cena předplatného pro rok 1997 je 564 Kč.

Aims and scope: The journal Veterinární medicína original publishes papers and reviews from all fields of veterinary medicine written in Czech, Slovak or English.
The journal is cited in the bibliographical journal Current Contents – Agriculture, Biology and Environmental Sciences, abstracts from the journal are comprised in the databases: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Toxline Plus, WLAS.

Periodicity: The journal is published monthly (12 issues per year), Volume 42 appearing in 1997.

Acceptance of manuscripts: Two copies of manuscript should be addressed to: Ing. Zdeňka Radošová, editor-in-chief, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, tel.: 02/24 25 79 39, fax: 02/24 25 39 38, e-mail: fofo@uzpi.cz. Applications for detailed instructions for authors should be sent to the editorial office.

Subscription information: Subscription orders can be entered only by calendar year (January-December) and should be sent to: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2. Subscription price for 1997 is 132 USD (Europe), 138 USD (overseas).

F 4884

ISOLATION AND IDENTIFICATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN CELL CULTURES*

IZOLACE A IDENTIFIKACE REPRODUKČNÍHO A RESPIRAČNÍHO SYNDROMU PRASAT NA BUNĚČNÝCH KULTURÁCH

L. Valíček, I. Pšikal, B. Šmíd, L. Rodák, R. Kubalíková, E Kosinová

Veterinary Research Institute, Brno, Czech Republic

ABSTRACT: Three strains of porcine reproductive and respiratory syndrome virus (PRRSV) were isolated in porcine lung macrophage (PLM) cultures from three swine herds. This has been the first successful isolation of PRRSV in the Czech Republic and the strains received the designations CAPM V-501, CAPM V-502 and CAPM V-503, respectively. All the three isolates in PLM were identified by immunofluorescence and immunoperoxidase tests and the strain CAPM V-502 also by electron microscopy using the ultrathin section technique. The strain CAPM V-502 has been adapted to the cell line MARC-145. Viral RNA in PLM cultures infected with any of the isolated PRRSV strains was demonstrated by RT-PCR targeted to the more conserved ORF 7 genomic region encoding the nucleocapsid protein. The assessment of PCR products in agarose gel revealed a uniform size of 394 bp in all the three isolates and the European prototype strain Lelystad used as positive control.

swine; PRRS; virus isolation; lung macrophages; cell line MARC-145; RT-PCR; electron microscopy

ABSTRAKT: Ve třech chovech prasat v České republice byly poprvé izolovány na buněčných kulturách prasečích plicních makrofágů (PLM) tři kmeny viru reprodukčního a respiračního syndromu prasat (PRRSV), které byly označeny CAPM V-501, CAPM V-502 a CAPM V-503. Izoláty byly v PLM identifikovány nepřímým imunofluorescenčním a imunoperoxidázovým testem a kmen CAPM V-502 navíc ještě elektronově mikroskopicky v ultratenkých řezech. Kmen CAPM V-502 byl adaptován také na buněčnou linii MARC-145. Virová RNA byla v kulturách PLM infikovaných izolovanými kmeny PRRSV prokázána také metodou RT-PCR, která byla zaměřena k amplifikaci konzervativního úseku genu (ORF 7) kódujícího virový nukleoprotein. Posouzení PCR produktů v agarózovém gelu ukázala jednotnou velikost 394 bp u všech izolovaných kmenů PRRSV a pozitivní kontroly (evropského prototypového kmene Lelystad).

prasata; PRRS; izolace viru; plicní makrofágy; buněčná línie MARC-145; RT-PCR; elektronová mikroskopie

INTRODUCTION

Viral aetiology of the porcine reproductive and respiratory syndrome (PRRS) was demonstrated for the first time in the Netherlands in 1991 by virus isolation in cultures of porcine lung macrophages (Wensvoort et al., 1991). This success was followed by further reports of isolations not only in European countries, but also in the United States, Canada, Mexico, Japan and other regions, and instigated the development of serological and virological diagnostic methods for PRRS (Meredith, 1995). In the Czech Republic, PRRS was diagnosed in swine herds by serological methods and reverse transcription with polymerase chain reaction (RT-PCR) for the first time in 1995 (Valíček et al., 1995a, b).

Antibodies to PRRSV were assayed by ELISA using the IDEXX kit and RT-PCR was used to demonstrate viral RNA in blood serum samples and lung macrophages collected from serologically positive sows and a dying piglet, respectively. RT-PCR was further used within the control of PRRS to demonstrate viral RNA in blood serum and semen of serologically positive boars (Valíček et al., 1997).

Although PRRS was diagnosed by serological methods and RT-PCR in a number of swine herds in the Czech Republic, the isolation and identification of the causative agent in PLM cultures, as described in this paper, was successful as late as in 1996.

MATERIAL AND METHODS

Animals

The isolation attempts were made in pigs (Landrace x White Improved crosses) coming from three

* Supported by the Grant Agency of the Czech Republic (Grant No. 508/95/0377) and Ministry of Agriculture of the Czech Republic (Grant No. RE 5556)

herds (N, B, and S) affected by reproductive disorders in sows and showing an increased mortality rate in pre-weaning piglets. Antibodies to PRRSV were demonstrated in all the three herds.

Experimental groups in the herds B and S were the progeny of the own parent stock, while in the herd N, experimental pigs free of antibodies to PRRS were purchased elsewhere and exposed to contact infection. This group of five 4-week-old pigs was monitored serologically and PLM were collected from one of them after seroconversion for the isolation of PRRSV (Tab. I).

Antibodies

ELISA (IDEXX kit) was used as a tool for serological screening and for the demonstration of antibodies to PRRSV.

Cell cultures

Porcine lung macrophages (PLM) flushed from the lungs of pigs suspected of PRRSV infection were used. After flushing and centrifugation, PLM were either frozen to be used as inoculum later, or were incubated for 48 to 72 h in the medium RPMI-1640 with antibiotics (400 U penicillin and 0.4 mg streptomycin per 1 ml) supplemented with 10% bovine foetal serum. PLM collected from conventional piglets free of antibodies to PRRSV and younger than 6 weeks were used in further passages of the virus. The collection by pulmonary lavage and preparation of PLM were described in detail elsewhere (Šmíd et al., 1977). A 10% suspension of lung and spleen tissues collected from dying piglets was used as the inoculum for PLM in some experiments. In addition to PLM, the established cell line MARC-145 (Kim et al., 1993) was used for the culture of the reference strain Lelystad and for the propagation of our PRRSV isolates (the strain was obtained as the 5th passage in PLM by courtesy of Dr. Wensvoort). The growth and maintainance medium for MARC-145 was Eagle's minimal essential medium supplemented with 8% and 4% bovine foetal serum, respectively, and penicillin + gentamycin (400 U and 0.4 mg per 1 ml, respectively). Infected PLM and MARC-145 cultures were examined daily for the development of CPE and the presence of PRRSV was confirmed by indirect fluorescent antibody (IFA) test, immunoperoxidase monolayer assay (IPMA) and RT-PCR. In the case of the strain CAPM V-502, the tests were completed with electron microscopic examination.

Indirect fluorescent antibody test (IFA)

Infected PLM or MARC-145 cells were incubated for 12 to 72 hours and fixed with cold acetone for 10 min. After the addition of PRRS-positive porcine blood serum diluted 1 : 10, the cultures were incubated at 37 °C for another 45 min. Unbound antibodies were removed by washing with phosphate-buffered saline (pH 7.2) and fluorescein-conjugated rabbit anti-porcine IgG was added. After another 45 min of incubation, the cells were washed as above and viewed with a fluorescent microscope. Positive controls (PLM or MARC-145 cells infected with the Lelystad reference strain) and non-infected cells were included into each test. IFA was also used for the titration of the PRRSV isolates and the strain Lelystad in PLM.

Indirect immunoperoxidase monolayer assay (IPMA)

Infected PLM or MARC-145 cells grown on slides were incubated for 12 to 72 hours, rinsed, dried at 37 °C and fixed with cold acetone at 20 °C for 10 min. After 20 min preincubation in PBS, containing 0.1% sodium azide and 0.3% hydrogen peroxide to inhibit the activity of endogenous peroxidase (Li et al., 1987), the cells were incubated at 20 °C for 30 min with PRRS-positive porcine blood serum diluted 1 : 100 with PBS containing 0.05% Tween and 1% lactalbumin hydrolysate (DIFCO) (PBST/LAH). Then conjugate solution (rabbit antibodies to porcine IgG purified by affinite chromatography and conjugated with horseradish peroxidase) diluted 1 : 100 with PBST/LAH was added and the incubation was repeated under the same conditions. Each incubation was followed by thorough washing (4 times 5 min in PBS). Finally, the cells were incubated for 5 to 10 min with a substrate solution using DAB (3,3'-diaminobenzidine, Merck) as the chromogen

I. Formation of antibodies to and isolation of PRRSV after contact infection of sentinel pigs included into the herd "N"

Pig. No.	Specific antibodies								
	26. 4.	9. 5.	15. 5.	25. 5.	30. 5.	16. 6.	3. 7.	18. 7.	25. 7. (90 days after contact)
11	–	–	–	–	–	–	*+	*+	virus isolation
12	–	–	–	–	–	–	+	+	0
13	–	–	–	–	–	–	–	+	0
14	–	–	–	–	–	–	–	+	0
15	–	–	–	–	–	–	+	+	0

– = absence of specific antibodies; + = presence of specific antibodies; * = viral RNA detected in blood serum by RT-PCR; 0 = virus isolation not attempted

and the reaction was stopped by the addition of 0.5% sodium azide and assessed microscopically.

Plaque test (PT)

Plaque test was used for the titration of the PRRSV strain CAPM V-502 that had been successfully adapted to the cell line MARC-145. Confluent cell monolayers grown in Petri dishes (50 x 15 mm) were used. PT and visualization of plaques by permanent staining with 1% crystal violet were done as described by Park et al. (1996).

Antisera

Two different porcine hyperimmune anti-PRRSV sera were used for the identification of the isolates by IFA and IPMA. One of them was the reference serum obtained by courtesy of Dr. Wensvoort and the other was prepared in a conventional pig free of specific antibodies by inoculation with the Lelystad strain propagated in PLM. The pig received two doses of 5 ml (10^5 $TCID_{50}$ per ml) intranasally at a three-week interval and blood was collected 43 days after the first inoculation. The presence of specific antibodies was confirmed by ELISA.

Electron microscopy

PLM and MARC-145 cell cultures infected with the PRRSV strain CAPM V-502 or the reference strain Lelystad were incubated 48 to 72 hours, fixed in 1% OsO_4 in phosphate buffer, dehydrated in acetone and embedded into the EPON 812 Araldite C4212 mixture. Ultrathin sections, prepared using the LKB Ultrotome III, were double-stained with uranyl acetate and lead citrate and examined with a Tesla BS 500 electron microscope.

RNA extraction

Total RNA was isolated from 50 ml aliquots of the infected PLM or MARC-145 cells by the guanidinium isocyanate-silica particles method (Boom et al., 1990) and resuspended in 20 µl of DEPC-treated water. Total RNA was isolated from non-infected PLM and from primary calf kidney cells infected with bovine viral diarrhoea virus (BVDV) to be used as negative controls. Checks for cross contamination with PCR products were done using negative controls free of RNA and DNA templates (distilled water).

Primers

The design of primers was based on sequence alignment of nucleocapsid protein gene (N) of European and Canadian strains of PRRSV (Lelystad and IAF-exp91, respectively; Mardassi et al., 1994a). Their sequences are as follows: Forward primer LV-607 5' – CCAGCCAGTCAATCAACTGT- 3', reverse primer LV-981 5'- TCGCCCTAATTGAATAGGTG-3'. Oligonucletides used in RT-PCR were synthesized by Genset (France).

Reverse transcription (RT)

Viral RNA (8 µl) was reverse transcribed, using 50 pmol of reverse primer LV-981, 500 mM (each) deoxynucleoside triphosphates (d NTP', Pharmacia), 40 U of rRNasin ribonuclease inhibitor (Promega), and 200 U of M-MLV reverse transcriptase (Gibco BRL) in a final volume of 20 µl containing 67 mM Tris-HCl (pH 8.8), 6.7 mM EDTA; 16.6 mM $(NH_4)_2SO_4$; 1.5 mM $MgCl_2$; 0.17 mg/ml of bovine serum albumin and 10 mM dithiothreitol. The mixture was incubated at 37 °C for 60 min.

Polymerase chain reaction (PCR)

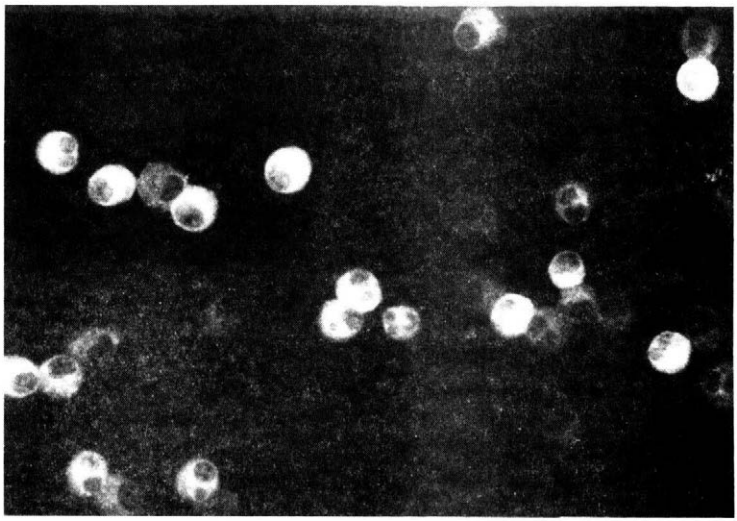
For the PCR step, the reverse transcription reaction volume (20 µl) was brought to 100 µl with PCR buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 100 mM of each dNTP', 2.5 U of Taq DNA polymerase (Promega) and 50 pmol of the corresponding primers.

The reaction mixtures were overlayered with 50 µl of light mineral oil (Sigma). PCR was started with the first cycle consisting of 3 min at 94 °C, 1.5 min at 49 °C and 1 min. at 72 °C and followed by 34 cycles: 45 sec at 94 °C, 30 sec at 59 °C, and 45 sec at 72 °C. After the 35 cycles, the reaction was held at 72 °C for 7 min. in order to elongate any incomplete product. All the PCRs were performed in an automated DNA thermal cycler (Perkin Elmer). The analysis of PCR products was undertaken routinely by electrophoresis in 2% agarose gels followed by staining with ethidium bromide (0.5 µg/ml).

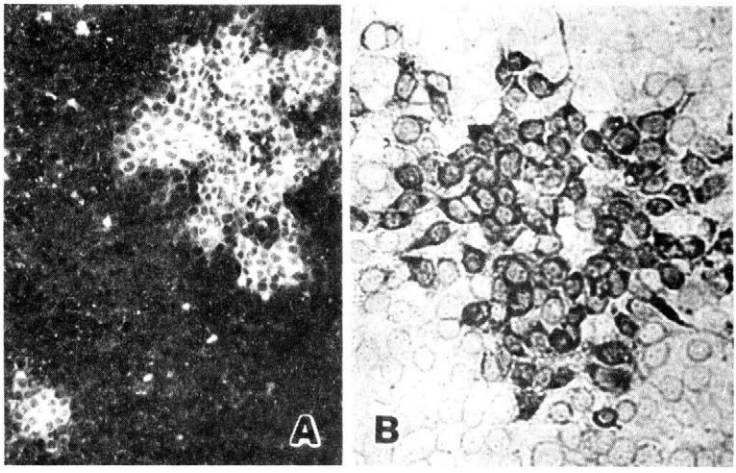
RESULTS

Three strains of PRRSV were isolated in PLM cultures in three serologically positive swine herds in the Czech republic. The isolates were designated CAPM V-501, CAPM V-502 and CAPM V-503 (CAMP = Collection of Animal Pathogenic Microorganisms, Veterinary Research Institute, Brno, Czech Republic).

The strain CAPM V-501 was isolated in the herd "N" affected by a low pregnancy rate, premature births and increased mortality in pre-weaning piglets reaching up to 40%. Antibodies to PRRSV were detected sporadically for the first time in February 1995, but later were found in a high percentage of animals of all age groups. The virus was circulating in the herd for a prolonged period as demonstrated by seroconversion in serologically negative gilts purchased elsewhere and included into the herd. PRRSV was isolated 17 months after the first detection of antibodies from lung washings of one of the five purchased experimental weaners



1. Specific fluorescence in cytoplasm of a large number of PLM 24h after infection with virus strain CAPM V-502 (5th virus passage in PLM)

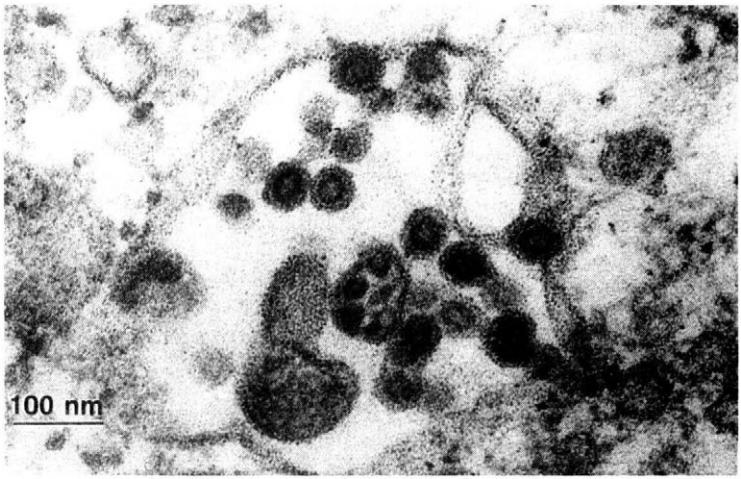


2. Foci of IFA-positive (Fig. 2A) and IPMA-positive (Fig. 2B) cells. Cell line MARC-145 48 h after infection with virus strain CAPM V-502

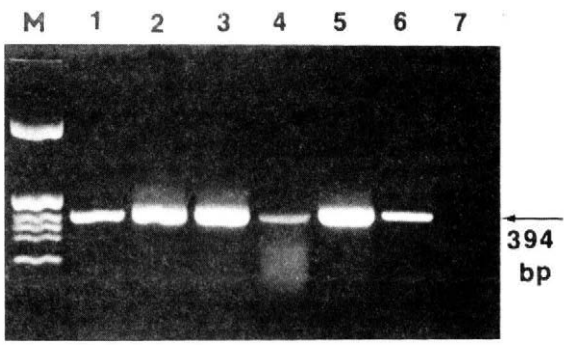
included among weaners reared in the herd and monitored serologically (Tab. I). Pig No. 11, in which serum antibodies to PRRSV and viral RNA (RT-PCR) were demonstrated 90 days after the first contact with the herd, was sacrificed. PLM obtained by lung lavage were incubated for 72 h and frozen to be used for further passages in cultures of PLM collected from a serologically negative conventional animal. PRRSV was demonstrated in cytoplasm of PLM by indirect immunofluorescence (IFA) and immunoperoxidase (IPMA) tests 24 h after the inoculation. No virus propagation was detectable throughout nine passages of the strain CAPM V-501 in MARC-145 cells.

The strain CAPM V-502 was isolated from a 4-week-old piglet born in the herd "S" affected by increased mortality rate in pre-weaning piglets, diarrhoea and respiratory diseases. PLM obtained from the sacrificed piglet by lung lavage were frozen to be used later as an inoculum for PLM cultures collected from a conventional piglet free of specific antibodies. PRRSV was demonstrated in the inoculated cultures of PLM by IFA (Fig. 1), IMPA and by electron microscopy in ultrathin sections (Fig. 3). Spherical enveloped virus particles were observed 48 h after the infection of PLM. Virions with a diameter of approx. 55 nm, containing 30–35 nm capsids, were observed in cytoplasmic vesicles and also in cell fragments and outside the destroyed cells, but not in nuclei.

The strain propagated in PLM was also used as an inoculum for the cell line MARC-145 co-cultivated with PLM for 8 days. Thereafter the cells were frozen and a cell suspension was prepared to be used as an inoculum for the cell line MARC-145 alone. PRRSV induced specific cytopathic effect (CPE) in this cell



3. PRRSV particles in a culture of PLM 48 h after infection with virus strain CAPM V-502 (Ultrathin section)



4. Comparison of PCR products of PRRSV isolates after electrophoresis in agarose gel

M = molecular standard pBR 322/Hinf I; 1 = reference strain Lelystad; 2, 3, 4 = various passages of CAPM V-501; 5 = CAPM V-502; 6 = CAPM V-503; 7 = negative control, noninfected PLM; arrow indicates a 396 bp fragment

line. The CPE started as small, rounded clumps raised above the uninfected cells. The number of rounded cells increased and cells became pyknotic and detached from the monolayer within 3–5 days. The virus was identified by IFA and IMPA (Fig. 2), as well as by electron microscopy. Infectivity titres in the MARC-145 cells reached 3 x 10^5 PFU per 1 ml on the twelfth passage of the virus.

The strain CAPM V-503 was isolated in the herd "B" showing high losses of pre-weaning piglets in 1995. Antibodies to PRRSV were demonstrated by ELISA in all age groups and also in boars used for natural breeding. The circulation of PRRSV in the herd was indirectly confirmed by demonstration of seroconversion in serologically negative gilts coming from another herd. The strain was isolated from lungs of a 33 days old weaned piglet. PLM obtained by pulmonary lavage were frozen to be used later as an inoculum for PLM collected from a conventional piglet free of antibodies to PRRSV. The virus was demonstrated in PLM by IFA and IPMA. No propagation of the strain in the cell line MARC-145 was demonstrable during nine passages.

RT-PCR

Genomic RNA of each of the three tested strains (CAPM V-501, CAPM V-502, and CAPM V-503) and the strain Lelystad (LV) was amplified using the primer pair of LV-607 and LV-981. The primers span a segment of 394 base pairs in a region coding for the nucleoprotein (N gene) of the LV strain (Fig. 4, lane 1), and the isolates CAPM V-501 (Fig. 4, lanes 2, 3, and 4), CAMP V-502 (Fig. 4, lane 5), and CAPM V-503 (Fig. 4, lane 6). No specific PCR products were detectable when total RNA of non-infected PLM, or heterologous RNA (BVD virus) were tested by RT-PCR (Fig. 4, lane 7).

DISCUSSION

Antibodies to PRRSV and viral RNA were demonstrated for the first time in swine herds in the Czech Republic by ELISA and RT-PCR, respectively, in 1995 (Valíček et al., 1995a). Limited serological examinations of 105 blood serum samples collected in

11 herds revealed the presence of antibodies in three sows of two herds and in a piglet born by one of them. RNA of PRRSV was detected by RT-PCR in blood serum samples collected from 2 serologically positive sows and in PLM of a dying piglet.

The subsequent more extensive serological screening, carried out in collaboration with the State Veterinary Institute, Jihlava, covered 1991 porcine blood sera collected in 70 herds and 1490 blood sera collected from adult boars. Specific antibodies were detected in 17% samples collected in 21 (30%) herds and in 1.25% boar sera. Viral RNA was demonstrated by RT-PCR in blood sera of ten out of the serologically positive boars.

RNA was also detected in semen of one of them (Valíček et al., 1997). In addition to the standard RT-PCR technique, amplicons of two positive samples, of the strain Lelystad and of four American isolates were analysed using non-radioactive hybridization based on the immunoenzymatic reaction with digoxigenin-labelled cDNA (PCR-ELISA). The results (Valíček et al., 1996) support the earlier finding of Meng et al. (1995) that the tested American and European PRRSV isolates represent two distinct genotypes. Although the infection by PRRSV was diagnosed in Czech swine herds by the above methods already in 1995, attempts to isolate the causative agent in cell cultures were successful only one year later. All the three strains of PRRSV (CAPM V-501, CAPM V-502, and CAPM V-503) were isolated from PLM obtained by pulmonary lavage from pigs coming from three serologically positive herds. The earlier finding that PLM obtained by pulmonary lavage were suitable for the isolation of PRRSV (Mengeling et al., 1995) could be thus confirmed. The identity of the isolated strains was confirmed by immunostaining (IFA, IPMA) using specific porcine sera.While the strains CAPM V-502 and CAPM V-503 were isolated from pigs born and reared in the herds under study, the strain CAPM V-501 was isolated from a sentinel pig purchased at the age of four weeks and included, along with another four serologically negative pigs, into the serologically positive herd "N". The first antibody responses were recorded as late as 83 days after their arrival into the herd. As a matter of fact, the antibodies became detectable only after the sentinel pigs had been included among older finishing pigs housed in a separate barn. The isolation of PRRSV 17 months after the first seroconversion had been detected in the herd, has confirmed not only the prolonged persistence of the virus, but also the already known fact that PRRSV can be isolated from serologically positive animals (Weensvoort, 1994).

The strain CAPM V-502 has been identified not only by IFA and IPMA, but also by electron microscopy. Morphological characteristics of virus particles, including spherical shape, diameter of approx. 55 nm and the presence of envelope, corresponded to the descriptions published by Wensvoort (1993), Botner et al. (1994) and Mardassi et al. (1994b). The isolate did not differ from the European prototype strain Lelystad, processed at the same time by the same technique, as far as its size, structure and localization in the cyoplasm were concerned. The presence of viral RNA in PLM cultures infected with any of the three isolates or the strain Lelystad was confirmed by RT-PCR. Products of 394 bp size were resolved by electrophoresis in agarose gel when target sequences prepared from PLM infected with any of our isolates or the strain Lelystad were used (Fig. 4). Products of the same size of 394 bp had also been demonstrated in our earlier attempts to detect PRRSV in clinical samples (blood serum, semen, tissue suspensions) collected from swine in the Czech Republic. On the other hand, the same primers used for the amplification of the American isolates tested in different studies yielded a 429 bp product (Valíček et al., 1995a, 1996). The results of RT-PCR and specific positive reactions of polyclonal immune sera to the strain Lelystad with our isolates justify the classification of the latter with the subgroup A (Wensvoort, 1994) including the European strains of PRRSV.

The attempts to adapt our PRRSV isolates to the cell line MARC-145 were successful only with the strain CAPM V-502 and only if the MARC-145 cells were co-cultivated with PLM in the first virus passage. However, the virus propagated well in subsequent passages in MARC-145 without PLM. The attempts to adapt the remaining two isolates (CAPM V-502 and CAPM V-503) to the MARC-145 cells failed after nine blind passages. However co-cultivation experiments with the cell line MARC-145 and PLM with the aim to adapt these isolates are ongoing.

Acknowledgements

The authors wish to thank Dr. Wensvoort for the supply of the reference strain Lelystad and the respective porcine antiserum, Mrs. J. Mikulská, Z. Mikulášková and A. Farníková for their excellent skilful assistance in virus isolation and culture and Ing. A. Paravanová for the technical processing of the manuscript.

REFERENCES

BOOM, R. – SOL, C. J. A. – SALIMANS, M. M. M. – JANSEN, C. L. – WERTHEIM Van DILLEN, P. M. E. – van der MOORDAA, J. (1990): Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol., *28*, 495–503.

BØTNER, A. – NIELSEN, J. – HANSEN, V. B. (1994): Isolation of porcine reproductive and respiratory syndrome (PRRS) virus in a Danish swine herd and experimental infection of pregnant gilts with the virus. Vet. Microbiol., *40*, 351–360.

KIM, H. S. – KWANG, J. – YOON, I. J. – JOO, H. S. – FREY, M. L. (1993): Enhanced replication of porcine repro-

ductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line. Arch. Virol., *133*, 477–483.

LI, C. Y. – ZIESMER, S. C. – LAZCANO-VILLAREAL, O. (1987): Use of azide and hydrogen peroxide as an inhibitor for endogenous peroxidase in the immunoperoxidase method. J. Histochem. Cytochem., *35*, 1457–1460.

MARDASSI, H. – WILSON, L. – MOUNIR, S. – DEA, S. (1994a): Detection of porcine reproductive and respiratory syndrome virus and efficient differentiation between Canadian and European strains by reverse transcription and PCR amplification. J. Clin. Microbiol., *32*, 2197–2203.

MARDASSI, H. – ATHANASSIOUS, R. – MOUNIR, S. – DEA, S. (1994b): Porcine reproductive and respiratory syndrome virus: Morphological, biochemical and serological characteristics of Quebec isolates associated with acute and chronic outbreaks of porcine reproductive and respiratory syndrome. Can. J. Vet. Res., *58*, 55–64.

MENG, X. J. – PAUL, P. S. – HALBUR, P. G. – LUM, M. A. (1995): Phylogenetic analysis of the putative M (ORF6) and N (ORF7) genes of porcine reproductive and respiratory syndrome virus (PRRSV): implication for the existence of two genotypes of PRRSV in the U.S.A. and Europe. Arch. Virol., *140*, 745–755.

MENGELING, W. L. – LAGER, K. M. – VORWALD, A. C. (1995): Diagnosis of porcine reproductive and respiratory syndrome. J. Vet. Diagn. Invest., *7*, 3–16.

MEREDITH, M. J. (1995): Porcine Reproductive and Respiratory Syndrome (PRRS). 1th Eur. ed. University of Cambridge, Pig Disease Information Centre. 78 pp.

PARK, B. K. – YOON, I. J. – JOO, H. S. (1996): Pathogenesis of plaque variants of porcine reproductive and respiratory syndrome virus in pregnant sows. Am. J. Vet. Res., *57*, 320–323.

ŠMÍD, B. – VALÍČEK, L. – MENŠÍK, J. (1977): Obtaining pig pulmonary macrophages for the cultivation of pig cytomegaloviruses. (In Czech). Vet. Med. – Czech., *21*, 589–595.

VALÍČEK, L. – PŠIKAL, I. – ŠMÍD, B. – RODÁK, L. – KUBALÍKOVÁ, R. (1995a): First detection PRRS in the Czech Republic. In: Abstr. 2nd Int. Symp. PRRS, Copenhagen Denmark, p. 46.

VALÍČEK, L. – PŠIKAL, I. – ŠMÍD, B. – RODÁK, L. – KUBALÍKOVÁ, R. (1995b). First detection of porcine reproductive and respiratory syndrome in the Czech Republic (In Czech). Veterinářství, *45*, 486–488.

VALÍČEK, L. – PŠIKAL, I. – ŠMÍD, B. – RODÁK, L. – KUBALÍKOVÁ, R. (1996): Detection and epidemiology of PRRS in the Czech Republic. In: Proc. 14th IPVS Congr., Bologna, Italy, p. 87.

VALÍČEK, L. – PŠIKAL, I. – BARTÁK, P. – KUBALÍKOVÁ, R. – KOSINOVÁ, E. – ŠMÍD, B. (1997): Porcine reproductive and respiratory syndrome (PRRS). The role of the boars in virus transmission (In Czech). Veterinářství, *47*, 158–160.

WENSVOORT, G. (1993): Lelystad virus and the porcine epidemic abortion and respiratory syndrome. Vet. Res., *24*, 117–124.

WENSVOORT, G. (1994): Porcine reproductive and respiratory syndrome. In: Proc. 13th IPVS Congr., Bankok, Thailand, pp. 11–14.

WENSVOORT, G. – TERPSTRA, C. – POL, J. M. A. – Ter LAAK, E. A. – BLOEMRAAD, M. – de KLUYVER, E. P. – KRAGTEN, C. – van BUITEN, L. – den BESTEN, A. – WAGENAAR, F. – BROEKHUIJSEN, J. M. – MOONEN, P. L. J. M. – ZETSTRA, T. – de BOER, E. A. – TIBBEN, H. H. – de JONG, M. F. – van't VELD, P. – GROENLAND, G. J. R. – van GENNEP, J. A. – VOETS, M. Th. – VERHEIJDEN, J. H. M. – BRAAMSKAMP, J. (1991): Mystery swine disease in the Netherlands: the isolation of Lelystad virus. Vet. Quart., *13*, 121–130.

Received 97-06-11
Accepted after corrections: 97-06-20

Contact Address:

MVDr. Lubomír Valíček, DrSc., Výzkumný ústav veterinárního lékařství, Hudcova 70, 621 32 Brno, Česká republika
Tel. 05/41 32 12 41, fax 05/41 21 12 29, e-mail: l.valicek<kahr@vuvel.anet.cz>vuvel/uluva

ÚSTŘEDNÍ ZEMĚDĚLSKÁ A LESNICKÁ KNIHOVNA, PRAHA 2, SLEZSKÁ 7

Ústřední zemědělská a lesnická knihovna v Praze (dále jen ÚZLK), která je jednou z největších zemědělských knihoven na světě, byla založena v roce 1926. Již od počátku šlo o knihovnu veřejnou. Knihovna v současné době obsahuje více než jeden milion svazků knih, cestovních zpráv, dizertací, literatury FAO, svázaných ročníků časopisů z oblasti zemědělství, lesnictví, veterinární medicíny, ekologie a dalších oborů. Knihovna odebírá 750 titulů domácích a zahraničních časopisů. Informační prameny získané do fondu jsou v ÚZLK zpracovávány do systému katalogů – je budován jmenný katalog a předmětový katalog jako základní katalogy knihovny a dále různé speciální katalogy a kartotéky. Počátkem roku 1994 přistoupila ÚZLK k automatizovanému zpracování knihovního fondu v systému CDS/ISIS.

Pro informaci uživatelů o nových informačních pramenech ve fondech ÚZLK zpracovává a vydává knihovna následující publikace: Přehled novinek ve fondu ÚZLK, Seznam časopisů objednaných ÚZLK, Přehled rešerší a tematických bibliografií z oboru zemědělství, lesnictví a potravinářství, AGROFIRM – zpravodaj o přírůstcích firemní literatury (je distribuován na disketách), AGROVIDEO – katalog videokazet ÚZLK.

V oblasti mezinárodní výměny publikací knihovna spolupracuje s 800 partnery ze 45 zemí světa. Knihovna je členem IAALD – mezinárodní asociace zemědělských knihovníků. Od září 1991 je členem mezinárodní sítě zemědělských knihoven AGLINET a od 1. 1. 1994 je depozitní knihovnou materiálů FAO pro Českou republiku.

Knihovna poskytuje svým uživatelům následující služby:

Výpůjční služby

Výpůjční služby jsou poskytovány všem uživatelům po zaplacení ročního registračního poplatku. Mimopražští uživatelé mohou využít možností meziknihovní výpůjční služby. Vzácné publikace a časopisy se však půjčují pouze prezenčně.

Reprografické služby

Knihovna zabezpečuje pro své uživatele zhotovování kopií obsahů časopisů a následné kopie vybraných článků. Na počkání jsou zhotovovány kopie na přání uživatelů. Pro pražské a mimopražské uživatele jsou zabezpečovány tzv. individuální reprosložby.

Služby z automatizovaného systému firemní literatury

Jsou poskytovány z databáze firemní literatury, která obsahuje téměř 13 000 záznamů 1 700 firem.

Referenční služby

Knihovna poskytuje referenční služby vlastních databází knižních novinek, odebíraných časopisů, rešerší a tematických bibliografií, vědeckotechnických akcí, firemní literatury, videotéky, dále z databází převzatých – Celostátní evidence zahraničních časopisů, bibliografických databází CAB a Current Contents. Cílem je podat informace nejen o informačních pramenech ve fondech ÚZLK, ale i jiné informace zajímající zemědělskou veřejnost.

Půjčování videokazet

V AGROVIDEU ÚZLK jsou k dispozici videokazety s tematikou zemědělství, ochrany životního prostředí a příbuzných oborů. Videokazety zasílá AGROVIDEO mimopražským zájemcům poštou.

Uživatelům knihovny slouží dvě studovny – všeobecná studovna a studovna časopisů. Obě studovny jsou vybaveny příručkovou literaturou. Čtenáři zde mají volný přístup k novinkám přírůstků knihovního fondu ÚZLK.

Adresa knihovny:

Ústřední zemědělská a lesnická knihovna
Slezská 7
120 56 Praha 2

Výpůjční doba:

pondělí, úterý, čtvrtek:	9.00–16.30
středa	9.00–18.00
pátek	9.00–13.00

Telefonické informace:

vedoucí:	24 25 50 74, e-mail: IHOCH@uzpi.agrec.cz
referenční služby:	24 25 79 39/linka 520
časopisy:	24 25 66 10
výpůjční služby:	24 25 79 39/linka 415
meziknihovní výpůjční služby:	24 25 79 39/linka 304
Fax:	24 25 39 38
E-mail:	ÚZLK@uzpi.agrec.cz

EFFECT OF AN ADRENERGIC AGONIST AND A CHOLINERGIC ANTAGONIST ON THE AIRWAY EPITHELIUM*

ÚČINEK LÉKŮ ZE SKUPINY SYMPATOMIMETIK A PARASYMPATOLYTIK NA EPITEL DÝCHACÍCH CEST

V. Konrádová[1], J. Uhlík[1], L. Vajner[1], J. Zocová[2]

[1] *Institute of Histology and Embryology, 2nd Faculty of Medicine, Charles University, Praha, Czech Republic*

[2] *Department of Applied Mathematics and Computer Science, Faculty of Science, Charles University, Praha, Czech Republic*

ABSTRACT: The ultrastructure of the rabbit tracheal epithelium was studied 30 minutes after intratracheal administration of two puffs of salbutamol and ipratropium bromide, respectively. The injury to the tracheal epithelium due to the treatment with both bronchospasmolytic drugs was considered moderate to severe. In both experimental groups, the degree of goblet cells' stimulation did not differ significantly, the ciliated cells were less damaged compared with the goblet ones and the morphological signs of the impaired self-cleaning ability were revealed.

airways; epithelium; bronchospasmolytics; ultrastructure

ABSTRAKT: Studovali jsme ultrastrukturu epitelu dýchacích cest králíků 30 minut po intratracheální aplikaci dvou dávek aerosolu salbutamolu a ipratropium bromidu. Poškození tracheálního epitelu po aplikaci obou bronchospasmolytik jsme hodnotili na hranici mezi středně závažným a těžkým. Stupeň stimulace pohárkových buněk se po podání obou látek statisticky významně nelišil. Řasinkové buňky byly ve srovnání s pohárkovými buňkami méně poškozeny. Nalezli jsme morfologické známky narušení samočisticí schopnosti epitelu.

dýchací cesty; epitel; bronchospasmolytika; ultrastruktura

INTRODUCTION

Using a light microscope, Spahr-Schopfer and Shorten with their fellow workers (Spahr-Schopfer et al., 1994; Shorten et al., 1995) have recently described the adverse effect of a β_2-adrenergic agonist – salbutamol – on the airway epithelium. The authors employed rather high doses of this substance (2 mg and 0.5 mg) in their experiments. We therefore decided to study the effect of a therapeutic dose of salbutamol (Ventolin) and also to compare its effect with that of a cholinergic antagonist – ipratropium bromide (Atrovent) – on the ultrastructure of the tracheal epithelium.

MATERIAL AND METHODS

In our nexperiments, nine healthy rabbits (body weight 1 500–3 000 g) were used. Three of them served as untreated controls, the remaining ones were divided into two groups of three animals each. The metered-dose inhaler containing either salbutamol (Ventolin, Glaxo, Aranda de Duero, Spain) or ipratropium bromide (Atrovent, Boehringer, Ingelheim am Rhein, FRG) was connected with a long thin catheter that was inserted into the mouth of a rabbit under general anesthesia and two puffs of aerosol were administered into its airways. For general anaesthesia, we used a mixture of ketamine (Narkamon 5% inj., Léčiva Praha, 35 mg/kg), and xylazine (Rometar 2% inj. ad usum vet., Spofa Praha, 5 mg/kg), administered intramuscularly. Three rabbits were thus treated with 0.2 mg of salbutamol and 0.04 mg of ipratropium bromide, respectively. The material for the electron microscopic examination was collected 30 minutes post exposure. Tiny fragments of the tracheal mucous membrane were processed using standard methods, and the ciliary border and the functional state of the goblet cells were evaluated quantitatively using our methods described in a previous paper (Konrádová and Šrajer, 1987). In controls and after administration of two puffs of salbutamol and ipratropium bromide 1 058 μm^2, 2 145.25 μm^2 and 2 657.75 μm^2 of ciliary border with

* Supported by the IGA, Ministry of Health of the Czech Republic (Grant No. 2859).

10 252, 11 092 and 13 628 kinocilia were evaluated, respectively. In those experimental groups, also a total of 186, 384 and 427 goblet cells were studied, respectively. The goblet cells were classified into three groups: mucus-filled, mucus-discharging and exhausted, degenerated ones. Kinocilia were classified into four groups: intact 9 + 2 cilia, slightly damaged pathological cilia with local swellings of the ciliary membrane or with tiny vacuoles situated in their shafts, degenerating cilia, represented by axonemes incorporated into the cytoplasmic blebs, and malformed cilia with either abnormal arrangement or number of microtubules in their axonemes. The used statistical methods were described in detail previously (K o n r á d o v á et al., 1996b).

RESULTS

In control rabbits, the tracheae were lined with the ciliated pseudostratified columnar epithelium. The ciliated cells of standard ultrastructure were the most numerous in the epithelium. Goblet cells, mostly filled with mucus, were scattered among the ciliated ones. Only 3 ± 1% of them discharged their secretion by means of gradual evacuation of the individual apical mucous granules (Tab. I). In the regular ciliary border, 9.7 ± 0.3 cilia per 1 μm^2 were found. 98.8 ± 0.1% of cilia were intact. The proportions of pathological, degenerating and malformed cilia are given in Tab. I.

In isolated voluminous protrusions, a great number of axonemes of degenerating cilia was encountered. In the deeper portions of ciliated cells' cytoplasm, a slight increase in the number of small vacuoles and tiny lysosomes, together with the dilatation of the cisternae of granular endoplasmic reticulum and of the Golgi complex were observed. Inhalation of 0.04 mg of ipratropium bromide caused only very slight apical blebbing and increase in the number of tiny vacuoles and lysosomes (Fig. 2).

In both experimental groups, the goblet cells were found as isolated elements among the ciliated ones. Only 9 ± 1% and 7 ± 3% of them were arranged in tiny groups, respectively. More than 90% of goblet cells were stimulated to discharge mucus (Tab. I). The stimulated goblet cells communicated widely with the lumen of the respiratory passages. Mucus was evacuated simultaneously from numerous apical mucous granules and packets of mucous granules were also detached from the goblet cells. In some stimulated goblet cells, chain fusion of the adjacent mucous granules' membranes was noticed. After salbutamol and ipratropium bromide administration, the completely exhausted degenerated secretory cells amounted to 79 ± 3% and 85 ± 2%, respectively (Fig. 3). The exhausted degenerated elements protruded above the level of the surrounding cells and after sloughing off, remnants of their electron-dense degenerated cytoplasm were observed in the area of the ciliary border.

I. Quantitative evaluation of the goblet cells (GC) and ciliary border in the trachea of rabbits 30 minutes after inhalation of 2 puffs of two bronchospasmolytics

	Controls	Salbutamol	Ipratropium bromide
Nonstimulated GC	97 ± 1%	*9 ± 1%	*5 ± 2%
Mucus-discharging GC	3 ± 1%	*12 ± 3%	10 ± 2%
Degenerated GC	0	*79 ± 3%	*85 ± 2%
Stimulated GC total	3 ± 1%	*91 ± 1%	*95 ± 1%
GC arranged in groups	6 ± 3%	9 ± 1%	7 ± 3%
Number of cilia per 1 μm^2 of ciliary border	9.7 ± 0.3	*5.2 ± 0.2	*5.1 ± 0.2
Intact cilia	98.8 ± 0.1%	*91.4 ± 2.9%	*97.4 ± 1.1%
Pathological cilia	0.5 ± 0.2%	0.8 ± 0.4%	*1.6 ± 1.0%
Degenerating cilia	0.3 ± 1%	*7.4 ± 2.1%	0.4 ± 0.2%
Malformed cilia	0.4 ± 0.2%	0.4 ± 0.5%	0.6 ± 0.3%
Altered cilia total	1.2 ± 0.1%	*8.6 ± 2.9%	*2.6 ± 1.1%

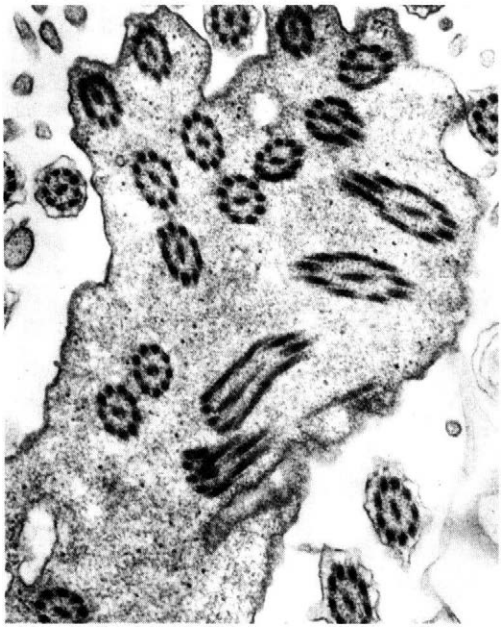
N = 3, mean ± SD, * values differ significantly ($p < 0.01$) from controls, ⌊⌋ values differ significantly ($p < 0.01$) from each other

Thirty minutes post exposure of both bronchospasmolytic drugs, rabbits' tracheae were lined by an altered pseudostratified ciliated epithelium with narrow intercellular spaces and intact apical junctional complexes.

Due to the salbutamol administration, rather numerous, small to medium sized cytoplasmic protrusions were developed on the apical portions of the ciliated cells. Inside those cytoplasmic blebs, usually only a few intact or disintegrating axonemes of kinocilia were revealed (Fig. 1).

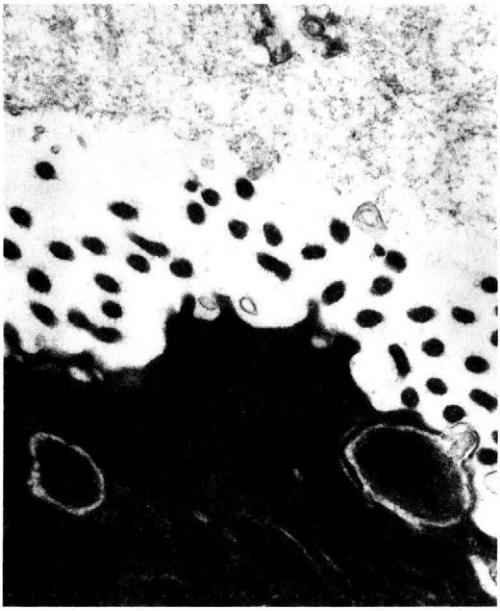
Isolated cells equipped with short irregular microvilli containing a few small rather electron-dense secretory granules in their undifferentiated cytoplasm, or secretory elements filled with small mucous granules separated by voluminous cytoplasmic septa were encountered in the tracheal epithelium after administration of both drugs.

As a result of both salbutamol and ipratropium bromide administration, the regular arrangement of the ciliary border was slightly impaired. The mean numbers

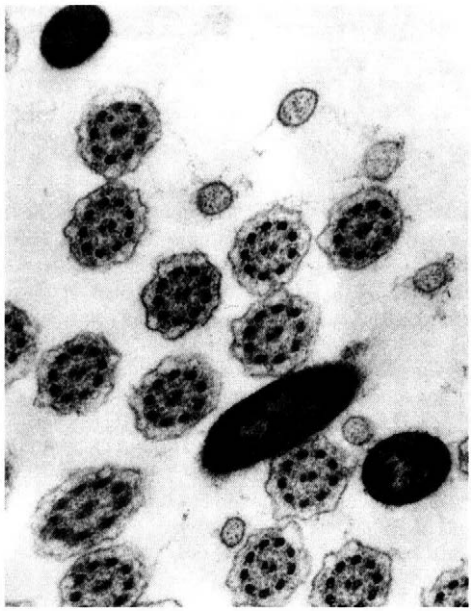


1. Cytoplasmic bleb on the apical portion of the ciliated cell containing axonemes of degenerating kinocilia. Rabbit tracheal epithelium 30 min after administration of Ventolin, 37 500x

2. Small secondary lysosome and tiny vacuoles in the cytoplasm of the ciliated cell. Rabbit tracheal epithelium 30 min after administration of Atrovent, 50 000x



3. Layer of inspissated secretion above the exhausted, degenerated goblet cell. Rabbit tracheal epithelium 30 min after administration of Atrovent, 37 000x



4. Bacteria in the area of the ciliary border. Rabbit tracheal epithelium 30 min after administration of Ventolin, 50 000x

of cilia per 1 μm^2 were 5.2 ± 0.2 and 5.1 ± 0.2, the altered elements represented 8.6 ± 2.9% and 2.6 ± 1.1%, respectively. The proportions of the individual types of altered cilia are given in Tab. I. In the area among the kinocilia, not very numerous bacteria and clumps or layers of inspissated mucus were observed (Figs. 3, 4).

DISCUSSION

The target cells for the function of both bronchospasmolytics were the goblet cells. The degree of goblet cells' stimulation caused by salbutamol and ipratropium bromide administration did not differ significantly. Inhalation of aerosols of both bronchospasmolytics significantly accelerated mucus release and influenced the mechanism of secretion. As a sign of apocrine type of secretion, the detachment of whole packets of mucous granules was frequently encountered. The disappearance of the adjacent mucous granules membranes, which enabled almost instantaneous evacuation of the whole mucus load, was also noticed in numerous cells. This most rapid way of mucus discharge was described in acetylcholine stimulated goblet cells not only in the small intestine (Neutra and Schaeffer, 1977; Specian and Neutra, 1980; Kurosumi et al., 1981; Roumagnac and Laboisse, 1987), but also in the airways (Konrádová et al., 1996a).

Two puffs of both bronchospasmolytic drugs also damaged the secretory elements. After a rapid release of mucus, the goblet cells did not mostly take part in further secretory cycles, but the exhausted cells degenerated. 30 minutes post exposure of both the adrenergic agonist and the cholinergic antagonist, the degree of secretory elements' degeneration did not differ significantly. The degenerated goblet cells represented about 80% of all secretory elements in both experimental groups.

In our previous study (Konrádová et al., 1990), we demonstrated that degeneration of more than a half of the goblet cells stimulated massive differentiation of new secretory elements resulting in their hyperplasia and changes in their distribution with the occurrence of intraepithelial mucous glands. As the first sign of this process, rather numerous differentiating secretory cells were noticed in the epithelium 30 minutes after the contact with aerosols of both bronchospasmolytic drugs, but the goblet cells' distribution in the epithelium was not yet influenced. In both experimental groups, the proportion of goblet cells arranged in groups did not differ significantly from that in healthy control rabbits.

As a result of bronchospasmolytics administration, the ciliated cells were less damaged compared with the goblet ones. After salbutamol inhalation, alteration and oedema of the cortical portions of the ciliated cells' cytoplasm reflected by the apical blebbing was revealed. The formation of voluminous apical cytoplasmic projections was associated with destruction of rather numerous kinocilia. Signs of the pathological alteration of the deeper portions of their cytoplasm were also encountered. On the other hand, only a few tiny apical cytoplasmic blebs and a small increase in the number of vacuoles resulted from the contact of the ciliated cells with ipratropium bromide aerosol.

The alteration of the ciliated cells was reflected in the impairment of the ciliary border. After salbutamol administration, a significant decrease in the mean number of kinocilia to 5.2 per μm^2 accompanied by the increased percentage of altered cilia was recorded. The alteration of the ciliary border was significantly less pronounced due to the administration of ipratropium bromide. Decrease of the average number of kinocilia was noticed, but the proportion of altered kinocilia did not differ significantly compared with healthy control rabbits.

In both experimental groups, the proportion of malformed cilia, containing axonemes that differ in number or arrangement of microtubules from the typical 9 + 2 inner pattern, did not differ significantly from that found in healthy control rabbits showing thus that the process of ciliogenesis was not affected by bronchospasmolytic drug administration.

After both salbutamol and ipratropium bromide inhalation, layers of condensed mucus and bacteria were discovered in the area of the ciliary border. In agreement with our previous studies (Konrádová, 1991; Konrádová et al., 1990, 1996a, b) and also with other authors who studied the relation of the cilia to the layer of mucus in the airways (Yoneda, 1976; Hulbert et al., 1982; Menco, 1989; Stratmann et al., 1991; Sturgess, 1977; Wanner et al., 1996), we regarded the appearance of inspissated secretion with numerous entrapped bacteria embedding free kinocilia as morphological signs of impaired self-cleaning ability of the tracheal epithelium. These changes were probably primarily due to the increased amount of mucus. The altered ciliated cells were apparently not capable of managing the large amount of secretion released all at once by the overstimulated goblet cells.

On the basis of our previous experiments, a classification of the degree of injury to the airway epithelium was proposed (Konrádová, 1991) – Tab. II. To estimate the airway epithelium, percentage of the stimulated goblet cells, degree of acceleration of mucus evacuation, mean number of cilia per 1 μm^2 of the ciliary border, proportion of altered cilia and appearance of the morphological signs of the impairment of the self-cleaning ability of the epithelium were taken into the consideration. According to this classification, the injury to the tracheal epithelium caused by the treatment with two puffs of both salbutamol and ipratropium bromide was considered moderate to severe (Tab. II).

Our results are in accordance with those described at the level of light microscopy by Spahr-Schopfer and Shorten with their fellow workers after administration of an adrenergic agonist – salbutamol (Spahr-Schopfer et al., 1994; Shorten et al., 1995). We demonstrated that, from the morphological point of view, there were only slight differences in the injury to the airway epithelium due to the administration of the selective β_2-adrenergic agonists and the cholinergic antagonists.

II. Evaluation of the degree of damage to the airway epithelium of rabbis 30 minutes after inhalation of 2 puffs of two bronchospasmolytics

	Control rabbits	Degree of damage			Salbutamol	Ipratropium bromide
		mild	moderate	severe		
Stimulated GC	<3%	3–50%	50–90%	>90%	91%	95%
Ratio discharging GC / degenerated GC	degenerated GC not found	>1	0.1–1	<0.1	0.15	0.12
Number of cilia per μm^2	>9	7–9	3–7	<3	5.2	5.1
Altered cilia	<1.2%	1.2–2.0%	2.0–10.0%	>10.0%	8.6%	2.6%
Signs of impairment of the self cleaning ability	0	0	+	++	+	+

GC = goblet cells

REFERENCES

HULBERT, W. C. – FORSTER, B. B. – LAIRD, W. – PIHL, C. E. – WALKER, D. C. (1982): An improved method for fixation of the respiratory epithelial surface with the mucous and surfactant layers. Lab. Invest., *47*, 354–363.

KONRÁDOVÁ, V. (1991): Quantitative evaluation of the degree of damage to tracheal epithelium. Funct. Develop. Morphol., *1*, 47–50.

KONRÁDOVÁ, V. – ŠRAJER, J. (1987): Quantitative evaluation of the ciliary border of the epithelium of the rabbit trachea and the human primary bronchus. Folia Morphol., *35*, 67–74.

KONRÁDOVÁ, V. – KANTA, J. – ŠULOVÁ, J. (1990): Effect of bronchoalveolar lavage on the ultrastructure of the tracheal epithelium in rabbits. Respiration, *57*, 14–20.

KONRÁDOVÁ, V. – UHLÍK, J. – VAJNER, L. – ZOCOVÁ, J. (1996a): Reaction of the goblet cells to the cholinergic stimulation. Acta Vet., Brno, *65*, 175–180.

KONRÁDOVÁ, V. – UHLÍK, J. – ZOCOVÁ, J. – ZAJÍCOVÁ, A. (1966b): Comparison of the effect of a high and low dose of atropin on the ultrastructure of the tracheal epithelium. Respiration, *63*, 150–154.

KUROSUMI, K. – SHIBUICHI, I. – TOSAKA, K. (1981): Ultrastructural studies on the secretory mechanism of goblet cells in the jejunal epithelium. Arch. Histol. Jpn., *44*, 263–284.

MENCO, B. P. M. (1989): Olfactory and nasal respiratory epithelia and foliate taste buds visualized with rapid-freeze drying and freeze-substitution and Lowicryl K 11 M embedding. Scan. Microscopy, *3*, 257–272.

NEUTRA, M. R. – SCHAFFER, S. (1977): Membrane interactions between adjacent secretion granules. J. Cell Biol., *74*, 983–991.

ROUMAGNAC, I. – LABOISSE, C. (1987): A mucus-secreting human colonic epithelial cell line responsive to cholinergic stimulation. Biol. Cell, *61*, 65–68.

SHORTEN, G. D. – DOLOVICH, M. – ENG, P. – LERMAN, J. – CUTZ, E. (1995): Metered-dose inhaler salbutamol–induced tracheal epithelial lesions in intubated rabbits. Chest, *108*, 1668–1672.

SPAHR–SCHOPFER, I. A. – LERMAN, J. – CUTZ, E. – NEWHOUSE, M. T. – DOLOVICH, M. (1994): Proximate delivery of a large experimental dose from salbutamol MDI inducers epithelial airway lesions in intubated rabbits. Am. J. Resp. Crit. Care Med., *150*, 790–794.

SPECIAN, R. D. – NEUTRA, M. R. (1980): Mechanism of rapid mucus secretion in goblet cells stimulated by acetylcholine. J. Cell Biol., *85*, 626–640.

STRATMANN, U. – LEHMANN, R. R. – STEINBACH, T. – WESSLING, G. (1991): Effect of sulfur dioxide inhalation on the respiratory tract of the rat. Zbl. Hyg., *192*, 324–335.

STURGESS, J. M. (1977): The mucous lining of major bronchi in the rabbit lung. Am. Rev. Resp. Dis., *115*, 819–827.

WANNER, A. – SALATHÉ, M. – O'RIORDAN, T. G. (1996): Mucociliary clearance in the airways. Am. J. Resp. Crit. Care Med., *154*, 1868–1902.

YONEDA, K. (1976): Mucous blanket of rat bronchus. Am. Rev. Resp. Dis., *14*, 837–842.

Received: 97–06–16

Accepted after corrections: 97–07–07

Contact Address:

Prof. MUDr. Václava Konrádová, DrSc., Ústav histologie a embryologie 2. LF UK, V úvalu 84, 150 18 Praha, Česká republika

Tel. 02/24 43 59 80–2, fax 02/24 43 58 20, e-mail: jiri.uhlik@lfmotol.cuni.cz

Nejčerstvější informace o časopiseckých článcích poskytuje automatizovaný systém

Current Contents

na disketách

Ústřední zemědělská a lesnická knihovna odebírá časopis **„Current Contents"** řadu **„Agriculture, Biology and Environmental Sciences"** a řadu **„Life Sciences"** na disketách. Řada „Agriculture, Biology and Environmental Sciences" je od roku 1994 k dispozici i s abstrakty. Obě tyto řady vycházejí 52krát ročně a zahrnují všechny významné časopisy a pokračovací sborníky z uvedených oborů.

Uložení informací z Current Contents na disketách umožňuje nejrozmanitější referenční služby z prakticky nejčerstvějších literárních pramenů, neboť báze dat je **doplňována každý týden** a neprodleně expedována odběratelům. V systému si lze nejen prohlížet jednotlivá čísla Current Contents, ale po přesném nadefinování sledovaného profilu je možné adresně vyhledávat informace, tisknout je nebo kopírovat na disketu s možností dalšího zpracování na vlastním počítači. Systém umožňuje i tisk žádanek o separát apod. Kumulované vyhledávání v šesti číslech Current Contents najednou velice urychluje rešeršní práci.

Přístup k informacím Current Contents je umožněn dvojím způsobem:

1) **Zakázkový přístup** – po vyplnění příslušného zakázkového listu (objednávky) je vhodný především pro mimopražské zájemce.
   Finanční podmínky: – použití PC – 15 Kč za každou započatou půlhodinu
   – odborná obsluha – 10 Kč za 10 minut práce
   – vytištění rešerše – 1 Kč za 1 stranu A4
   – žádanky o separát – 1 Kč za 1 kus
   – poštovné + režijní poplatek 15 %
2) **„Self-service"** – samoobslužná práce na osobním počítači v ÚZLK.
   Finanční podmínky jsou obdobné. Vzhledem k tomu, že si uživatel zpracovává rešerši sám, je to maximálně úsporné. (Do kalkulace cen nezapočítáváme cenu programu a databáze Current Contents.)

V případě Vašeho zájmu o tyto služby se obraťte na adresu:

Ústřední zemědělská a lesnická knihovna
Dr. Bartošová
Slezská 7
120 56 Praha 2
Tel.: 02/24 25 79 39, l. 520, fax: 02/24 25 39 38

Na této adrese obdržíte bližší informace a získáte formuláře pro objednávku zakázkové služby. V případě „self-servisu" je vhodné se předem telefonicky objednat. V případě zájmu je možné si objednat i průběžné sledování profilu (cena se podle složitosti zadání pohybuje čtvrtletně kolem 100 až 150 Kč).

POKYNY PRO AUTORY

Časopis uveřejňuje původní vědecké práce, krátká sdělení a výběrově i přehledné referáty, tzn. práce, jejichž podkladem je studium literatury a které shrnují nejnovější poznatky v dané oblasti. Práce jsou uveřejňovány v češtině, slovenštině nebo angličtině. Rukopisy musí být doplněny krátkým a rozšířeným souhrnem. Časopis zveřejňuje i názory, postřehy a připomínky čtenářů ve formě kurzívy, glosy, dopisu redakci, diskusního příspěvku, kritiky zásadního článku apod., ale i zkušenosti z cest do zahraničí, z porad a konferencí.

Autoři jsou plně odpovědni za původnost práce a za její věcnou i formální správnost. K práci musí být přiloženo prohlášení o tom, že práce nebyla publikována jinde.

O uveřejnění práce rozhoduje redakční rada časopisu, a to se zřetelem k lektorským posudkům, vědeckému významu a přínosu a kvalitě práce. Redakce přijímá práce imprimované vedoucím pracoviště nebo práce s prohlášením všech autorů, že se zveřejněním souhlasí.

Rozsah původních prací nemá přesáhnout 10 stran psaných na stroji včetně tabulek, obrázků a grafů. V práci je nutné používat jednotky odpovídající soustavě měrových jednotek SI.

Rukopis má být napsán na papíře formátu A4 (30 řádek na stránku, 60 úhozů na řádku, mezi řádky dvojité mezery). K rukopisu je vhodné přiložit disketu s textem práce, popř. s grafickou dokumentací pořízenou na PC s uvedením použitého programu. Tabulky, grafy a fotografie se dodávají zvlášť, nepodlepují se. Na všechny přílohy musí být odkazy v textu.

Pokud autor používá v práci zkratek jakéhokoliv druhu, je nutné, aby byly alespoň jednou vysvětleny (vypsány), aby se předešlo omylům. V názvu práce a v souhrnu je vhodné zkratek nepoužívat.

Název práce (titul) nemá přesáhnout 85 úhozů a musí dát přesnou představu o obsahu práce. Jsou vyloučeny podtitulky článků.

Krátký souhrn (Abstrakt) musí vyjádřit všechno podstatné, co je obsaženo v práci, a má obsahovat základní číselné údaje včetně statistických hodnot. Nemá překročit rozsah 170 slov. Je třeba, aby byl napsán celými větami, nikoliv heslovitě.

Rozšířený souhrn prací v češtině nebo slovenštině je uveřejňován v angličtině, měly by v něm být v rozsahu cca 1–2 strojopisných stran komentovány výsledky práce a uvedeny odkazy na tabulky a obrázky, popř. na nejdůležitější literární citace. Je vhodné jej (včetně názvu práce a klíčových slov) dodat v angličtině, popř. v češtině či slovenštině jako podklad pro překlad do angličtiny.

Literární přehled má být krátký, je třeba uvádět pouze citace mající úzký vztah k problému. Tato úvodní část přináší také informaci, proč byla práce provedena.

Metoda se popisuje pouze tehdy, je-li původní, jinak postačuje citovat autora metody a uvádět jen případné odchylky. Ve stejné kapitole se popisuje také pokusný materiál a způsob hodnocení výsledků.

Výsledky tvoří hlavní část práce a při jejich popisu se k vyjádření kvantitativních hodnot dává přednost grafům před tabulkami. V tabulkách je třeba shrnout statistické hodnocení naměřených hodnot. Tato část by neměla obsahovat teoretické závěry ani dedukce, ale pouze faktické nálezy.

Diskuse obsahuje zhodnocení práce, diskutuje se o možných nedostatcích a výsledky se konfrontují s údaji publikovanými (požaduje se citovat jen ty autory, jejichž práce mají k publikované práci bližší vztah). Je přípustné spojení v jednu kapitolu spolu s výsledky.

Literatura citovaná v textu práce se uvádí jménem autora a rokem vydání. Do seznamu se zařadí jen publikace citované v textu. Citace se řadí abecedně podle jména prvních autorů.

Klíčová slova mají umožnit vyhledání práce podle sledovaných druhů zvířat, charakteristik jejich zdravotního stavu, podmínek jejich chovu, látek použitých k jejich ovlivnění apod. Jako klíčová slova není vhodné používat termíny uvedené v nadpisu práce.

Na zvláštním listě uvádí autor plné jméno (i spoluautorů), akademické, vědecké a pedagogické tituly a podrobnou adresu pracoviště s PSČ, číslo telefonu a faxu, popř. e-mail.

Podrobné pokyny pro autory lze vyžádat v redakci.

Applications for detailed instructions for authors should be sent to the editorial office.

INSTRUCTIONS FOR AUTHORS

Original scientific papers, short communications, and selectively reviews, that means papers based on the study of technical literature and reviewing recent knowledge in the given field, are published in this journal. Published papers are in Czech, Slovak or English. Each manuscript must contain a short or a longer summary. The journal also publishes readers' views, remarks and comments in form of a text in italics, gloss, letter to the editor, short contribution, review of a major article, etc., and also experience of stays in foreign countries, meetings and conferences.

The authors are fully responsible for the originality of their papers, for its subject and formal correctness. The authors shall make a written declaration that their papers have not been published in any other information source.

The board of editors of this journal will decide on paper publication, with respect to expert opinions, scientific importance, contribution and quality of the paper. The editors accept papers approved to print by the head of the workplace or papers with all the authors' statement they approve it to print.

The extent of original papers shall not exceed ten typescript pages, including tables, figures and graphs.

Manuscript should be typed on standard paper (quarto, 30 lines per page, 60 strokes per line, double-spaced typescript). A PC diskette with the paper text or graphical documentation should be provided with the paper manuscript, indicating the used editor program. Tables, figures and photos shall be enclosed separately. The text must contain references to all these annexes.

The **title** of the paper shall not exceed 85 strokes and it should provide a clear-cut idea of the paper subject. Subtitles of the papers are not allowed either.

Abstract. It must present information selection of the contents and conclusions of the paper, it is not a mere description of the paper. It must present all substantial information contained in the paper. It shall not exceed 170 words. It shall be written in full sentences, not in form of keynotes and comprise base numerical data including statistical data.

Introduction has to present the main reasons why the study was conducted, and the circumstances of the studied problems should be described in a very brief form. This introductory section also provides information why the study has been undertaken.

Review of literature should be a short section, containing only literary citations with close relation to the treated problem.

Only original method shall be described, in other cases it is sufficient enough to cite the author of the used method and to mention modifications of this method. This section shall also contain a description of experimental material and the method of result evaluation.

In the section **Results**, which is the core of the paper, figures and graphs should be used rather than tables for presentation of quantitative values. A statistical analysis of recorded values should be summarized in tables. This section should not contain either theoretical conclusions or deductions, but only factual data should be presented here.

Discussion contains an evaluation of the study, potential shortcomings are discussed, and the results of the study are confronted with previously published results (only those authors whose studies are in closer relation with the published paper should be cited). The sections Results and Discussion may be presented as one section only.

References in the manuscript are given in form of citations of the author's name and year of publication. A list of references should contain publications cited in the manuscript only. References are listed alphabetically by the first author's name.

Key words should make it possible to retrieve the paper on the basis of the animal species investigated, characteristics of their health, husbandry conditions, applied substances, etc. The terms used in the paper title should not be used as keywords.

If any abbreviation is used in the paper, it is necessary to mention its full form at least once to avoid misunderstanding. The abbreviations should not be used in the title of the paper nor in the summary.

The author shall give his full name (and the names of other collaborators), academic, scientific and pedagogic titles, full address of his workplace and postal code, telephone and fax number, or e-mail.

VETERINARY MEDICINE – CZECH

Volume 42, No. 10, October 1997

CONTENTS

VETERINÁRNÍ MEDICÍNA

Ročník 42, č. 10, Říjen 1997

OBSAH

Vědecký časopis VETERINÁRNÍ MEDICÍNA ● Vydává Ústav zemědělských a potravinářských informací ● Redakce: Slezská 7, 120 56 Praha 2, tel.: 02/24 25 79 39, fax: 02/24 25 39 38 ● Sazba: Studio DOMINO – ing. Jakub Černý, Bří. Nejedlých 245, 266 01 Beroun, tel.: 0311/229 59 ● Tisk: ÚZPI Praha ●

Rozšiřuje Ústav zemědělských a potravinářských informací, referát odbytu, Slezská 7, 120 56 Praha 2