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Állatorvos-tudományi Doktori Iskola

Some aspects of urogenital tract diseases of female breeding swine

Theses

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1. Introduction and study goals

Urogenital tract diseases of female breeding swine are well-known problems in all major swine producing areas of the world, and reportedly account for significant losses in the industry. Our previous practical experience suggested that Hungarian herds are not free from the negative effects of urogenital tract diseases either. However, this subject did not receive enough attention in recent domestic veterinary literature. One of our goals was to draw attention to this subject.

As revealed by the literature survey, *Actinobaculum suis* is widely indicated as a leading cause of urinary tract disease in female swine. However, its occurrence has not been reported before in Hungary. A bacteriological and histopathological study was undertaken to prove the presence of this bacterium in Hungarian swine herds.

Therapy of urogenital tract infections, although many times rather frustrating, almost solely utilizes different antimicrobials. Information on antibiotic sensitivity patterns of facultatively pathogenic bacteria of the urinary tract, and especially of *Actinobaculum suis* strains is quite limited. Very few recent studies on the *in vitro* sensitivity of *A. suis* strains are available. Novel antibiotics with a possible use in the treatment of urinary tract infections were introduced to the market recently, for which effectiveness against *A. suis* has not been determined yet. An *in vitro* study was performed to determine the antibiotic sensitivity of Hungarian isolates of *A. suis*, with a special reference to novel antimicrobials, including but not limited to florfenicol, marbofloxacin, enrofloxacin, and doxycycline. As the disk diffusion method would be a more practical choice for determining *in vitro* sensitivity of *A. suis* isolates than the agar dilution method, we have also ventured to compare these two methodologies. Another aim of this study was to compare sensitivity patterns of our own isolates and of the type strain of *A. suis*.

Slaughterchecks are integrated part of reproductive problem solving in many countries. Their use is especially indicated in cases of noninfectious reproductive failure and urogenital tract problems. There are quite a few reports available from other countries on this subject, whereas Hungarian publications are scarce. As a part of our diagnostic work directed to solve reproductive problems of some Hungarian swine farms, we have conducted slaughterchecks and performed supplementary examinations on the urogenital apparatus of culled sows and gilts.

One of the aims of this study was to report prevalence values of urogenital tract lesions of sows and gilts culled for reproductive failure in Hungary. Also, we have tried to estimate the magnitude of agreement between results of macroscopic and/or bacteriological diagnosis and histopathology in case of urocystitis and endometritis, in order to provide sensitivity and specificity estimates. Our another goal was to prove or disprove putative associations between urocystitis and non-specific endometritis, with controlling for a possible major confounding factor, parity.

2. Materials and Methods

2. 1. First detection of *Actinobaculum suis* in Hungary

The aim of this investigation was detection of *A. suis* in the prepuce of boars on some Hungarian farms and its isolation from clinical cases of urocystitis in sows.

Samples were collected in the course of our diagnostic work on reproductive problems in several swine herds. Three farms were involved in the present examination. On the first farm six, on the second ten preputial swab samples were taken from actively used healthy mature boars. Swabs were transported cooled and in appropriate transport medium to the laboratory. All samples were processed within 4 h after collection.

At an abattoir whole urogenital tracts (both kidneys, ureters, urinary bladder, ovaries, oviducts, uterine horns, cervix and vagina) were removed from five sow carcasses originating from the third farm. The neck of urinary bladders and the terminal portion of the vagina were tied with a string to prevent fecal contamination of the upper urogenital tract during transportation. All samples were transported cooled and were processed within 4-5 h.

The preputial swab samples were streaked individually on blood agar plates containing 5% defibrinated sterile horse blood and colistine-nalidixic acid-metronidazole supplemented Columbia agar plates (CCNAM). They were cultured at 37°C for 6-7 days under anaerobic conditions. Urinary bladder samples, taken with sterile cotton tipped swabs were inoculated on two horse blood agars, a CCNAM plate and a crystal violet-lactose-bromthymol blue agar plate. In the case of each bladder sample, one blood agar and the crystal violet-lactose-bromthymol blue agar were incubated under aerobic conditions at 37°C for 24 h. The isolated bacteria were identified using standard methods. Reference strain of *A. suis* DSM 20.639 = ATCC 33144 purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) was used in the bacteriological examinations.

Characteristic colonies were subcultured on horse blood agar plates under anaerobic conditions at 37°C for 4-5 days. Morphological and biochemical characteristics of cultured bacteria were determined using standard methods and were compared to the type strain of *A. suis*.

Oxygen tolerance was examined by inoculating 4 own isolates and the type strain onto horse blood agar plates and incubating them aerobically at 37°C for 10 days. Aerobically grown bacteria were inoculated onto horse blood agar plates and were incubated afterwards at 37°C for 4-5 days in anaerobic atmosphere again.

All urinary bladder, ureter and kidney samples were processed for histopathological evaluation and were examined for the presence of inflammatory reactions.

2. 2. In vitro sensitivity of Hungarian *Actinobaculum suis* strains to selected antimicrobials

The main aim of this study was to determine in vitro antimicrobial sensitivity pattern of recently isolated Hungarian *A. suis* strains. In vitro sensitivity of anaerobic bacteria is generally determined using the agar dilution method however, valuable results were gained even in the case of obligate anaerobic bacteria using the disc diffusion method. Since *A. suis* is reportedly not a true obligate anaerobic bacterium, and its propagation is not as slow as of most obligate anaerobic bacteria, we also intended to assess the feasibility of using the disc diffusion method as a possible practical alternative for the determination of sensitivity of *A. suis* isolates. Furthermore, we have intended to compare resistance patterns of our own isolates with the type strain of *A. suis*.

The type strain and twelve Hungarian *A. suis* strains were used in this study. Eight of the latter were isolated in a previous investigation, two were isolated later from the prepuce of healthy boars, two were cultured from cases of haemorrhagic cystitis of sows. All of the isolates were identified by biochemical methods and were compared to the type strain.

Antibiotic sensitivity testing was done by both the agar diluton and the disc diffusion method. Twenty one antimicrobials: penicillin, ampicillin, ceftiofur, gentamicin, neomycin, streptomycin, spectinomycin, oxytetracycline, doxycycline, lincomycin, tylosin, erythromycin, tiamulin, valnemulin, chloramphenicol, florfenicol, nalidixic acid, flumequine, enrofloxacin, ofloxacin, sulfamethoxazole + trimethoprim were tested in the agar dilution method. The same antimicrobials, plus clavulanate potentiated amoxicillin, cefotaxim, and marbofloxacin were used in the disc diffusion method.

For the agar dilution method, twofold serial dilutions of each antimicrobial compound were prepared to yield twelve final test concentrations, ranging from 0,05 to 100 μg/ml. In case of each bacterial strain, 5 μl of an approximately 1,5 x 10^4 CFU/ml bacterial suspension were streaked on Westphal agar plates containing 5% defibrinated sterile sheep blood and the tested concentration of each antimicrobial. A similar plate without antimicrobials was used as a control for the presence of bacterial growth. The minimal inhibitory concentration was determined as the lowest dilution of the antimicrobial where bacterial growth was not visible.

For the disc diffusion method, approx. 10 μl of a McFarland No. 1. suspension of each bacterial strain was streaked on a Westphal agar plate containing 5% defibrinated sterile sheep blood and four commercially obtained sensitivity discs were placed on each agar plate at maximum. Sensitivity of the strains to a given antibacterial compound was evaluated following general guidelines in the disc manufacturer's instructions.

The culture media was selected as being the most suitable for growth of *A. suis* according to our previous experiences. Plates were cultured at 37°C for 3-4 days under anaerobic conditions.

A Spearman's correlation coefficient was determined for paired MIC and disc diffusion results of each strain. For this purpose, MIC values were categorized as indicating "sensitivity" (0.05-3.125 μg/ml), "intermediate sensitivity" (6.25-12.5 μg/ml) and "resistance" (25-100 μg/ml). The boundaries of these categories were chosen arbitrarily. Mean MIC value of the type strain were compared to mean MIC_{50} value of our own isolates by the two sample Mann-Whitney test.

2. 3. Association between endometritis and urocystitis in culled sows

We estimated the prevalence of different reproductive-tract lesions found at swine reproductive slaughterchecks in Hungary. Furthermore, we assessed the sensitivity and specificity of macroscopic and/or bacteriological examinations in the diagnosis of endometritis and urocystitis. We also examined the association between these two conditions accounting for the possible confounding effect of parity, by using Mantel-Haenszel analysis. Examinations on the reproductive organs of culled sows and gilts collected at slaughter were performed over a 6-year period (1995-2000). Twenty-one large swine herds from the main pig producing regions of Hungary were included. Sow herd size ranged from 300 to 2000 (median:

750). A total of 499 sows were examined (range 4-53/farm). Out of this sample, due to loss or unavailability, data from only 353 animals (range 1-49/farm) were analyzed.

Method of slaughterhouse sample collection was similar to that described by several authors. Whole reproductive tracts (ovaries, oviducts, uterine horns and body, cervix, vagina, vestibule, and vulva) and the urinary bladder with the urethra were collected from the viscera trays into clean plastic boxes. Kidneys and ureters were not examined, because their safe collection was prevented by the high line speed. The neck of the bladder and the vagina were tied with a string to lessen their bacterial contamination during transport. Samples were then transferred to clean plastic bags, and were transported to our laboratory in cooling boxes.

All urogenital tracts were completely dissected after collecting samples for bacteriology. Lesions were described by the same person in all cases. Endometritis was diagnosed macroscopically when any thick, purulent material, or thin, turbid fluid without developmental anomaly of the tubular genital tract was present in the lumen of at least one of the horns. Content of the bladder, thickness of its wall, and the state of urothelium (integrity, fluid content, and vascularization) were recorded. Urocystitis was diagnosed macroscopically upon the following lesions: 1.) at least moderate diffuse congestion of the urothelium, or 2) substantial thickening of the wall or 3.) presence of mucosal oedema and hemorrhages, or 4.) presence of purulent material or fibrin flecks in the lumen.

Full-thickness samples (about 4 x 1 cm) from the midportion of both uterine horns, and from the fundus of the bladder were cut and fixed in 8% formaldehide solution for histopathological examinations. Hematoxylin-eosin stained slides were examined for the presence of inflammatory lesions under light microscope by the same person. Endometritis was diagnosed histopathologically: 1.) when the endometrium contained large number (>5/high-power field [400 X, HPF]) of neutrophil granulocytes and/or plasma cells in the epithelium and stratum compactum; 2.) when there was more than one focal periglandular or perivascular inflammatory cell accumulation in the stratum spongiosum; or 3.) when diffuse leucocytic infiltration of the stratum spongiosum was observed. Urocystitis was diagnosed when the mucosa contained more than one inflammatory focus subepithelially or in the propria, or more than 5 inflammatory cells/HPF were seen.

Bacterial examination of samples from both uterine horns, and from the mid-cervix and urinary bladder were processed as described previously. The same person evaluated all cultures; isolated bacteria were identified using standard methods. Cultures showing > 100 colonies/plate (pure or mixed culture) were considered positive; all other culture results were considered negative. Positive culture of one or both uterine horn samples or bladder sample indicated endometritis or urocystitis, respectively.

All data recorded and generated as above were checked twice for errors and were collected in Microsoft Excel spreadsheets. We regarded histopathology results as "Gold Standards" to which macroscopic diagnostic and bacteriological culture results were compared. Sensitivity and specificity for culture result of uterine samples and/or macroscopic evaluation of endometrium; or culture result of bladder samples and/or macroscopic evaluation of bladder mucosa in relation to presence of endometritis and urocystitis, respectively, were calculated from 2x2 contingency tables.

Data were stratified on parity (number of recorded farrowings) and stratum specific odds ratios (ORs) were calculated for the urocystitis (presumed "exposure") and endometritis ("disease") relationship. A cut-off point of 5 was used for differentiation between "high" and "low" parity. A crude overall odds ratio and the Mantel-Haenszel odds ratio, along with their confidence intervals were calculated to assess confounding possibly related to parity. Results were regarded significant at the $p < 0.05$ level.

3. Results

3. 1. First detection of *Actinobaculum suis* in Hungary

Four *A. suis* strains were isolated from six preputial swab samples from the first farm and three out of ten samples from the second. Morphological and biochemical characteristics of the isolated bacteria were identical to those of the type strain.

Growth could not be seen on the aerobically incubated plates inoculated with *A. suis* before the 8^{th} day of incubation. After 10 days of incubation, three out of four own *A. suis* strains and the reference strain

formed colonies morphologicaly distinct from what is seen after anaerobic incubation. When these bacteria were grown under anaerobic conditions, the colonies became typical again.

A. suis together with *Proteus mirabilis* was isolated from a case of subacute haemorrhagic-necrotizing cystitis without noticeable involvement of the upper urinary tract.

3. 2. In vitro sensitivity of Hungarian *Actinobaculum suis* strains to selected antimicrobials

In the agar dilution study, "low" MIC_{50} values were determined in case of penicillin, ampicillin, ceftiofur, doxycycline, tylosin, pleuromutilines, chloramphenicol, florfenicol, enrofloxacin, erythromycin and lincomycin. "Moderate" MIC_{50} values were determined for oxitetracycline and spectinomycin. We obtained "high" MIC_{50} values in case of ofloxacin, flumequine, neomycin, streptomycin, gentamicin, nalidixic acid, and sulfamethoxazole + trimethoprim.

In the disc diffusion study, all of the strains proved to be sensitive to penicillin, cephalosporins tested, doxycycline, tylosin, pleuromutilines, chloramphenicol, florfenicol and lincomycin. For the exception of one, all strains were sensitive to ampicillin and clavulanate potentiated amoxicillin. Variable sensitivity was observed for fluoroquinolones (flumequine, enrofloxacin, ofloxacin), 84% of the strains were susceptible to marbofloxacin. Almost all strains were resistant to aminoglycosides tested but most of them were sensitive to spectinomycin. One strain showed a partially distinct resistance pattern, being moderately sensitive to amoxicillin, clavulanate potentiated amoxicillin, oxytetracycline, and being resistant to erythromycin. The same strain proved to be moderately sensitive to nalidixic acid, aminoglycosides tested and sulfachlorpiridazin + trimethoprim.

High level of correlation was determined between the results of the two techniques (Spearman's rho: 0.789; $p < 0.0001$). However, in case of a few strains which seemed susceptible for ampicillin, lincomycin or erythromycin with disc diffusion, we determined high MIC values for the given antimicrobials. The determined MIC_{50} values were not different from MIC values of the type strain ATCC 33144 (two sample Mann-Whitney test, $p = 0.9$).

3. 3. Association between endometritis and urocystitis in culled sows

The prevalence of different urogenital tract lesions was similar to what has been reported in the literature. Prevalence of endometritis diagnosed with macroscopic observation, bacteriology and histopathology was 3%, 25% and 16%, respectively. Corresponding values for urocystitis were 13%, 38% and 27%. Cystic ovaries, urocystitis and urinary calculi were the most frequent abnormalities encountered during macroscopic examination. In positive cases bacteriologic examination yielded pure or mixed cultures of enterobacteria, *Streptococcus* spp., *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp., *Corynebacterium* spp. In three instances *Eubacterium suis* was detected in mixed culture.

Sensitivity and specificity of macroscopic and/or bacteriological diagnosis of endometritis, compared to histopathologic diagnosis were 18.1% and 31.8%, respectively. Sensitivity values were somewhat higher in case of urocystitis: 47.9% and 63.0% for macroscopic diagnosis and bacteriology, respectively.

Data used in the analysis of the urocystitis – endometritis association, stratified for parity are presented in Table 3. 6. The stratum-specific ORs could be combined into one summary OR because of the nonsignificance of the Breslow-Day test for similarity of stratum-specific ORs. There was no evidence of confounding as the crude summary OR and the Maentel-Haenszel OR did not differ. The MHOR was 3.44 (95% CI: 2.85 , 4.13). Similar results were obtained when we defined different cut-off points, from parity 3, 4 and 6 and more, and when performed a detailed stratification.

4. Conclusions, suggestions

4. 1. First detection of *Actinobaculum suis* in Hungary

The seven strains isolated from preputial swab samples of boars in two farms in Hungary and the one isolated from the urinary bladder of a sow having subacute haemorrhagic-necrotizing cystitis proved to be *A. suis*. It was confirmed by comparing them to the type strain of the bacterium (ATCC 33144). **This was the first isolation of *A. suis* in Hungary**.

Although *A. suis* is regarded as an anaerobic bacterium, three out of four of our isolates and the type strain **could be cultured under aerobic**

conditions, suggesting that *A. suis* was not a strictly anaerobic microorganism.

4. 2. In vitro sensitivity of Hungarian *Actinobaculum suis* strains to selected antimicrobials

We were the **first to report *in vitro* sensitivity of Hungarian *A. suis* isolates** to different antimicrobials. Sensitivity of *A. suis* for **florfenicol, marbofloxacin, tiamulin, valnemulin, and tylosin have not been reported before**. This was the **first comparison** of the agar dilution technique and the disc diffusion method in case of *A. suis*.

Considering the often mixed bacterial flora present in cases of urocystitis and pyelonephritis of swine, treatment of these conditions requires the use of broad spectrum antimicrobials or antimicrobial combinations. Based on these and on our *in vitro* results, where available, **semisynthetic penicillines** or a potentiated form of them, as **clavulanate potentiated amoxicillin**; furthermore **ceftiofur**, **florfenicol**, **doxycycline**, and possibly **marbofloxacin** and **enrofloxacin** can potentially be useful in treating mixed urinary tract infections of swine involving *A. suis*. However, **some *A. suis* strains might considerably differ in susceptibility from the above pattern**. These data should be regarded as guidelines only when choosing antimicrobials for the treatment of *A. suis* infections in swine, since the pharmacokinetics of the drugs, especially the production of effective concentrations on the mucus membranes of urogenital organs must be considered.

As our results indicate, the **disc diffusion method** as performed **might be a practical alternative of the agar dilution technique** in case of determining *in vitro* susceptibility of *A. suis* isolates.

4. 3. Association between endometritis and urocystitis in culled sows

We were among the first to report detailed prevalences of urogenital tract lesions in a non-random sample of culled sows **in Hungary**. Prevalence of detected lesions was similar in our study to what has been reported in the literature. Apart from ovarian cysts, urocystitis and concretions accounted for the majority of lesions. **Our data indicates that urocystitis occurs frequently** in Hungarian sows culled for reproductive reasons.

Both bacteriology and macroscopic observation alone were found to detect only a small proportion of animals with urocystitis or endometritis; the chance for false negative diagnosis was particularly high for macroscopic diagnosis of endometritis. **These are the first reported estimates of sensitivity and specificity regarding bacteriologic and macroscopic diagnosis of urocystitis and endometritis.**

These results further emphasize the **necessity of histopathology** in the correct diagnosis of endometritis and urocystitis, and the beneficial effect of **considering macroscopic and bacteriological test results together** in order to estimate the true disease status of these organs.

We were the first to report a strong epidemiological association between urocystitis and endometritis, which apparently was not confounded by parity. This supports the presumed association between urocystitis and endometritis. However, as it was a cross-sectional study, it was not possible to determine temporal relationships between the two conditions. Longitudinal studies would be required to clearly answer this question.

5. Related publication list of author

5. 1. Refereed full-text research papers published (or accepted for publication) in scientific journals

5. 1. 1. In domestic journals, in foreign languages

Biksi, I., Takács, N., Vetési, F., Fodor, L., Szenci, O., Fenyő, E.: Association between endometritis and urocystitis in culled sows. Acta Vet. Hun., accepted for publication.

Biksi, I., Major, A., Fodor, L., Szenci, O., Vetési, F. In vitro sensitivity of Hungarian Actinobaculum suis strains to selected antimicrobials. Acta Vet. Hun., submitted for publication.

5. 1. 2. In (national or international) foreign journals, in foreign languages

Biksi, I., Fodor, L., Szenci, O., Vetési, F. The first isolation of *Eubacterium suis* in Hungary. J. Vet. Med. B, 1997. 44, 547-550.

5. 2. (Chapters of) books, university textbooks and other reports published in edited non-periodicals

Biksi I., Harmath S., Steiner A. (szerk). Állatorvosi terápiás útmutató I. Pet Press, Budapest, 1995.

5. 3. Further full-text professional papers published in non-scientific journals

Biksi I., Takács N., Vetési F. Nemi szervi váladékozással járó kórképek elkülönítő kórjelzése sertésben. A Sertés, 1996 1 (2), 42-49.

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