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Állatorvos-tudományi Doktori Iskola

Some aspects of urogenital tract diseases of female breeding swine

Doktori értekezés

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List of abbreviations

AI	Artificial insemination
ADV	Aujeszky's disease virus
ATCC	American Type Culture Collection
BE	Base excess
CCNAM	Colistin-nalidixic acid-metronidazol supplemented Columbia blood agar
CI	Confidence interval
HPF	High power field
IF	Immunofluorescence
MHOR	Mantel-Haenszel odds ratio
MIC	Minimum inhibitory concentration
MMA	Metritis-mastitis-agalactia syndrome
NPD	Non productive days
OR	Odds ratio
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFD	Post farrowing discharge
PHS	Postparturient hypogalactia syndrome
PMD	Post mating discharge
PPDS	Postparturient dysgalactia syndrome
PPV	Porcine parvovirus
PRRSV	Porcine reproductive respiratory syndrome virus
RBC	Red blood cell
SG	Specific gravity
SEM	Scanning electronmicroscope
TEM	Transmission electronmicroscope
TDS	Total dissolved solids
UTI	Urinary tract infection

This dissertation is dedicated to my niece, Virág.

"So it goes"
Kurt Vonnegut: Slaughterhouse-Five

Summary

Reproductive failure accounts for substantial losses in the swine industry worldwide. Urogenital tract infections are among the most important causes of reproductive failure. Studies performed in the framework of this dissertation were directed to facilitate the understanding of some aspects of urinary tract disease in female breeding swine. Two studies were related to *Actinobaculum suis*, a "specific" urinary tract pathogen of swine. One other report was made on the prevalence of urogenital tract lesions in culled female breeding swine and the statistical and epidemiological associations between urocystitis and non-specific endometritis.

An extensive literature survey on urogenital tract diseases of female breeding swine was made. Anatomy and physiology of the urinary and reproductive system, predisposing factors, pathogenesis, diagnosis, therapy and prevention of lower urinary and genital tract disorders, with particular references to urocystitis and non-specific endometritis were discussed in detail. The survey provided practical references to less often discussed subjects like economic importance of urogenital tract diseases, production data analysis, and herd inspection in relation to reproductive problem solving. It became apparent from the literature survey, that the putative predisposing effect of urocystitis to non-specific endometritis, although biologically plausible, is insufficiently backed by statistical analyses. Moreover, magnitude of such associations, and the effect of possible confounders were not discussed yet in the available literature. It also became apparent, that urogenital tract diseases of swine were not discussed yet in detail in the Hungarian scientific literature.

Actinobaculum suis is widely indicated as a leading cause of urinary tract disease in female swine. However, its occurrence has not been reported previously in Hungary. In a study directed to prove the presence of *Actinobaculum suis* in Hungarian swine herds, we have isolated seven strains from preputial swab samples of boars in two farms and one from the urinary bladder of a sow having subacute haemorrhagic-necrotizing cystitis. This was the first isolation of *A. suis* in Hungary. We have also proven that *A. suis* is not a strictly anaerobic microorganism.

Therapy of urogenital tract infections frequently 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. An *in vitro* study was performed to determine the antibiotic sensitivity of 12 Hungarian isolates of *A. suis*, with a special reference to novel antimicrobials. A comparison of disc diffusion and agar dilution methods was also made. Twenty one and twenty four antimicrobials were tested in the two methods, respectively. Sensitivity of all Hungarian strains was not different from the type strain, results yielded by the two methods were closely correlated. Some strains showed partially distinct resistance patterns. Based on the current literature recommendations and our *in vitro* results, where available, semisynthetic penicillines, ceftiofur, florfenicol, tetracyclines, and possibly some of the quinolones can be useful in treating urinary tract infections of swine involving *A. suis*.

Slaughterchecks are integrated part of reproductive problem solving in many countries, including Hungary. Their use is especially indicated in cases of noninfectious reproductive failure and urogenital tract problems. Slaughterhouse sampling and subsequent detailed laboratory examination of urogenital tracts of sows and gilts culled for reproductive failure were performed in a study. 499 animals from 21 farms over a six-year period were sampled. We could conclude, that the prevalence of major urogenital tract lesions was similar to what reported in foreign studies. This was among the first detailed reports on the prevalence of urogenital tract lesions of sows and gilts in Hungary.

A comparatively large set of data on macroscopical, histopathological and bacteriological results of sow and gilt urogenital organs was collected in a study described above. This dataset was subjected to a number of epidemiological analyses. We determined sensitivity and specificity of macroscopic and bacteriologic diagnosis of urocystitis and endometritis as compared to the results of histopathology (Gold Standard). Sensitivity of macroscopic and bacteriologic diagnoses appeared quite low. Thus, macroscopic or bacteriological examination of the urinary bladder or the uterus alone is likely to be not sufficient to arrive at a correct diagnosis of urocystitis or endometritis. Histology should be utilized whenever possible for establishing such a diagnosis. We have also examined whether the presence of urocystitis and endometritis are related when the effect of parity is accounted for. Our results indicated that urocystitis is indeed positively associated with endometritis, regardless of the parity of the animal. The odds ratio of approximately 3.5 indicated a strong positive association between these two conditions.

1. Literature survey

1. 1. Sow reproductive failure - economic aspects

Reproductive failure accounts for substantial losses in the swine industry worldwide. Reproductive failure on a herd level can be defined as failure to reach the optimal number of liveborn piglet/sow/year. This simple term is well under the influence of a multitude of factors. The liveborn piglet/sow/year figure can be split into two main constituents: litter/sow/year and liveborn piglet/litter (Figure 1. 1.).

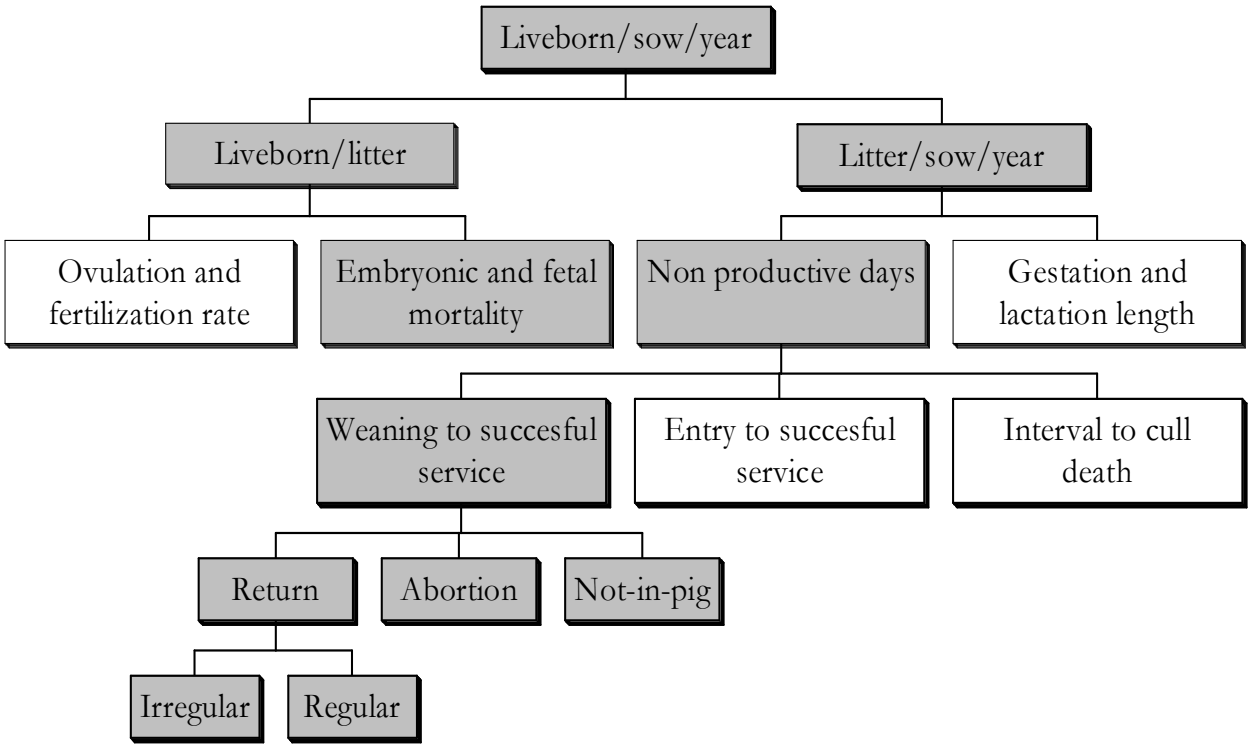

Figure 1. 1.: Factors influencing the number of liveborn piglets/sow/year (modified after Cameron, 1998)

Litter/sow/year is determined by gestation and lactation length and by the number of non-productive days/litter. A non-productive day (NPD) by definition is any day a sow or gilt of breeding age is present in the breeding herd and is not either gestating or lactating. Gestation length can be regarded as constant; lactation length cannot be substantially decreased under 21 days without a toll on reproductive efficiency in the next parity, thus the number of litters weaned from a sow annually depend on the number of NPDs. A portion of NPD - the period from weaning to the first (successful) service, usually 4 to 7 days - cannot be avoided. Other components of NPDs are related to different causes of reproductive failure (Table 1. 1.).

Table 1. 1.: Causes of non-productive days in different periods of the breeding cycle

Period		Reason
From	**To**	
entry to the sow herd	successful service or culling	anestrus, prolonged puberty
weaning	successful service or culling	partly unavoidable, post weaning anestrus, late estrus
service	return between 18-24 days	"regular return", fertilization failure, early embryonic death etc.
service	return 24 < days	"irregular return", early embryonic death, implantation failure, abortion
service	fail-to-farrow	"sow not in pig", complete loss of litter detected at or close to the expected time of farrowing

The period from entry to the herd or weaning to the next successful service is a major component of NPD. Factors interfering with conception, implantation, or causing embryonic or fetal death are responsible for the elongation of this period. **Urogenital tract infections**, which are in the focus of this review, are among such factors. Areas where urogenital tract infections can possibly contribute to increased number of NPDs (and, although less likely, to low liveborn litter size) are shaded in the flowchart of Figure 1.

There are various approaches to calculate **the amount of financial losses** caused by NPD, with an aim of estimating the magnitude of loss due to reproductive failure. It has been estimated that a NPD costs USD 1.1, and a 21 days return to service adds USD 19.80 to the cost of production of the next litter, whereas USD 119.70 is added to the cost of the next litter of a 16 week fail-to-farrow female (Cameron, 1998). Applied for current Hungarian circumstances, calculations yield a conservative estimate of **HUF 500/NPD/sow**, an approx. USD 1.8/NPD/sow (Biksi and Biró, 1999). As it is common to find sow herds in Hungary with 24 NPD/litter and 2 litters/sow/year, the total estimated loss in such herds could reach a substantial amount. In some Western-European countries the average number of NPD/litter can be much lower than the Hungarian figures (Biksi et al., 1999). Determination of the number of NPDs is usually done by **specialized software packages**, which often allow for further analyses, like paritywise distribution of NPDs for a given period. Results of such analyses can alert producers and their consultants and provide information on the magnitude of losses; however, the exact causes of sow reproductive failure usually require further investigations.

Few reports are available on the financial losses resulting from particular disease conditions of the urogenital tract. Parsons et al. (1988b) reported the economic impact of vaginitis and endometritis in the gilt. They calculated a USD 4.78 break-even cost for the treatment of vaginitis and a USD 6.78 for endometritis. (Break even cost is the maximum amount of money that should be spent on a 100% effective prophylactic treatment.) The cost per affected animal was determined for endometritis and vaginitis as USD 46.92 and USD 9.20, respectively. The authors suggested that the actual financial losses associated with vaginal discharge syndrome are due **to increased days open and reduced litter size**, but also dependent on prevailing market conditions and the biological variability of a given epizootic. Decision tree analysis was used to determine the optimum time for culling affected gilts in the absence of an effective therapeutic option. It was shown that the time of culling is dependent on the pathological diagnosis and the producer's attitude toward risk.

1. 2. Causes of sow reproductive failure

Reproductive failure in swine can have either **infectious** or **noninfectious** etiology. There is a great overlap in their clinical presentation; both can present as reduced performance in only one reproductive parameter or as a syndrome in which several reproductive measures are compromised. Usually, in clinical presentations when the unfavorable changes in certain production parameters are abrupt and of high magnitude, infectious causes are suspected. On the other hand, when herd productivity deteriorates over a longer time period, management practices are considered suspect. These preconceptions might not hold true, as changes for example in feed composition can lead to rapid decline in reproductive efficiency, and urogenital tract infections can lead to smaller scale but steady reduction in e. g. farrowing rate. Moreover, clinical illness is not consistently observed following infection with any of the major agents causing reproductive disease in swine.

The overlap in clinical signs and pathology of infectious and noninfectious causes of reproductive failure is due to the fact that they both mainly interfere with embryonic and fetal development. From this regard, the **time of insult** is more important than its infectious or noninfectious nature. Two days after fertilization, the conceptus lies free in the uterine lumen until implantation, which occurs around day 14 post service. Exposure of a sow or gilt to a pathogen or other harmful stimulus before this time followed by early embryonic death and resorption, results in pregnancy termination and **regular return to estrus** 18-24 days post service (Christianson, 1992). "**Ovulation failure"** and **conception failure** will have the same effect (Straw et al., 1999). Exposure after 14 days but prior to the time of onset of fetal calcification at approximately 30-40 days post service, results in complete resorption of fetuses and **irregular return to estrus** (> 24 days post mating), usually 5 to 10 days post pregnancy loss (Christianson, 1992). In some instances sows will **fail to farrow**, i. e. will not show signs of estrus after the loss of their whole litter until close to or at term (90 to 115 days of pregnancy). Death of fetuses after the onset of fetal calcification results in fetal **mummification**. Infections during the last few days of gestation may result in either fetal death during late gestation (prepartum deaths, autolytic fetuses) or failure to survive the birth process (intrapartum deaths) (Dial et al., 1992). **Abortion**, expulsion of fetuses before their expected time of viability (i. e. before approx. day 110 of gestation) can occur from day 14 onwards, and follow the death of the whole litter. When at least four embryos remain alive until the time of implantation, and at least one fetus remains alive after that, pregnancy is carried until term, but the result will be a **decreased liveborn litter size**.

1. 2. 1. Infectious causes

The main focus of this literature survey is on urinary and genital tract infections caused by opportunistic bacteria, and their association with reproductive failure. Infectious causes of other origin and noninfectious ones will be dealt here only briefly, mainly for the sake of differential diagnosis.

Generally, infectious reproductive diseases can be categorized as **systemic** (hematogenous) and **genital infections**, which can both occur in epizootic and enzootic forms. Reproductive infections most commonly occur via the systemic route, when female ingests or inspires a pathogenic virus or bacterium to which she is immunologically naive. The pathogen then travels via bloodstream to its place of effect, which can be the embryo, the fetus, the placenta or the uterus. Although occasionally of hematogenous origin, genital diseases usually result from ascending infections by opportunistic bacteria. In pigs, there are no "venereal" diseases that are specifically harbored in the male or female reproductive tract and primarily cause infertility, except for brucellosis (Thacker and Gonzalez, 1988). Seminal shedding may occur transiently with pseudorabies virus, porcine parvovirus or enteroviruses, leptospira may appear in semen as contaminants from the urinary tract (Thacker et al., 1984).

Specific infectious agents most frequently causing reproductive failure include porcine parvovirus (PPV), Aujeszky's disease virus (ADV), Porcine Reproductive and Respiratory Syndrome virus (PRRSV), Encephalomyocarditis virus, leptospira, *Brucella suis*, and *Eperythrozoon suis*. Pathogenesis, clinical features and pathology of such infections have been subject to numerous reviews (Cutler, 1986; Mengeling, 1986; Thacker and Gonzalez, 1988; Christianson, 1992; Dial et al., 1992; Dee, 1995a, b; Clark, 1996; Straw et al., 1999). Other viruses or bacteria causing systemic infection (septicaemia or viraemia) in a pregnant female can cause fetal or embryonic loss and a herd scale decrease in reproductive efficiency.

1. 2. 2. Noninfectious causes

The reproductive inefficiency on commercial swine farms is more often noninfectious than infectious in origin, having its etiology in **bad management practices** and/or in **environmental factors**. Noninfectious causes of fetal death often are multifactorial and difficult to diagnose. Many of the major noninfectious factors share clinical signs in common with each other and with infectious etiologies, although they usually have a different clinical presentation. **Lactation length** is positively related to fertility and total born litter size, it is inversely related to weaning-to-service interval. **Parity** influences most measures of reproductive performance including fertility, litter size parameters (total born litter size, number of mummies, stillbirths, and pigs weaned/litter), and various sources of NPDs (e. g. weaning to service interval). In general, mid parity females (3-5) have higher fertility and born alive litter sizes than younger or older parity females. Low ovulation rate, poor conception with progressive embryonic mortality in first parity females can result from inadequate **breeding gilt management**. On commercial farms it is not uncommon for lactation length and parity distribution to shift frequently and relatively abruptly, leading to marked changes in herd productivity.

Breeding management factors that may influence a herd's reproductive performance include the frequency and duration of boar exposure, the time of service during the estrus cycle, the number of matings per service and the quality of mating or artificial insemination (AI). **Boar usage** and variations in boar fertility potentially contribute to breeding inefficiency. **Inadequate nutrition** of the female in all production phases can be associated with anestrus, early embryonic death and decreased liveborn litter size. High energy feeding during early pregnancy is often associated with increased embryo mortality (Einarsson and Rojkittikhun, 1993; Muirhead and Alexander, 1997); however, it is still a controversial issue. **Transfer** of mated females between days 2 to 28 of pregnancy, or keeping them in **large groups** during the same period might result in early embryonic loss and return to service. The **summer season, high ambient temperatures** and increasing photoperiod are associated with lower total born litter size and farrowing rate, and increased weaning to service interval. **Poor environmental conditions** (dampness, draught, low light intensity post weaning) increase the chance for early embryo loss. Other factors, as mycotoxicosis, genetic defects of embryos, developmental anomalies of the female genital tract, other sources of stress, can all contribute to increased return rates and reproductive failure (Hurtgen, 1986b, Christianson, 1992; Muirhead and Alexander, 1997; Table 1.3.).

1. 3. Diagnostics of swine reproductive failure

Background, usefulness and limitations of some diagnostic methods used in detecting urogenital diseases are discussed herein, with a particular reference to slaughterhouse examinations.

Diagnosis of swine reproductive failure is often a difficult task. As reproduction is a complex series of events, a specific reproductive problem, e. g. repeat breeding may have **many possible causes**. Failure to determine and control all of these could result in no improvement; success is often determined by the most limiting factor (Thacker, 1986). As it was shown, reproductive failure is often from noninfectious causes, laboratory methods to support the

clinical diagnosis of such conditions are not readily available. Moreover, some reproductive problems are detected long after the specific failure has occurred (e. g. sows not in pig, low litter size).

1. 3. 1. Record analysis

Computerized herd record keeping systems are widely utilized in today's swine industry (Templeton, 1998). They dispersion was driven partly by veterinary consultants, whose work is almost impossible without appropriate herd record databases. The more advanced information systems provide great opportunity for investigating reproductive problems. In that case, herd records should allow for retrospective epidemiological investigations to determine the stage of gestation at which the failure occurred, the group affected, and the magnitude of losses (Thacker, 1986; Tubbs, 1996; Biksi and Biró, 1999). Consultants and producers will also benefit from historical comparisons or comparisons to industrial production averages. However, one should keep in mind that the accuracy of herd records is based on the ability and willingness of the breeding herd personnel to observe events and put that information into a written record or into a computerized system (Thacker, 1986). Discrepancies between records and true state of matters are found on swine farms in every country.

At first, when a producer or consultant suspects a reproductive problem, it is important to examine certain parameters to see, whether there is a real change in herd productivity. Important to note, that the majority of production parameters show seasonal fluctuations, so historical comparison to previous data is inevitable. Moreover, short term changes in some figures might just be due to normal sample variations, calculation of a rolling average or standard deviation of such figures is advised (Tubbs, 1995a). Main parameters used to detect reproductive problems are presented in Table 1.2. Urogenital infections are usually associated with parameters in bold letterface.

Table 1. 2.: Main herd record parameters used in reproductive problem solving

Sow inventory	**Sows not-in-pig**
Gilt inventory	**Farrowing rate**
Parity distribution	**Farrowing rate after repeat**
Boar inventory	**Liveborn/litter**
Number of weekly, monthly matings	Dead/litter
Weaning to estrus interval	Mummified/litter
Regular returns	Litter scatter (< 8 pigs/litter)
Irregular returns	**Litter/sow/year**
Abortion	**Number of NPDs/litter**

Target figures or different production schemes are reported in the literature (Thacker, 1986; Kiss Tiborné et al., 1996; Tubbs, 1996; Muirhead and Alexander, 1997). However, a thorough understanding of each farm's potential is needed when comparing its production parameters to such benchmarks (Tubbs, 1995a).

1. 3. 2. Clinical signs

Evaluating reproductive efficiency of a swine operation includes examining the herd, observing facilities and reproductive management practices (Tubbs, 1995a). This usually provides chance to observe some of the clinical signs associated with the particular problem (e. g. vaginal discharges). Moreover, observing the facilities might tell more about the true nature of the problem than records and herd history. The following areas should be considered while inspecting the breeding herd. The presence of **over- or underconditioned animals** indicates improper feeding practices (Thacker, 1986; Tubbs, 1995a). Animals appearing **not to be pregnant** in groups due to farrow soon may indicate embryonic resorption. Sows and gilts

should be checked for the presence of **vaginal discharges**; differential diagnosis of such conditions is presented in the followings (Table 1.7.). Examining sows and boars for signs of **clinical lameness** should deserve special attention. A walk-through on the farm is usually suitable to perform a **physical inventory** count - the result of which might differ from available records. **Environmental conditions**, such as barn temperature, lighting, ventilation and sanitation should be thoroughly inspected (Muirhead and Alexander, 1997). During a farm visit, the following **specific areas** should be observed or discussed with breeding herd personnel: gilt pool management, weaning-to-breeding management, estrus detection, mating technique and hygiene, postbreeding management (feeding, movements), postbreeding estrus detection and pregnancy diagnosis, observation of abnormal situations (discharges, abortions), and disease control (Thacker, 1986; Tubbs, 1995a, b; Muirhead and Alexander, 1997).

Clinical patterns of some conditions responsible for reproductive failure are presented in Table 1.3. Such information should be regarded as guideline only, as disease presentation might vary farm to farm, depending on preventive measures, management, and concomitant disease conditions, etc.

Table 1. 3.: Clinical patterns of some conditions responsible for reproductive failure (Thacker, 1986; Tubbs, 1987; Tubbs, 1995a, b; Dial et al, 1992; Muirhead and Alexander, 1997; Straw et al., 1999)

	Regular return	Irregular return	Mummi-fication	Abortion	Stillbirths	Low live litter size	Postnatal death
Parvovirus infection	+	++	+++	-	++	++	-
Aujeszky's disease virus infection	+	+	+	++	+	+	+++
PRRSV-infection	-	+	++	++	+++	+	+++
Leptospirosis	+	+	+	+++	++	+	+
Non-specific endometritis	++	+	-	+	-	+	-
Zearalenone toxicosis	++	+	+	-	+	++	+
Conception failure (boar power, usage, age, maturity, AI technique, multiple or single matings)	+++	-	-	-	-	++	-
Seasonal effects	+	++	-	+	-	+	-
Short lactation, excessive lactational weight loss	++	+	-	-	-	+	-
Overfeeding post mating	+	++	-	-	-	+	-
Stress < 30 days post mating	+	++	-	-	-	+	-

-: not likely; + - ++ - +++: increasing likelihood

1. 3. 3. Slaughterhouse examinations / pathology

Slaughterhouse sampling and subsequent examination of female reproductive organs is a valuable tool in swine reproductive management (Almond and Richards, 1992; Tubbs, 1995b). Examinations of reproductive organs of sows culled for infertility provide useful hints on the possible causes of reproductive disorders of swine (Almond and Richards, 1992; Dalin et al., 1997; Einarsson et al., 1974; Heinonen et al., 1998; Straw et al., 1986; Ványi et al., 1995). Such examinations considered particularly useful in case of non-infectious reproductive problems and urogenital tract infections where serological and epidemiological investigations are of limited value. Slaughterhouse examinations are indicated in cases of delayed puberty, failure to return to estrus after weaning, **regular and irregular returns to estrus**, **pseudopregnancies**, and **vaginal discharges**. Results of these surveys might reveal abnormalities in the urogenital tract, being responsible for reproductive failure, or might illustrate shortcomings in breeding management (Almond and Richards, 1992; Straw et al., 1986). Examination of the female reproductive system of swine is a useful diagnostic method of assessing ovulation rate, and determining the accuracy of estrus detection. Slaughterhouse examinations were used to assess the prevalence of developmental anomalies in female swine (Einarsson and Gustafsson, 1970).

A slaughtercheck is usually a "one time" examination directed to reveal causal factors in case of a herd problem. This method, however, poses some statistical drawbacks. Sows culled for reproductive reasons are usually **not representing the whole sow herd**, due to their non-random selection and to the usually low sample size (Martin et al., 1987; Almond and Richards, 1992). Thus, generalization of its results should be done carefully. On the other hand, findings coupled with individual history and clinical signs might provide clearer understanding of an **ongoing reproductive problem**. Examining large number of culled sows from several farms over a longer period of time can provide an insight on the prevalence of certain reproductive tract lesions on a regional or country level and can help to reveal cause-effect relationships and seasonal fluctuations (Geudeke et al., 1992). Larger sample size allows for more valid generalization of data, although this type of cross-sectional studies also have inherent limitations.

Technical details of reproductive slaughterchecks are described in the literature (Almond and Richards, 1992; Dial et al., 1992) and in this thesis. Important factors to consider are the **selection of sows** and their **proper identification** at slaughter. Unfortunately, consultants cannot always influence producer's decision about which animals to be culled for such examinations and when. Many times only the regular monthly batch of culled sows is available for examination, which might include animals not representative for the observed reproductive problem. Proper identification at slaughter is very important if one attempts to relate findings to individual records. Sows are preferably identified by tattoos, metal or plastic eartags; their identity should be checked at several places at the slaughterline when a change in the sequence of carcasses is possible. Cooperation among the veterinarian, swine producer and packing plant manager is necessary to conduct slaughterhouse examinations (Almond and Richards, 1992).

Apart from obtaining the urogenital tract for further examinations, condition of culled females (backfat thickness), their leg problems and parasitic infestation can also be assessed. In a laboratory, samples can be obtained for bacteriology, histopathology and for other complementary examinations from the urogenital tract. Reproductive slaughterchecks should always include collection of detailed production data pertaining to the reproductive performance of the individual.

A brief description of the main urogenital tract lesions encountered during slaughterhouse examinations will be presented in the followings.

A thorough understanding of changes in **ovarian morphology** during the estrus cycle, pregnancy, lactation and after weaning is paramount in accurate assessment of the reproductive tracts (Almond and Richards, 1992). Akins and Morrissette (1968), Schnurrbusch et al. (1985), and Leiser et al. (1988) describe ovarian changes during sexual cycle in detail. Abnormal findings in the ovaries are cystic degeneration, arrested development and atrophy. Classification

of ovarian cysts and their possible effect on reproductive performance were discussed by, among others, Wrathall (1980), Miller (1984), McEntee (1990), Almond and Richards (1992), Ebbert and Bostedt (1993). Macroscopic appearance of ovaries of anestrous sows and gilts can be found in references cited above. Important to note, that about 40% of all sows in the temperate zone have inactive ovaries from July to October, whereas 10% have inactive ovaries during the winter months (Straw et al., 1986). Uni- or bilateral agenesia of the ovaries is extremely rare. Macroscopic changes due to ovarian inflammation are rare, except for porcine brucellosis (McEntee, 1990). Microscopic inflammatory changes, characterized by mononuclear cell infiltration can accompany virus infections (Bolin et al., 1985). Occasionally, papillary cystadenoma, cystadenocarcinoma, granulosa cell tumor, thecoma and leiomyoma were reported in sows. Haemangioma appears to be the most common ovarian neoplasm in aged sows (Hsu, 1983; McEntee, 1990).

Developmental anomalies of **uterine tubes** are rare. Absence of uterine tubes usually accompany severe developmental anomalies of the uterine horns (Einarsson and Gustafsson, 1970), segmental aplasia can also occur in the oviduct (McEntee, 1990). Hydrosalpinx, salpingitis and pyosalpinx are comparatively frequent abnormalities of the female genital tract. Salpingitis is usually not detectable macroscopically, and its milder forms can be easily overlooked even in histopathology (Jubb et al., 1993). Prevalence of such lesions differs considerably among reports, ranging from 1.5 to 58.1% (McEntee, 1990). Information on the pathogenesis of inflammatory lesions of the oviduct is quite limited, they believed to be consequences to ascending non-specific bacterial uterine infections, however, experimental viral infections can induce similar changes (Bolin et al, 1985). The role of chlamydial or mycoplasmal infection in salpingitis in swine is yet to be confirmed. Squamous metaplasia of the uterine tubes can occur in association with inflammatory conditions, zearalenone toxicosis or vitamin A-deficiency (McEntee, 1990). Neoplasia of the uterine tubes is extremely rare in swine.

Congenital anomalies of the **uterus** are quite frequent in swine, major abnormalities account for 0.98 to 1.4% of samples in different studies (Einarsson and Gustafsson, 1970; McEntee, 1990; Heinonen et al., 1998). Total or segmental aplasia of the uterus, uterus unicornis, partial duplication of one uterine horn and uterus dydelphis were found in a survey of Teige (1957). Inflammatory changes found with varying frequency in uterine samples, from 1.4% (Heinonen et al., 1998) to 27% (Dalin et al., 1997). Gross and histopathological lesions of non-specific endometritis are described in detail elsewhere in this chapter (1. 5. 6. 3.), lesions indicative of specific inflammatory conditions are reported in the literature (McEntee, 1990; Jubb et al., 1993). Cysts in the endometrium of sows can be attributed to chronic inflammation (Almond and Richards, 1992), which is likely to induce squamous metaplasia at the same time. Phytoestrogens can induce cystic endometrial hyperplasia in sheep, the cystic endometrial hyperplasia - pyometra complex is well described in the dog and cat, such hormonal etiology of cystic endometrial hyperplasia in the sow is yet to be proven. There is a report describing simultaneous occurrence of cystic endometrium and multiple large ovarian cysts (Thain, 1965). Lymphangiectasia can occur occasionally in the endometrium of aged sows. Zearalenone can induce squamous metaplasia of the endometrial lining and endometrial hyperplasia of progestational type (Jubb et al., 1993; Ványi et al., 1995). Adenocarcinoma, leiomyoma and lymphoma were reported in swine, although they occurrence is very low (McEntee, 1990; Buergelt, 1997).

Developmental anomalies of the **cervix, vagina, vestibule and vulva** are rarely reported for swine. Partial and total duplications of the cervix account for the majority of such conditions (Teige, 1957; Einarsson and Gustafsson, 1970), rectovaginal or rectovestibular fistulas can be found occasionally in the pig (McEntee, 1990). Inflammatory lesions of the lower genital tract are discussed briefly elsewhere in this thesis. It appears that cervicitis is usually a sequel to vaginitis or endometritis; vaginitis can occur independently of endometritis (Dial and MacLachlan, 1998b). Estrogenic mycotoxins can induce squamous metaplasia of the cervical

mucosa, severe hyperemia and edema of the vaginal mucosa with subsequent prolapse. Parturition- or copulation induced lacerations can be present in the cervical or vaginal wall. Tumors of the cervix and the rest of the lower genital tract are rare; there are reports on papillomas, and embryonal sarcomas of the vagina (McEntee, 1990; Jubb et al., 1993).

Developmental anomalies of the **lower urinary tract** are rare, with ectopic ureters and urethrovaginal fistulas being the most frequent conditions (Jubb et al., 1993). There are only a few reports available on the prevalence of lower urinary tract diseases in slaughtered sows. Geudeke et al. (1992) found cystitis on macroscopic examination in 11% of over 11.000 culled sows from 151 herds, with a variation of 0 to 35% among herds. Tumors of the urinary tract are rare, mainly involving the kidneys (nephroblastoma); neoplasms of the bladder are exceedingly rare (Drolet and Dee, 1999).

There are few reports on complex studies, involving slaughterhouse sampling, detailed examination of the urogenital tract and evaluation of production records. Dalin et al. (1997) examined 115 genital organs from sows and gilts culled for reproductive reasons from a Swedish sow pool. They also collected anamnestic data as parity number of the sow, date of farrowing, dates of weaning, estrus and service, and cause of culling. Macroscopic examination of the urogenital tract and also histopathological examination of the endometrium were performed. They revealed that the most common reason for culling was repeat breeding (67%), in most cases at irregular intervals. In 49.6% of the sows no pathological lesions were found. Macroscopic examinations of the ovaries in 108 animals showed that 69% were cycling normally, 17% were anestral and 14% had multiple follicular cysts. On histopathology, 27% of the animals had endometritis, classified as mild in 50% of them. Anestral animals had a higher incidence of endometritis (61%) than animals showing cyclic ovarian activity.

Einarsson et al. (1974) examined the genital organs from 54 gilts, slaughtered for anestrus. The ovaries of 35.2% of these animals did not contain luteal tissue. Bacteriological and histopathological examination did not indicate an infectious cause of the condition. Endometrial samples taken for histopathology did not show inflammatory changes.

Einarsson and Gustafsson (1970) performed a post mortem examination on the reproductive tract of 1000 (non-breeding) gilts in order to estimate the prevalence of developmental anomalies. The total number of developmental anomalies in this sample was 22.1%. Paraovarian cysts were responsible for 14%, partial duplication of the vagina for 4.1%. 3.7% of the anomalies was due to maldevelopments in the Müllerian duct system, 0.3% was due to general developmental defects (hermaphroditism).

Kjelvik et al. (2000) examined 114 sows at the slaughterhouse for the presence of macroscopic and histologic lesions of urocystitis. Based on histology, 22.8% of the bladders showed signs of slight to severe cystitis. Severe macroscopic alterations correlated fairly well with histological changes.

1. 3. 4. Other investigation methods

Other laboratory diagnostic methods include serology, virology, immunohistochemical studies and nucleic acid hybridization assays. These all can have an important role in ruling out primary infectious etiologies. Freedom from major genital tract pathogens is routinely checked serologically at breeding farms. Serological investigations also have value in assessing the effectiveness of vaccination programmes (ADV, PPV). There are numerous reports on the detection of special genital tract pathogens (like PRRSV) using polymerase chain reaction (PCR). These techniques does not have implication in the detection of non-specific urogenital infections rather that ruling out primary infectious causes.

Diet analysis can identify errors in ratio formulation, inadequate mixing or can detect mycotoxin contamination (Thacker, 1986). It should be an integral part of reproductive problem solving.

1. 4. Urinary tract infections

The porcine urogenital tract is divided into the urinary and reproductive system. Normal anatomy and physiology of these will be addressed separately in the followings. This overview is necessary to understand the pathomechanism of inflammatory changes in the urogenital tract.

Generally, urogenital infections are the result of a complex of social, environmental, and hormonal stress factors that lead to an imbalance of the normal microflora of the urogenital tract, enabling commensal organisms to become pathogens (Dee, 1992). Inflammatory diseases of the upper genital tract (oviduct and uterus) will lead to short term or permanent infertility, characterised mainly by regular returns to estrus. Urinary tract diseases are not thought to be responsible directly for embryonic or fetal losses, they are regarded as predisposing factors for upper genital tract infections (see 1. 6.).

Urogenital tract disorders of sows are important causes of sow reproductive failure in many countries; however, they did not receive much attention yet in the Hungarian veterinary literature.

1. 4. 1. Anatomy and physiology of the urinary system in female swine

The porcine urinary tract consists of kidneys, ureters, ureteric valves, bladder, and urethra. Urine is produced by the kidneys and passed through the ureters into the bladder and through the urethra to the outside world. Pigs have multipyramidal kidneys without external lobation. The medullar portion of each lobe is called a pyramid. Pyramids can be simple or compound, the latter being formed from the fusion of two or more primitive pyramids. The apical portion of the pyramid, called the papilla projects into the renal pelvis or its ramifications, called the calyces. Papillae of solitaer pyramids are conical and narrow, whereas those of compound pyramids, usually located at the poles of the kidneys are broad and flattened. There are 8-12 papillae per kidney. Collecting ducts of the kidneys have their opening at the tips of the papillae (Henrikson, 1993).

Urine is moved to the bladder by peristaltic contractions of the smooth muscle of the ureter. The ureters, being continuous with the renal pelvis leave the kidneys in a sharp caudal curve. They ultimately reach the dorsolateral sides of the bladder neck area, penetrating its muscular coat at almost right angles and pass obliquely through the submucosa, raising the mucosa slightly before ending at the ureteric orifices. The ureteric orifices and the urethral outlet form the trigon of the bladder. The intramural portion of the ureters are relatively short, whereas the intravesical part is quite long. The **ureterovesical junction** of pigs is acting as a one-way valve to prevent reflux of urine from the bladder, yet allows urine to continue to enter and fill the bladder. Carr et al. (1993) reported morphological characteristics of ureterovesical junction of 177 pigs of various ages and bodyweight. They found that the length of the intravesical ureter increases with age, from 5 mm at birth to 36 mm at maturity. The width of the ureteric orifice also increases with age. The ratio between the length of the intravesical ureter and width of the ureteric orifice was 8.3:1. In man, vesicoureteric reflux is more likely if this ratio is below 4.5:1. The ureteric orifice was mainly horseshoe shaped, less frequently of stadium shape. The width of the ureter at the intravesical/intramural portion was greater than at the ureteric orifice in 75% of the cases. The authors suggested that these types of ureters might provide continued protection against ureterovesical reflux even when their orifice is damaged.

Urinary bladder of the pig is large and has a long neck. The bladder is supported by two lateral and one ventral ligaments. The urethra of adult female swine is approx. 7-8 cm long. Its external ostium is located ventrally, at the junction of the vagina and vestibule. Beneath it is a small depression, called the suburethral diverticulum, lined by transitional epithelium and having an underlying loose connective tissue layer (Henrikson, 1993).

The mucosa of the calyces, the pelvis, the ureters, the bladder and proximal urethra is lined with transitional stratified epithelium, commonly referred as urothelium (tunica mucosa).

All the mentioned structures also have an underlying loose connective tissue layer (propria-submucosa); a tunica muscularis of smooth muscle forming inner longitudinal, middle circular, and outer longitudinal layers, a tunica adventitia of loose connective tissue or a tunica serosa of mesothelium and connective tissue when a visceral peritoneal covering is present (Henrikson, 1993). The caudal portion of the urethra is lined by stratified squamous epithelium. The lamina propria contains small lymphoid follicles. The normal mucosa of the lower urinary tract is smooth, grayish white and glistening (Confer and Panciera, 1995).

Scanning electron microscopic studies of the normal porcine bladder revealed that its lining was characterized by regularly arranged large polygonal superficial cells, their surface being covered with an irregular network of microplicae. All cells showed tight cell to cell contact margins. Cells of the intermediate layer are smaller, with bleblike, stubby processes, which form microplicae by merging. This is thought to be part of the maturation process of bladder epithelial cells (Wendt et al., 1992). The intact porcine urothelium does not contain goblet cells (Liebhold et al., 1995).

1. 4. 2. Characteristics of normal porcine urine

The **volume** of urine produced daily depends on several factors, like diet, fluid intake, ambient temperature and humidity, the size and weight of the animal, and the water distribution system used (Bollwahn et al., 1988; Drolet and Dee, 1999). Urine is normally **transparent**; its **color** is usually yellow to amber, depending on the concentration of urochromes. The urine **specific gravity** of adult swine is about 1.020, one of the lowest in domestic animals (Drolet and Dee, 1999). Other authors found even lower mean values; in 1397 samples from 22 breeding herds Almond and Stevens (1995) detected a range of specific gravity values between 1.000 to 1.036, with a mean value of 1.009. A mild ammoniacal **odor** in sow urine is normal. Urinary **pH** is usually between 5.5 and 7.5 (Drolet and Dee, 1999). One investigation on random urine samples revealed pH between 5.0 to 8.5, with a mean of 6.93 (Almond and Stevens, 1995). **Glucose, acetone, bilirubin, occult blood, urobilinogen and nitrite** are not found in normal porcine urine samples. Colorimetric reagent strips in the urine of healthy sows can detect only traces of protein. Zero to five **red blood cells** and/or **white blood cells** per high power field (HPF) are acceptable as normal in porcine urine samples (Almond and Stevens, 1995). **Sediment** of normal urine samples consists of white blood cells, red blood cells, epithelial cells of bladder or renal origin, casts, crystals and bacteria. **Casts** are mainly granular. **Crystals** in the urinary sediment are mainly triple phosphate, calcium oxalate or calcium phosphate (Almond and Stevens, 1995; Wendt and Lappe, 1996b). A modest number of **bacteria** exist in normal urine.

Routh and Almond (1998) investigated the changes in urine composition in a cohort of 20 breeding sows during an 8-month period. Urinalysis was performed on urine samples collected during lactation, during the weaning-to-estrus interval and in gestation. Sows had access to ad libitum water from nipple waterers of at least 500 ml/min flow rate. No significant differences were noted in urine composition across different production phases. A transient water shortage during the study resulted in high proportion of abnormal urine samples (containing protein, white blood cells, crystals and bacteria). The authors concluded that urine abnormality, and presumably urogenital tract infection is infrequent in sows with adequate access to water.

Water and food deprivation for 48 hours in prepubertal gilts caused the production of urine with high specific gravity (Almond et al., 1996). Serum electrolyte concentrations of gilts did not change during the experiment. Feed restriction and associated catabolism contributed to increased creatinine concentration in the urine. It appears that pigs maintain a high level of renal function in order to limit detrimental changes in serum chemistry, and healthy animals tolerate brief periods of water and food deprivation. The authors concluded that the high prevalence of urinary tract infections might reflect extreme and chronic conditions placed upon sows in commercial farms.

1. 4. 3. Defense mechanisms of the lower urinary tract

One of the most important defence mechanisms of the lower urinary tract is **normal micturition**, the regular voiding of large amount of urine and emptying of the bladder. This process "washes" out bacteria from the lower urinary tract and lessens the chance of bacterial proliferation in the bladder (Dee, 1992; Wendt, 1998). For this mechanism to work efficiently there must be **adequate urine volume** and the bladder **must be emptied completely** and **at frequent enough intervals**. Insufficient water supply, lack of exercise, locomotory disorders and increasing residual volumes in older sows are the main reasons for dysfunction of this mechanism (Wendt, 1998).

In scanning electron microscopic studies a thin mucus layer could be detected on bladder epithelium, similar to other species (Wendt et al., 1992; 1994). This **glycosaminoglycan layer** covers the mucosa and binds with water to form a barrier to prevent urinary constituents from coming in contact with urothelium (Dee, 1992). This layer prevents adherence of bacteria through covering of possible receptor sites. Secretion of the glycosaminoglycan layer is under the influence of estrogen and progesterone. A defective layer can be observed in animals with urinary tract infection (Wendt, 1998; Liebhold et al., 1995). In cases of significant bacteriuria the rapid and massive **appearance of goblet cells** and the ensuing excessive mucus production can be interpreted as a non-specific local defence mechanism (Liebhold et al., 1995). Other substances, as **oligosaccharides** and **uromucoid** (Tamm-Horsfall mucoprotein) act by aggregating bacteria in the urine (Wendt, 1998). Oligosaccharides may also help detach bound bacteria from the bladder wall (Dee, 1992).

The bladder mucosa is known to have antibacterial activity due to several non-specific factors. Such factors are **high osmolality, urea concentration** and low **urine pH**. In addition, the presence of **immunoglobulins** such as IgG, IgA and secretory IgA in the urine and **exfoliation of epithelial cells** bound with bacteria aids in bacterial clearance (Wendt, 1998).

Normal bacterial flora of the vulva, vagina and distal urethra can inhibit colonisation by uropathogens by competition for nutrition and binding sites as well as by the presence of antibacterial agents (Wendt, 1998).

1. 4. 4. Facultative pathogens in urinary tract infections

Urocystitis is mainly the result of ascending bacterial infection from the urethra. The vulva, vagina and distal urethra have a normal bacterial flora comparable with the fecal microflora of the animal. Some of the bacterial species commonly found in the lower urogenital tract of female swine are listed in Table 1. 4. Population of these agents is at their highest concentration in the **vulvar and vaginal regions**. The bladder is usually regarded as sterile; bacteria found on urinalysis of voided urine from healthy animals presumably originate from the distal urethra or from the vulvar mucosa (Bollwahn et al., 1988).

Table 1. 4.: Normal bacterial flora of the female lower urogenital tract (Dial and MacLachlan, 1988b; Dee, 1992; Wendt, 1998)

*Actinomyces pyogenes**	*Enterobacter aerogenes*	*Pseudomonas aeruginosa**
(*Actinobaculum suis*)*	*Fusobacterium spp.*	*Staphylococcus albus*
Bacillus spp.	*Klebsiella spp.**	*Staphylococcus aureus*
Bacteroides fragilis	*Lactobacillus spp.*	*Staphylococcus epidermidis*
Chromobacterium spp.	*Mycoplasma spp.*	*Streptococcus faecalis**
Citrobacter spp.	*Neisseria catarrhalis*	*Streptococcus suis**
Clostridium spp.	*Pasteurella multocida**	*Streptococcus zooepidemicus*
*E. coli**	*Peptostreptococcus spp.*	Group A, G, L streptococci
Edwardsiella spp.	*Proteus spp.**	

* Denotes potential urinary tract pathogens

The urinary tract is a dynamic microbiological ecosystem, where dominant bacterial species can change spontaneously (Bertschinger, 1999). This occurs more frequently when sows are treated with antimicrobials (Berner, 1990). Urinary tract infections are seldom result from the overgrowth of only one bacterial species; **mixed infections** of the urinary tract are very common. Mainly for the ease of discussion, urinary tract infection will be classified here as specific, being caused by *Actinobaculum (Eubacterium, Actinomyces) suis* and as nonspecific, caused by a variety of microbes. It is important to note, that nonspecific urinary tract infection often (if not always) paves the way for *A. suis* infection (Bertschinger, 1999).

1. 4. 4. 1. Actinobaculum (Eubacterium, Actinomyces) suis

Actinobaculum suis is capable to cause chronic urocystitis and pyelonephritis with chronic weight loss and subsequent culling or death of the sow. It is also reported to induce acute uraemia and sudden death (Taylor, 1999). Morphological and biochemical properties of this pathogen are quite well understood and extensively studied, however, it remains quite unclear how it becomes pathogen in many cases.

Actinobaculum suis was first isolated by Soltys and Spratling (1957) in England from the urinary tract and urine of sows with cystitis and pyelonephritis. Later it was isolated from clinical cases of urocystitis and pyelonephritis in many countries, among others in Australia, Canada, Finland, Hong Kong, The Netherlands, Switzerland and in the United States (Glazebrook et al., 1973; Percy et al., 1966; Kauko et al., 1977; Munro and Wong, 1972; Frijlink et al., 1969; Schällibaum et al., 1976; Walker and MacLachlan, 1989).

The nomenclature of this bacterium has changed several times since its first isolation. Originally described as *Corynebacterium suis* (Soltys and Spratling, 1957), later was assigned to the genus *Eubacterium* (Wegienek and Reddy, 1982); subsequently it was suggested to be transferred to the genus *Actinomyces* (Ludwig et al., 1992), and finally to the genus *Actinobaculum* (Lawson et al., 1997).

A. suis is a slender, nonmotile, 0,5 x 1-3 μm pleiomorphic rod, arranged singly, in pairs (often found at an angle to each other), in palisades, or in small clusters. Gram positive but rather easily decolorized, especially in old cultures. Not acid fast, non spore forming. It can be isolated and cultured under anaerobic conditions. On blood agar it forms 2-3 mm wide characteristic dry, grayish, smooth colonies with a crenated edge and a slightly elevated, shiny center in 3 days. After one week of incubation colonies are 3-5 mm in diameter and flatter. There is no haemolysis. The organism is not strictly anaerobic; its prolonged aerobic incubation on blood agar results in the development of colonies within 5-10 days. Such colonies are pinpoint, shiny, round with entire edge (Wegienek and Reddy, 1982).

Peptone-yeast extract-starch broth supports excellent growth of *A. suis*, which is enhanced by the addition of urea to a final concentration of 1.2% (w/v). Optimal growth occurs at pH 7-8, there is no growth at pH 5 or less. Optimal culture temperature is 37°C, growth occurs from 30 to 43°C (Wegienek and Reddy, 1982).

A. suis is quite inactive in different biochemical tests: the catalase, metilred, Voges-Proskauer, indol- and nitrate reduction tests are negative. Does not produce hydrogen sulfide, lipase or lecithinase, ammonia is not produced from peptone and does not hydrolyze esculin and gelatin. Urease is produced and hippurate is hydrolyzed by every strain of the bacterium, it ferments only maltose, glycogen and starch (Soltys and Spratling, 1957; Wegienek and Reddy, 1982; Moore and Moore, 1986). Growth in urea greatly enhances the activity of urease.

No demonstrable exotoxin is produced by the bacterium (Wegienek and Reddy, 1982). Larsen et al. (1986) demonstrated that some strains are heavily fimbriated and are able to adhere to epithelial cells of the porcine bladder; glycoconjugates are the presumed receptor sites for the attachment of *A. suis*. Isolates of *A. suis* haemagglutinate cattle, sheep and human erythrocytes, there is no haemagglutination using horse or pig erythrocytes. The velocity of the haemagglutination was enhanced by the presence of 1% urea; this might be an important feature

in the pathogenesis of disease (Carr and Walton, 1990). Strains examined by Carr and Walton (1990) possessed two plasmids of 2 and 1.5 Kbp.

Strains of *A. suis* on covered colistin nalidixic acid plates remained viable for four days when held at 20 °C, and for 17 days at 4 °C. *A. suis* was capable of surviving only up to 24 hours when held at -20 °C in PBS. It was capable to survive for one hour on concrete surface when mixed with semen, for 12 hours when mixed with urine and for 24 hours when covered with manure at 20 ºC. At this temperature, it was capable to survive on the surface of latex gloves for 24 hours when mixed with semen or urine. Survival lasted for two to five hours in solutions with pH 3 and 4, respectively; at pH 5 to 7 the bacterium remained viable up to 18 hours (Dee et al., 1993). Commonly used disinfectants can kill the bacterium at regular concentrations. Dee (1991) suggests disinfectant sensitivity tests to be conducted in order to choose disinfectants effective against *A. suis*. Based on such tests he found phenols, formaldehyde based compounds and quaternary ammonium compounds to be effective against *A. suis*.

A. suis is a normal inhabitant of the prepuce and preputial diverticulum of mature hogs (Jones et al., 1982; Pijoan et al., 1983; Taylor, 1999). *A. suis* was successfully isolated from the prepuce of 5-15-week-old (Jones and Dagnall, 1984) and 20-day-old male piglets, from the stall floor and from the footwear of hog attendants (Carr and Walton, 1990). It is only occasionally isolated from the urogenital tract of healthy females; however, Dee et al. (1993) detected presence of the bacterium in the vagina of female piglets, immediately after being born to a sow with urinary tract infection. In the same study, they detected *A. suis* in the vagina of 27% of sampled female swine aged from 14 days to 6 months. *A. suis* cannot be found in other organs but the urinary tract, and is not pathogenic to other species except the pig (Jones, 1987). It was detected in the preputial diverticulum of wild boars (Jones et al., 1982).

In an extensive survey by Høgh et al. (1984) in Denmark, 999 organs or samples were examined for the presence of *A suis*. It was isolated in 33% of kidneys and bladders originating from slaughterhouses and was detected in 25% of urine samples, but only in 3% of vaginal discharges or uteri (the latter originating from slaughter plants). *A. suis* was present in approx. 24% of the 243 boar semen samples tested. The presence of the bacterium in semen did not seem to reduce its quality and fertility. No seasonal distribution of the disease has been observed. The bacterium was detected in 116 herds, evenly distributed in Denmark.

Wendt and Vesper (1992) found *A. suis* with indirect immunofluorescence in 11.4% of 943 urine sediment samples of sows from 21 breeding herds in Germany.

1. 4. 4. 2. Other bacteria

Escherichia coli is the most frequent isolate of unspecific urinary tract infection (Wendt, 1998). In humans and in dogs colonization of the lower genital tract and of the urinary tract by *E. coli* is greatly facilitated by adhesive fimbriae. Characterization of *E. coli* isolates from sows for detection of potential urinary virulence factors demonstrated the presence of type 1 fimbriae in 41% of the strains. P fimbriae (important in *E. coli* causing human pyelonephritis) could only be found in one of 66 isolates (Carr and Walton, 1992a). According to Wendt (1998), expression of type 1 fimbriae might be an important virulence factor, for it enables *E. coli* to adhere to the surface of superficial cells and intermediate cells of the urothelium. It is not currently possible to characterize uropathogenic *E. coli* strains based on a single virulence characteristic. 31 *E. coli* strains isolated from cases of significant bacteriuria by Brito et al. (1998) possessed a variety of virulence factors as serum resistance, aerobactin, colicin and hemolysin production in various combinations. 23% of their isolates produced LT and VT, but none of them produced STa. Various types of fimbriae (F41, F42, and F1) were observed. Characterization of porcine uropathogen *E. coli* isolates warrants further studies. Much less is known about other potential urinary tract pathogens (Table 1.4.), their virulence factors have not been studied yet in swine.

1. 4. 4. 3. Mixed infections

As stated earlier, mixed infection with several facultatively pathogenic bacteria is the commonest form of urocystitis in swine. Carr and Walton (1992b, 1993) performed bacteriological examinations on the urinary tract of 23 sows died of cystitis and pyelonephritis. Gram negative isolates from the upper urinary tract and bladder were *E. coli*, *Proteus spp*. and *Pseudomonas spp*., *E. coli* being the most frequent isolate. Apart from *A. suis* (21 of 23 cases), Gram positive isolates from the upper urinary tract were *Actinomyces pyogenes*, *Enterococcus faecalis*, different streptococci (e. q. *Streptococcus lactis*, *Aerococcus viridans*). *Bacillus spp*. and micrococci were isolated from the lower urinary tract only. Midstream urine samples of sows with asymptomatic bacteriuria contained a less complex bacterial flora, mostly consisting of pure culture of *E. coli*. *Proteus spp.*, *Klebsiella pneumoniae*, streptococci, *Enterococcus faecalis* have been also isolated in a few cases and in low numbers. Generally, obligate aerobic bacteria are not causing cystitis-pyelonephritis, as its anaerobic environment prevents their growth in the upper urinary tract (Carr and Walton, 1992b, 1993).

Stirnimann (1984; 1988b) isolated a broad spectrum of bacteria from the urine of sows showing signs of acute UTI, predominantly *E. coli*, streptococci, *Proteus spp*. and *A. suis*. These bacteria were present in mixed cultures or as a monoinfection.

1. 4. 4. 4. Bacterial content of boar genitalia and semen

The bacterial flora of male genital tract contains various nonpathogens, potential pathogens and might contain *Brucella suis* (Thacker et al., 1984). Bacterial flora of the male genital tract is compatible with bacteria found in the female (Table 1.4.). Semen from old boars has higher seminal bacterial counts. Retention of urine in the preputial diverticulum increases seminal bacterial counts (Thacker et al., 1984).

1. 4. 5. Predisposing factors of urinary tract infections

Upper urinary tract infections, especially pyelonephritis mainly result from ascending urocystitis. In this regard, predisposing factors of lower urinary tract diseases have primary importance. In the followings such factors are listed. Developmental anomalies of the urinary tract, parasitic and neoplastic diseases, which might also contribute to UTI, are not discussed.

In discussing predisposing factors of UTI perhaps the main point is, that the majority of the microorganisms involved in urogenital infections are members of the normal flora of the urogenital tract, as well as of the normal fecal microflora (Dee, 1992). Therefore, as the fecal contamination of the environment rises, so do the number of potential pathogens. **Excessive fecal contamination** of the environment, among others, can be a result of the following factors: **overcrowding**, **infrequent** and/or **inefficient cleaning of stalls**, **improper stall design**. During breeding or gestation, sows that are housed on solid floors with inadequate drainage or on slatted floors with insufficient waste removal have a higher prevalence of urogenital infections (Dial and MacLachlan, 1998a). Laxatives in the ration can produce an excessively **loose stool** and enhance contamination of the perineal region. Manure in a liquid state has a tendency to contaminate nearby animals, particularly if they are housed in stalls or crowded pens (Dee, 1992). Heavy bacterial contamination of the lower genital tract usually occurs at parturition, there is also a high risk of UTI in the postfarrowing period.

The popularity of **confinement gestation and breeding facilities** led to an increasing frequency of urogenital tract infections in many Western European countries (Madec, 1984; Dial and MacLachlan, 1988a; Dee, 1992). Confinement facilities predispose to UTI if their floor design allows fecal accumulation under the hindquarters of the animal, if they are too short and the sow forced to obtain a "dog sitting" position. Another factor associated with this type of accommodation is the lack of exercise. Restricted movement causes less frequent urination and thereby predisposes the sow to urine stasis, bacterial multiplication and subsequent UTI. Sows

housed in pens tend to have a lower morbidity than do those tethered or housed in crates. Chronic debilitating disease can also be a cause of lack of exercise.

Another frequently cited predisposing factor to UTI is **decreased water intake**, which leads to the reduction of the frequency of urination (urinary stasis), thus potentiating microbial growth in stagnating urine. Decreased water intake can result from lack of exercise, confinement, and weight excess of sows and also from **reduced water availability** or **poor palatability**. Water allowances for sows kept under intensive dry feeding conditions have been estimated as 10-18 L/pig/day for dry sows and 20-35 L/pig/day for lactating sows (Williams, 1998). Water requirements of sows published by the National Research Council (1998) are 10-20 L/pig/day for pregnant gilts and 12 to 40 L/pig/day for lactating sows. The actual water requirement is likely to depend on type and quality of food, water quality and the environment. If water is provided through nipple waterers, their flow rate should be at least 2 L/min for lactating and gestating sows (Muirhead and Alexander, 1997; Anonymous, 1999). Pregnant sows consumpting less than 11,5 L water daily had a 5.38 times higher chance to develop severe bacteriuria (Madec, 1984). Lowest rate of detection of *A. suis* in urinary sediment samples was in herds with ad libitum watering (Wendt and Vesper, 1992). The following factors can influence water intake in sows through poor palatability or reduced availability (Table 1.5.; Wendt, 1998).

Table 1. 5.: Factors that may affect water intake in sows

Water quality	low temperature, high concentration of dissolved solids, high mineral content, medications, deposits
Header tank	algae/bacterial growth, contamination, no lid, insufficient capacity, freezing up
Pipelines	incorrect pressure, freezing up
Drinker	insufficient drinker/animal ratio, wrong installation/position, type of drinkers and flow rates not suitable, defective or damaged drinkers, stray voltage
Through	pollution (feces, feed), water depth < 4 cm
Management	restricted access to water, inadequate maintenance of installations, slippery floor, liable to icing

Drinkers with **low flow rates** are often found in gestation and lactation stalls. There might be a great variety in drinker flow rates even within the same farrowing room. Although frequently cited as a possible cause for poor lactation performance, it is not clear yet to what extent drinkers with low flow rates contribute to urinary tract infections (Muirhead and Alexander, 1997; Anonymous, 1999). **Maintenance** of installations generally receives inadequate attention in practice (Carr, 1992). **Hardness** of water due to the presence of magnesium and calcium ions does not reduce palatability of water, but result in scale deposition and a subsequent decrease in water flow rate. **Water pH** has little or no effect on water quality and on UTI, although high water pH impairs the efficiency of chlorination and low water pH can cause the precipitation of antibacterial agents delivered via the watering system (National Research Council, 1998). **Total dissolved solids (TDS)**, a measure of the total inorganic matter dissolved in a sample of water (calcium, magnesium, sodium ions in the bicarbonate, chloride or sulfate form are the most common salts found in water with high TDS). A high (> 7000 ppm) TDS content of water can adversely affect water uptake through water refusal (National Research Council, 1998).

Urinary tract infections become increasingly more common with more parity (Madec, 1984; Dial and MacLachlan, 1988a; Wendt and Vesper, 1992; Dee, 1992). **Parity distribution** of the sow herd can affect the prevalence of urinary tract disorders; high proportion of older parity females can lead to herd level problems with UTI. This is possibly due to several factors. One is the **increasing residual volume** of urine in the bladder in higher parity females; also these animals are more frequently **overweight** and affected with **locomotion problems**. These

alterations will increase the chance for urine stasis and consequent microbial proliferation in the bladder. Many times **vulvar damage** can be observed in high parity sows - improper closure of the damaged vulva will increase the chance for bacterial contamination of the vagina and distal urethra. Risk factors for vulva biting (and resulting vulvar damage) in sows have been reported by Rizvi et al. (1998). Similarly to vulvar damage, partuition-associated injuries of the bladder and urethra can also be predisposing factors and are more frequent with advancing parities.

Not much is known yet about the associations between urolithiasis, cystitis and genital tract infections. Cross-sectional studies can provide data on such associations, but cannot reveal the temporal relationship between urolithiasis and cystitis. Generally, urolithiasis or crystalluria can be either a cause or consequence of UTI. **Urolithiasis** is the presence of calculi in the urinary passages. Calculi (uroliths) are macroscopic aggregations of precipitated urinary solutes, urinary proteins and proteinaceous debris; minerals predominate in calculi and matrix predominates in so called urethral plugs (Jubb et al., 1993). The latter is a mass of sandy sludge with a much higher organic component whose form is largely determined by the shape of cavity it fills. The term **crystalluria** is used for abnormal microscopic crystalloid precipitates in urine. The mineral composition of calculi found in pigs and their importance has not been extensively studied yet. Clinical problems associated with urolithiasis are mainly described in suckling piglets or in weaned pigs (Smythe et al., 1986). Similar reports in breeding animals are lacking in the available literature.

Although some authors does not attribute much importance to urolithiasis in pigs (Jubb et al., 1993), and the frequency of urolithiasis is reportedly lower than in other domestic animals, various types of calculi can be found in pigs, too. They include calcium carbonate, calcium phosphate, struvite (magnesium ammonium phosphate hexahydrate), uric acid, and urate (Drolet and Dee, 1999). Factors generally known to predispose to the formation of uroliths include the **diet, urinary pH, reduced water intake, urinary stasis and preexisting urinary tract diseases**. Theories of calculus formation in other species, mainly in companion animals is described in detail by Jubb et al. (1993), and other authors. Much less is documented about urolith development in pigs, but similar mechanisms should be expected. High mineral content of sow feed has a marked effect on renal excretion of the minerals. Feed **high in phosphorus concentrations** can be responsible for crystalluria, mainly consisting of calcium phosphate crystals. **Water restriction** and **alkaline urine pH** support the development of certain urinary concrements, however, sows show great individual differences (Wendt et al., 1996; Wendt and Lappe, 1996). These authors suggest that urinary sediment promote urinary tract infections by irritation of the bladder mucosa. According to Drolet and Dee (1999) infection induced calculi are observed occasionally in sows with cystitis and pyelonephritis, but the yellowish sediment often found in the bladder of sows do not seem to be of clinical significance. On postmortem examinations such sediments admixed with desquamated epithelial cells might give the false diagnosis of cystitis because of turbidity of the urine. The authors did not define the nature of this "sediment" more closely.

During estrus **estrogens** cause the urine pH to rise and remain slightly alkaline for up to three weeks postweaning, thus possibly enhancing the growth of *A. suis* (Dee, 1991).

Boars can play a role in the development of UTI, especially at farms where natural mating is practiced. *Actinobaculum suis* is described as the normal inhabitant of the preputial diverticulum of healthy boars, this and other bacteria can be transmitted to sows when they excessively multiplicate in the preputial sheath or when the preputial ostium is heavily soiled. Postcoital infections of the urogenital tract are seen more commonly on farms that have unhygienic breeding practices, such as manual manipulation and subsequent contamination of the boar's penis before mating, than on farms that follow hygienic breeding programs (Dial and MacLachlan, 1988a). The highest incidence of UTI can be observed usually within one month post mating. Beside microbial contamination, trauma to the urethral opening due to the penis of the boar or to the AI catheter is speculated as one possible predisposing factor.

As seen in other female mammals, the relatively **short and wide urethra** of the sow can predispose to ascending bacterial infections. **Atony of the muscle sphincter vesicae** in late pregnancy and post partum might further increase this possibility (Wendt, 1998).

Inflammatory conditions of the genital tract (vestibulitis, vaginitis, cervicitis or endometritis) can serve as a nidus for bacterial proliferation in the urinary tract (Wendt, 1998). However, the lack of long term prospective observational studies on the development of urinary and genital tract infections make true temporal relationships between the two conditions hard to reveal. Also, as both have a considerable overlap in predisposing factors, their relationship is likely to be confounded by this (1. 6. 2.).

As in other species, **cooling of the abdomen** by cold and wet floors might damage the non-specific defense mechanisms of the lower urinary tract, thus predisposing to urinary tract infections (Wendt, 1998).

Interestingly, Geudeke et al. (1992) did not found distinct predisposing factors during the analysis of a large dataset of slaughterhouse findings and management features, involving over 11.000 sows from 151 Dutch herds. However, they found that cystitis was correlated with decreased productivity.

1. 4. 6. Pathogenesis of urocystitis-pyelonephritis

Urinary tract infections can develop in sows due to hematogenous spread of bacteria and include embolic purulent interstitial nephritis and renal abcessation (Jubb et al., 1993). Ascending bacterial infections from the lower urethra resulting in urocystitis and pyelonephritis are much more common and are in the scope of this review.

Predisposing factors outlined in the previous paragraph (1. 4. 5.) are necessary for facultatively pathogen bacteria to overcome natural host defense mechanisms. The resulting bacterial multiplication will induce changes in urine characteristics, will injure urothelium and ureteric valves. Bacterial adherence to the urothelium is supposedly necessary to induce such changes. Urothelial damage can lead to necrosis, ulceration and sometimes extensive hemorrhages. Clinically, disuria will ensure, but fever, inappetence is not a usual feature in this stage. Sows with cystitis and acute pyelonephritis or chronic active pyelonephritis had statistically significant shortening of the intra-vesicular portion of the ureter (Carr et al., 1990). This shortening is believed to be due to bacterial products. Damage to the ureteric valves and the increased intraluminal pressure due to stagnation can result in vesicoureteric reflux. The retrograde flow of bacteria laden urine can induce inflammatory lesions in the kidney pelvis and subsequently, in the parenchyma. In cases of acute pyelonephritis sudden damage to the intravesicular ureter might allow large number of bacteria and bacterial products to reach the renal pelvis, with acute renal failure as a result (Carr et al., 1990). Fever, inappetence, uremia and sometimes sudden death characterize acute pyelonephritis.

A. suis infection of the porcine bladder reportedly causes more severe alterations than infections due to other bacteria. However, debilitation of the urothelium due to previous infection is necessary to support infection of the bladder with *A. suis* (Wendt et al., 1994). Experimental monoinfection with *A. suis* was not always successful to induce any change in the urothelium, otherwise healthy sows could clear off the infection.

Chronic urocystitis will result in thickening of the bladder wall, sometimes polypoid projections on the mucosa will also appear. Chronic follicular cystitis, a frequent finding in dogs has not been reported yet in swine. Chronic urocystitis might have an important role in predisposing sows to endometritis and/or to postparturient dysgalactia syndrome. Chronic pyelonephritis and the ensuing tubulointerstitial nephritis will lead to chronic kidney failure. Clinically, weight loss and uremia will be the most frequent findings in this case.

1. 4. 7. Diagnosis of urinary tract infections

1. 4. 7. 1. Record analysis

Production data analysis has limited value in diagnosing urinary tract infections as recording of supplementary information on sow health and morbidity is usually of low importance to producers. However, if the computerized herd management system is capable to handle such information and the attendants willing to record it, valuable data can be gathered. Information which might be relevant to urinary tract infections are morbidity and mortality reasons of sows, paritywise distribution of culling percentages, treatment for urinary tract infections, incidence of discharges in relation to farrowing, weaning or breeding and parity distribution of the breeding herd. Urinary tract infection and subsequent endometritis can be suspected when the decrease in farrowing rate is due to regular returns, and older parity sows are mainly affected (Tubbs, 1987; Dial and MacLachlan, 1988a). Sows that develop discharges after mating in which the discharge occur regardless of the stage of estrus cycle and do not influence fertility are most likely to have urinary tract (or vaginal) infections. However, such associations on a herd level cannot be determined with certainty without other diagnostic efforts.

1. 4. 7. 2. Clinical signs

In the majority of nonspecific urinary tract infections there are no observable clinical signs. There is usually no fever or inappetence; however, sows with cystitis may show abnormal urination. They void urine in small quantities with straining, they are more often seen in a dog-sitting position (Bertschinger, 1999). Urine can be turbid. Voiding of urine rich in crystals might result in chalky deposits on the vulva or on the pen floor. This might not be accompanied with lower urinary tract infection (Dee, 1992). **Vulvar discharge** associated with cystitis is usually small to moderate in amount (less than 20 ml), purulent, often mucoid or mucopurulent. It is evident at the end of urination and occurs independently of reproductive status or stage of estrus. This type of discharge may occur after mating, but can be seen anytime during pregnancy and lactation (Dial and MacLachlan, 1988a). UTI associated vulvar discharge is more frequent in older sows.

Clinically, **macrohaematuria** is the main sign of acute *A. suis* infection of the upper urinary tract. In these cases the vulvar discharge usually contains some blood. Some sows might die suddenly, due to acute renal failure. There is fever, or in some cases the rectal temperature is subnormal. In chronic cases there is weight loss and uraemia. Severe pyelonephritis manifested clinically during the first two weeks postpartum in 40% of the cases reported by Stirnimann (1984).

1. 4. 7. 3. Serum biochemistry

There are no observable metabolic alterations in cases of asymptomatic bacteriuria or in uncomplicated low-grade urocystitis. In sows suffering form acute pyelonephritis the following deviations were frequent: normocytary-normochromic anaemia, neutrophilia, uraemia, hypercreatininaemia (Stirnimann, 1988a). Reference values for serum urea and serum creatinine concentrations in sows kept under modern management conditions are 4.8 mmol/L (range 2.7 - 7.2) and 178.1 μmol/L (range 134-241), respectively (Elbers et al., 1994).

The first sign of renal lesions is a reduced glomerular filtration rate and raised plasma creatinine concentration. In sows without clinically manifested UTI plasma concentrations of sodium, potassium, phosphorus, calcium and urea are in the normal range. Parallel with developing tubular damage clinical signs appear and uraemia can be diagnosed upon raised urea and creatinine concentrations and haemoconcentration. Acute pyelonephritis or terminal chronic pyelonephritis will lead to uraemia, hyponatraemia, elevated plasma potassium and phosphorus concentrations (Wendt, 1998).

1. 4. 7. 4. Urinalysis

Early morning midstream urine samples can be collected from sows for urinalysis. Rousing the sows before feeding time can induce voiding. Catheterization is possible, but impractical and involves the risk of actually inducing UTI (Stirnimann, 1984). Urine samples can be collected from the bladder of culled or dead sows or gilts at necropsy. However, microbial contamination and degradation of urine constituents can make urinalysis results unreliable in these cases. When the bladder is dissected several hours post mortem, sloughing of epithelial cells will increase the turbidity of urine. In sows culled with electrocution, the bladder is frequently emptied, leaving only traces of urine for analysis.

Characteristics of normal porcine urine were given earlier (1. 4. 2.). Examination of urine samples involves gross examination, tests for chemical constituents and evaluation of urine sediment (Almond and Stevens, 1995).

On gross examination, the **color** of urine can change to dark yellow or yellowish brown in sows with restricted water intake and urinary stasis. It can turn colorless in sows with chronic kidney failure and consequent polyuria/polydipsia. Urine often appears light or dark red in cases of acute *A. suis* infection (haematuria). Red or brown urine indicates that haematuria or hemoglobin/myoglobin is present in concentrations of greater than 30-50 mg/dl (Almond and Stevens, 1995). **Transparency** of porcine urine can decrease due to the presence of red blood cells, white blood cells, crystals, epithelial cells, mucus, casts and bacteria. Crystals, sloughed cells, bacteria and white blood cells may clump and cause flocculation. **Viscosity** of urine can markedly increase in UTI, especially in *A. suis* infection (Walker and MacLachlan, 1989). This is presumably due to increased mucus production as a non-specific defence mechanism of the bladder mucosa. **Specific gravity** (SG) is inversely related to urine volume. Concentrated urine can result from diminished water intake, increased fluid loss via routes other than urination (diarrhea or high ambient temperature), or increased secretion of urine solutes. On the other hand, diluted urine is a frequent finding in gestating sows. Sows that consume water ad libitum produce large volumes of urine with low specific gravity. Urine abnormalities are common in samples with SG greater than 1.020 and rare in samples with SG less than 1.005 (Almond and Stevens, 1995). **Strong odor** of urine may result from breakdown of urea to ammonia, due to urease producing bacteria. A putrid odor is evident if there is extensive protein breakdown caused by bacteria.

Tests for chemical constituents utilize diagnostic test strips and involve determination of pH, nitrite, protein, and hemoglobin content of urine (Bertschinger, 1999). **Urinary pH** is influenced by the metabolism and the composition of the feed; starvation or high protein intake lowers urinary pH. Feed additives can also influence urine pH (Dee et al., 1994b; van Kempen et al., 1998). Infection with urease producing bacteria (e. g. *A. suis*) may result in significant alkalinization of urine. In *A. suis* infection urinary pH can exceed 8.2 (Liebhold et al., 1995) or 9 (Walker and MacLachlan, 1989). Generally, there is a positive correlation between high urinary pH and increased bacterial concentration in urine (Dee et al., 1994b). **Nitrite** can be present in urine due to bacterial reduction of urinary nitrate. It presumes the presence of nitrogen compounds, specific bacterial flora and a prolonged retention of urine in the bladder. Thus, early morning urine samples indicate nitrituria with a higher chance (Madec, 1984). Many Gram-negative bacteria and staphylococci share the ability to reduce nitrates. A positive reagent strip test indicates at least 10^5 organisms per ml of urine (Almond and Stevens, 1995). Nitrite detection can yield a false negative result when the SG or the ascorbic acid content of the urine is high. Bertschinger (1999) warns that the sensitivity of the test strip is too low due to the normally low nitrite concentration in porcine urine. **Protein** concentration of normal porcine urine is usually not detectable by commercial test strips (< 30 mg/dl). Fever, glomerulonephritis or inflammation anywhere in the urinary tract can lead to proteinuria. Vaginal contamination of the sample can also lead to positive results. If blood concentration is high in urine, haematuria (and haemoglobinuria/myoglobinuria) can cause a positive test. Alkaline urine or prolonged

dipping of the test strip may produce false positive results for protein. A high protein concentration is associated with high urinary pH (Madec, 1984) and with high SG (Almond and Stevens, 1995). **Hemoglobin** can usually be present in urine due to intravascular hemolysis (e. q. copper poisoning). Urine with low specific gravity can lyse red blood cells present in urine due to haematuria, thus a positive test for hemoglobin without RBCs in the sediment can result (Almond and Stevens, 1995). **Myoglobin** will appear in urine due to severe muscle damage.

Urinary sediment examination can reveal the presence of crystals, casts, cellular elements, and bacteria. Formation of **crystals** is under the influence of many factors, among them the composition of the feed and urinary pH. Wendt et al. (1996) and Wendt and Lappe (1996) concluded that high mineral content of sow feed have a marked effect on renal excretion of the minerals. Especially **feed high in phosphorus** concentrations can be responsible for calcium phosphate crystalluria. Walker and MacLachlan (1989) detected several types of crystalline sediment in sows with *A. suis* urocystitis, formed of struvite alone, or composed of struvite, apatite and calcium oxalate dihydrate. As struvite or calcium oxalate crystals can be abundant on microscopic examination of urine sediment of apparently healthy sows, the exact significance of crystalluria is not yet determined (Almond and Stevens, 1995). Wilson et al. (1972) reported high levels of oxalate crystals associated with proteinuria, even in absence of kidney damage in a slaughterhouse survey. Published reference photomicrographs should aid the identification of different types of crystals (Osborne et al., 1990). **Casts**, mainly granular ones can be found in the urine of normal pigs, a large number of them suggest tubular degeneration. From **cellular elements**, presence of high number of neutrophil granulocytes suggests UTI. Commercial test strips are reportedly having too low sensitivity to detect leukocytes in porcine urine samples (Almond and Stevens, 1995). In a study of Bollwahn et al. (1988) urine samples taken during estrus showed a high leukocyte content, a slightly increased pH and higher specific gravity. Urine taken during heat, therefore, should not be used to diagnose urinary tract infections. Over 5 red blood cell/HPF suggest **haematuria**. Renal or bladder epithelial cells can be present in normal urine, their increased number suggest degeneration of their tissue of origin. **Bacteria**, both bacilli and cocci are common in urinary sediment. In the absence of leukocyturia, it is challenging to decide whether bacteria indicate UTI or contamination (Almond and Stevens, 1995). **Bacteriuria** is generally considered significant when more than 10^5 colony forming units (CFU)/ml are present in urine.

Clinical signs of urinary tract infection do not always correlate with morphological findings in the bladder. Sows with macroscopically unchanged urine can show histological changes associated with cystitis. In these cases only the significant bacteriuria indicates the presence of a urinary tract disorder. Thus **bacterial culture** of the urine is very important for the diagnosis of urinary tract infections. However, other urinary characteristics should always be determined in order to improve the reliability of diagnosis (Liebhold et al., 1995). Bacteria in urine are identified by standard aerobic and anaerobic culture methods. Commercially available slides covered by bacterial culture media (dip slides) give satisfactory quantitative results (Both et al., 1980). They are not suitable, however, for the detection of *A. suis*. Laboratory diagnosis of *A. suis* infection can be based on isolation of the pathogen from urine or urinary bladder mucosa scrapings on horse blood agar or colistin-nalidixic acid-metronidazole supplemented Columbia blood agar (CCNAM) media under anaerobic conditions for 4-6 days at 37 °C (Dagnall and Jones, 1982).

Madec (1984) and Madec and David (1984) investigated several factors possibly related to urinary tract disease in sow herds. The investigation included sows of different parities and health status. He found that urine pH varied greatly among samples, but 85% of the samples ranged between 5.6 and 7.5. High values (> 8) were more often related to abnormalities (protein, blood, crystalline sediment) in the urine. Samples were positive for nitrites in 17.6% of the samples. Nitrite found to be a good indicator for significant bacteriuria, especially in the first urine samples collected in the morning. On the other hand, bacteria might be present without

nitrituria. Significant bacteriuria (>10^6) was present in 13.5% of the 1617 samples examined. Phosphate crystals were the most frequent forms of sediment found in urine samples, but oxalates were also observed. Fourth parity and older sows had significantly higher chance to have high nitrite, protein concentration or bacteria in their urine. Among herd level risk factors "bad hygiene" was associated with severe bacteriuria with much higher chance.

1. 4. 7. 5. Serology

Serology generally has little or no value in detecting nonspecific urinary tract infections. Serum antibody against the infecting *E. coli* strain can usually be detected in sows with pyelonephritis, less often in sows with cystitis and rarely in sows with asymptomatic bacteriuria (Bertschinger, 1999).

A. suis can be detected by indirect immunofluorescence (IF) (Schällibaum et al., 1976; Wendt and Amtsberg, 1994). Indirect IF found to be a highly specific test with lower sensitivity (78.9%). Seroconversion in sows occurs after 3 weeks at the earliest; however, it may take 6-8 weeks to develop. Serum antibody levels can remain elevated at least for 4 to 9 months. Serology can yield negative results in spite of *A. suis* infection if immunological response still not taken place or development of antibodies has failed to appear after short time infection of the bladder (Wendt and Amtsberg, 1994). As in other bacterial infections, antibiotic treatment may influence the magnitude of immune reaction. Serological examination in case of *A. suis* infection gives no information about renal involvement.

Rapid detection of *A. suis* can be achieved by the use of IF techniques (Schällibaum et al., 1976; Langfeld et al., 1990). In comparing indirect IF, Gram-stained stains of urine sediment, direct culture and culture after enrichment, Langfeld et al. (1990) concluded, that the indirect IF technique is the most reliable and time saving of these methods. By using indirect IF it is also possible to detect antibody coated bacteria in urine. Antibody coated bacteria in urinary sediment of other animals and humans are characteristic for pyelonephritis, they usually cannot be detected in uncomplicated cases of cystitis (Nicolet and Fey, 1979).

1. 4. 7. 6. Pathology

Cystoscopy proved to be of value in the diagnosis of lower urinary tract infections (Wendt and Aengenheister, 1989; Wendt et al., 1990), especially in subclinical cases, when changes in the urine are not evident. However, it is not a practical technique in field situations. Necropsies or slaughterhouse examination of the urogenital organs of culled sows or gilts will provide information on the prevalence of UTI (Almond and Richards, 1992).

On **gross examination**, focal or diffuse mucosal hyperaemia and congestion are the first signs of acute urinary tract infection. Mucosal irritation is first seen usually on the floor of the bladders (Wendt et al., 1990). A fibrinopurulent exudate can be observed over affected areas. There is also often edema of the bladder mucosa, especially in *A. suis* infections (Walker and MacLachlan, 1989). Thickening of the mucosa and polypoid projections are apparent in chronic cases of urocystitis. Urinary **calculi**, usually of struvite, calcium oxalate and calcium phosphate can be frequent finding in sows with urocystitis (see also 1. 4. 5.) (Dee, 1991).

Caution is needed when examining urinary bladders of culled sows, as electrocution and shock can induce mucosal hemorrhages and/or hyperaemia. The empty bladder of older sows can have a seemingly thickened wall, without apparent inflammatory lesions.

Bladders demonstrate an erosive and ulcerative, hemorrhagic cystitis on the whole mucosal surface in case of natural or experimental *A. suis* infection (Wendt et al., 1990). Occlusion of one or both ureters with inflammatory exudate can lead to uni- or bilateral hydronephros or pyonephros. Ascending infection of the bladder causes hemorrhagic to purulent ureteritis with dilatation and tortuosity. Examination of the **ureteric valve** and intravesicular ureters deserves special attention, as their abnormalities are frequently associated with pyelonephritis. The ureter is enlarged and the ureteric orifices are open with ragged edges in

acute pyelonephritis. In chronic pyelonephritis the orifice is shrunken and swollen (Almond and Richards, 1992). Both conditions cause shortening of the intravesicular portion of the ureter (Carr et al., 1990). Fibrinopurulent necrotizing pyelitis results when the infection ascends to the kidneys. In chronic cases severe kidney fibrosis can develop.

Light and electron microscopic studies of urinary bladder samples of sows with subclinical bacteriuria and without detectable *A. suis* infection revealed prominent **goblet cell proliferation** in the whole uroepithelial layer. Macroscopic lesions, like catarrhal- or catarrhal-purulent cystitis were accompanied by intraepithelial cyst formation, as a result of goblet cell necrosis. Single cell necrosis along with neutrophil granulocytic infiltration was observed in the epithelial layer. The lamina propria contained moderate to severe mononuclear cell infiltration (Liebhold et al., 1995). In similar cases the surface cells were slightly rounded and the number of desquamated cells increased, the bladder surface sometimes gave an irregular appearance (Wendt et al., 1992).

In the same study of Liebhold et al. (1995), examination of 12 sows with significant bacteriuria and *A. suis* isolation resulted in the detection of more severe and more widespread changes in the urothelium. The epithelial layer was massively interspersed with (often confluent) cysts, some of them contained neutrophil granulocytes, mucus and erythrocytes. Affected areas were almost completely void of epithelial cells. The epithelium contained moderate to large numbers of neutrophil granulocytes and showed mild to moderate mononuclear cell infiltration. Large ulcers and focal intraepithelial hemorrhages were also observed. Severe hyperaemia, edema, infiltration with lymphocytes, plasma cells and histiocytes could also be identified.

On SEM examinations irregular formation of surface cells was observed without tight cell to cell contact giving a cobblestone pattern to the epithelium in some instances. Funnel shaped orifices were present between epithelial cells with evidence of mucinous secretion. No mechanical defect of capillaries was revealed by TEM examinations. *A. suis* could be detected only on the surface of cells, no evidence was found for intracellular localisation of this bacterium (Wendt et al., 1992).

Epithelial loss with cellular infiltration and thrombus formation in the vessels of the submucosa can be seen in acute ureteritis. Severe pyelitis, papillary necrosis as well as interstitial and tubular nephritis characterize acute pyelonephritis. The papillary necrosis leads to hemorrhage with thrombus formation. In chronic active pyelonephritis renal changes include fibrotic interstitial nephritis and sometimes renal scarring (Wendt, 1998).

On ultrastructural examinations areas of renal pelvis without desquamation of the transitional epithelium reveal mucinous degeneration with prominent goblet cell proliferation as well as formation of cysts by dying goblet cells. Findings are very similar to what can be found in cases of *A. suis* cystitis and can be interpreted as local defence mechanism of the urothelium (Drommer et al., 1994).

1. 4. 8. Therapy of urinary tract infections

Most of the treatment strategies recommended in the literature are based on the use of antimicrobials. Generally, considering the broad spectrum of bacterial species possibly involved in urinary tract infections, one should choose broad spectrum or combined antimicrobials (Bertschinger, 1999). Administration of antibiotics is frequently effective, at least in the short term; however, relapses commonly occur. Culling of chronically affected animals is advised (Taylor, 1999). Success of antimicrobial therapy, among other factors, depends on the severity and extent of urinary tract lesions. Sows with cystitis can rapidly recover, but treatment of pyelonephritis is at best frustrating and often unsuccessful.

Dee (1991) suggested four key points to consider when choosing an antibiotic for treatment of *A. suis* infections in sows: effectiveness against the organism, toxicity to the renal system, excretion through the urinary tract and effectiveness at alkaline pH. These guidelines can

be extrapolated when one attempts to treat UTI of other bacterial origin. Dial and MacLachlan (1988b) suggested that treatment should follow antimicrobial sensitivity testing after the isolation of one of the more commonly observed pathogens. Practitioners are advised to maintain databases of sensitivity patterns of various pathogens over time (Dee, 1992). From the potentially effective drugs, one should choose an antimicrobial that is delivered to the urinary tract in concentrations exceeding the minimum inhibitory concentration of the pathogen.

There are few descriptions of the antibiotic susceptibility of bacterial pathogens of the porcine urogenital system, and no published information on the distribution of antimicrobials in the urinary and genital tracts is available (Dial and MacLachlan, 1988b). Following is a short description of certain antimicrobials, potentially useful in treating urinary tract infections (Dial and MacLachlan, 1988b; The Merck Veterinary Manual, 1991; Dee, 1992).

Tetracyclines are broad-spectrum antibiotics, therefore potentially effective for mixed infections of the urinary tract. They are rapidly absorbed following oral administration, which makes them suitable for in feed or water medication. Excretion is through the kidneys and to less extent through the gastrointestinal tract. Tetracyclines are active under anaerobic conditions and in the presence of pus and blood.

Penicillins are probably the most frequently used drugs to treat urogenital disease in swine. These β-lactam antibiotics have several subclasses based on antimicrobial spectrum and activity against pathogens possessing the penicillinase (β-lactamase) enzyme (e. q. *Staphylococcus spp*.). Narrow spectrum (e. q. benzylpenicillin) and broad-spectrum (e. q. ampicillin, amoxicillin) β-lactamase sensitive penicillins are available for veterinary use. β-lactamase protected broad spectrum penicillins, such as clavulanate-potentiated amoxicillin are active against β-lactamase producing, penicillin resistant bacteria. Classical penicillins are active against a wide variety of Gram-positive pathogens, among them, anaerobes; broad-spectrum semisynthetic penicillins are also active against most Gram-negative bacteria. Following absorption, penicillins are widely distributed in most tissues, among them, in kidneys. The activity of some penicillins is enhanced at alkaline pH, which occurs readily in infection with urease positive organisms. Penicillins after parenteral administration are secreted in high concentration via the kidneys, often sufficient to suppress both Gram-positive and -negative bacteria. Anuria may increase the half-life of benzylpenicillin substantially. Penicillins are mainly administered parenterally to swine, but water-soluble and feed-grade penicillins are also available. These are suitable for preventive mass medication against urogenital infections, although this treatment can involve substantial expense.

Cephalosporins are a class of β-lactam antibiotics similar to the penicillins in several respects. First generation cephalosporins (e. q. cephalexin) have high activity against many Gram-positive bacteria but are only moderately active against Gram-negatives. They are relatively susceptible to β-lactamases and are not as effective against anaerobes as are the penicillins. Third generation cephalosporins (e. q. ceftiofur) have generally only moderate activity against Gram-positive bacteria, but have an extended spectrum of Gram-negative activity, including in certain instances *Pseudomonas spp*, *Proteus vulgaris* etc. They are mainly also effective against anaerobes, except *Bacteroides fragilis*. They are usually β-lactamase resistant. These antibiotics are most stable and effective at a pH of 6-7. Cephalosporins are widely distributed through most body fluids and tissues, including kidneys. Most cephalosporins are excreted by renal tubular secretion, though glomerular filtration is important in some cases. In renal failure dose rates should be reduced. Generally, the cost of cephalosporins can prevent their extensive use in swine practice.

Fluoroquinolones (flumequine, norfloxacin, enrofloxacin, and marbofloxacin) are potent synthetic antibiotics. They are active against a wide range of Gram-negative and number of Gram-positive pathogens. Obligate anaerobes tend to be resistant to most quinolones as are most enterococcal group D *Streptococcus spp.* (e. q. *S. faecalis*). They are rapidly absorbed after parenteral administration and penetrate all tissues quickly. Particularly high levels are

encountered in the kidneys, and in the endometrium. Renal excretion is the main route for most quinolones, with both glomerular filtration and tubular secretion involved. In renal failure clearance is impaired and reduction in dose rates are essential.

Chloramphenicol is a highly effective bacteriostatic antibiotic. It is quickly absorbed, its highest concentration can be found in the kidneys, liver and bile, metabolized in the liver and excreted in the urine. Only 5-15% of the active compound is present in urine, but hematogenous delivery of the drug to the site of infection contribute to its effectiveness in treating urinary tract infections. Chloramphenicol is relatively stable up to pH 9. Its spectrum of activity includes many Gram-positive and Gram-negative bacteria, including anaerobes, but excluding *Pseudomonas spp*. Use of chloramphenicol is prohibited in food producing animals, but its congener, **florfenicol** is registered for treating respiratory tract infections in swine. This compound is significantly more active *in vitro* than chloramphenicol.

Other antimicrobials have limited value in treating urinary tract infections in swine. **Macrolides**, **lincosamides**, and **pleuromutilins** have a predominantly Gram-positive spectrum, their use is restricted to such monoinfections. These compounds, however, are usually highly active against *A. suis*. **Aminoglycosides** possess some favorable characteristics as wide spectrum of activity, but they are not active against anaerobes (*A. suis* is usually resistant to members of this group), require long withdrawal periods and are usually nephrotoxic. The **sulfonamides**, although having wide antimicrobial spectrum, are nephrotoxic, and inactive in pus and necrotic debris.

Actinobaculum suis is sensitive *in vitro* to several antibiotics, including penicillin and its semisynthetic derivates and tetracyclines. The original description of *A. suis* by Soltys and Spratling (1957) contains data on the *in vitro* sensitivity of three strains. The authors reported minimum inhibitory concentration ranges for penicillin (0.01 - 0.1 μg/ml), streptomycin sulphate (100 μg/ml), chloramphenicol (1 - 10 μg/ml), oxytetracycline-HCl (10 μg/ml), tetracycline-HCl (10 μg/ml), and chlortetracycline-HCl (100 μg/ml). The type strain (ATCC 33144) is susceptible to chloramphenicol (12 μg/ml), clindamycin (1.6 μg/ml), erythromycin (3 μg/ml), penicillin G (2 U/ml), tetracycline (6 μg/ml), ampicillin (4 μg/ml) and cephalothin (6 μg/ml) (Moore and Moore, 1986). Ten isolates of *Actinobaculum suis* tested by Jones et al. (1982) showed *in vitro* sensitivity to penicillin, ampicillin, chloramphenicol, tetracycline, erythromycin and nitrofurantoin; they were resistant to streptomycin, neomycin, nalidixic acid and trimethoprim. *A. suis* was found to be sensitive to penicillin, a great variation in its sensitivity to polymyxin and neomycin was observed (Høgh et al., 1984). Three *A. suis* strains isolated by Dreau and Laval (2000) were sensitive to ceftiofur, amoxicillin, penicillin and tetracycline, variably sensitive to enrofloxacin and sulfonamides, and resistant to flumequine. Testing method was not reported for these latter studies.

Walton (1984) had successfully used 20 mg/kg oxytetracycline im. at the time of service to stop deaths of recently farrowed sows due to cystitis and pyelonephritis and to control post mating discharges. Both conditions were shown previously to be caused by *A. suis*. Reproductive performance of the treated group of sows was superior to the control one. The age distribution of the treated and control sows, however, has not been reported.

Medicated early weaning techniques, designed for the elimination of *Mycoplasma hyopneumoniae* and other pathogens utilizing early weaning and heavy medication of dams and their progeny with therapeutic doses of tetracyclines, penicillins or enrofloxacin and tiamulin at two farms were not successful in eliminating *A. suis* from early weaned piglets (Dee et al., 1994a).

D'Estaintot et al. (1996) successfully decreased (but not eliminated) bacteriuria in sows using orally administered flumequine at a dose of 15 mg/kg/day for one week. In the same trial, similar duration of 20 mg/kg/day oxolinic acid treatment did not attain the same effect. The authors suggested that flumequine can be a preferably drug to treat urinary tract diseases over

oxolinic acid for its better tissue diffusion, and relative insensitivity to urine pH. *E. coli* is more susceptible to flumequine than to oxolinic acid; however, it is also known that quinolones are generally not the first drugs of choice for Gram-positive infections. Becker et al. (1988) were unsuccessful in eliminating bacteriuria from sows with different dosage regimes of parenteral gentamicin and a trimethoprim/sulfonamide combination.

Stirnimann (1988b) had successfully used ampicillin and an analgeticum - antiphlogisticum (Novaminsulfon) to treat sows with acute UTI caused by mixed bacterial flora. Dee (1991) proposed ampicillin at 11 mg/kg b. i. d. for three to five days im. as the drug of choice for treating acute cystitis-pyelonephritis.

1. 4. 9. Prevention of urinary tract infections

Since the **treatment** of urinary tract infections is generally frustrating, prevention, rather than therapy should be emphasized (Dial and MacLachlan, 1988b). **Proper hygiene** in all production phases, especially during breeding and farrowing is essential. Sows and boars should be bred in clean breeding pens washed at the end of each breeding period and disinfected as needed. Gestation stalls should be scraped frequently to reduce manure bildup around the perineal region (Dee, 1992). Mating hygiene, proper washing and cleaning of the perineum of mated or inseminated sows is a simple yet important preventive measure against urogenital tract infections (Dee, 1992). Holding the prepuce at hand mating increases the bacterial contamination of the lower urogenital tract (Dee, 1992; Muirhead and Alexander, 1997).

Proper facility design is a key point in the prevention of UTI. Sow and boar stalls should be constructed to facilitate manure removal. Ideally, sows should be kept in small groups in pens rather than in confinement. If kept in stalls, the floor of a 210 cm stall should consist of a front solid portion and a rear slatted portion of 120 - 150 cm. Slats should be 10 cm wide with 2,5 cm gaps. Slats running perpendicular to the direction the sow is standing are preferable. Slats should be made to avoid leg injuries. Fecal contamination of the perineal region is often excessive in stalls with a solid backboard, which drops down to ground level. In these facilities there should be a 10 cm gap between the bottom of the board and the floor to prevent manure bildup (Muirhead and Alexander, 1997). It is important to observe guidelines for pen area and/or group sizes to prevent overcrowding and excessive fecal contamination. Adequate ventilation is important to enhance drying of the environment.

Ensurance of adequate water intake and **minimization of sedentary behavior** may decrease the prevalence of urinary tract disease (Dial and MacLachlan, 1988b). Generally, good quality fresh water should be provided free choice for all breeding animals. There should be adequate number of drinkers with sufficient flow rates for sows in groups. Every stall should be fitted with a nipple waterer or other device. Every watering device should be checked daily and cleaned out thoroughly 2 to 3 times a year (Carr, 1992). Fitting of drinkers is also important as improper drinker placement and position can adversely affect water intake or can contribute to spillage. Water throughs with a minimum depth of 10 cm are preferably over nipple drinkers, especially in loose-housed sows. In pens, there should be one nipple drinker per 10-15 sows with water flow rate of 2 L/minute (Muirhead and Alexander, 1997). Throughs and bowls should be cleaned out regularly, as their soiling with manure might effect negatively the water intake of sows. Although severe problems with water quality are rare, in cases of UTI outbreaks drinking water should be checked by a certified laboratory chemically and microbiologically.

Nutritional modifications, such as adding salt (0,4-2%) to the ration can also increase water intake and urine output, assuming water is available (Seynaeve et al., 1996; Muirhead and Alexander, 1997; Wendt, 1998). Rations containing an excessive amount of phosphorus should be avoided to lessen the possibility for crystalluria. Twice-a-day feeding will promote activity, resulting in an increased frequency of urination (Dee, 1992). Proper feeding has a key role in avoiding excessive weight gain, ideally, sows should be fed according to their body condition.

Increasing crude protein in the ration will decrease urinary pH, however, one should closely observe nutritional requirements of different production phases before making alterations to the diet (Dee, 1992).

Regulation of urinary pH could be used as a prophylactic measure in herds where the incidence of alkaline urine in sows is high. Close relation exists between the urinary pH value and the base excess (BE) of the diet (pH = 6.5 + 0.0028 x BE). Calcium, magnesium, sodium and potassium are efficient in increasing the BE; phosphorus, chloride, methionine/cysteine having acidifying effects. A decrease of the urinary pH can be achieved by removing alkalizing substances from the diet or adding acidifyers like ammonium chloride, methionine or citric acid to the ration. In an uncontrolled trial, Dee et al. (1994b) successfully used 2 oz (approx. 60 g) anhydrous citric acid/sow/day for two-week periods in order to lessen losses due to urinary tract disease in a seedstock herd. They found significant decrease in urine pH and bacterial count during periods of citric acid supplementation. Urine acidification has no therapeutic effects in cases of urinary tract infection, but can decrease the bacterial count in the urine. It is recommended especially in the periparturient period to prevent urogenital tract infection (Dee et al., 1994b; Wendt, 1998). Decreasing urinary pH also has important implications in lowering ammonia emission from slurry. Such regulation of urinary pH was attempted in swine with several acidifying agents, among them adipic acid (van Kempen et al., 1998). More research on this subject should be anticipated in the near future.

As older sows are more prone to urinary tract disease **parity distribution** of the sow herd should be closely followed, preferably by the use of farm management software. In this regard, the average parity number is not enough to look at, as abnormalities at both slopes of the distribution curve may occur simultaneously (Muirhead and Alexander, 1997). Proper culling policies can ensure that an abnormal parity distribution does not occur; culling is often the most cost effective measure in preventing losses due to UTI (Dee, 1992).

Prophylactic treatment of the entire herd may decrease morbidity and transfer of pathogens between animals. On a herd basis, treatment can be carried out using either CTC or OTC at levels of 600 g/ton for a period of 14 days. It might be necessary to repeat this treatment every 4 to 6 weeks (Muirhead and Alexander, 1997). Others recommended to medicate all dry sows on problem farms for 7-10 days at 6 week intervals (Bertschinger, 1999). It might be advisable to treat groups of sows in periods of high risk, such as breeding and farrowing. Some authors suggest treating pregnant sows with signs of UTI with antimicrobials shortly before partuition. Sows could also be medicated from weaning to 21 days post mating (Muirhead and Alexander, 1997). Tetracyclines are the most frequently recommended antimicrobials for such mass medication (Dee, 1992; Dial and MacLachlan, 1988b), but broad-spectrum penicillins, as amoxicillin might also prove effective. Nevertheless, all these preventive treatment strategies incur considerable costs and their effectiveness is many times questionable (Bertschinger, 1999). There are reports on administration of different antimicrobials to boars as a **preputial infusion** with an aim to prevent urogenital tract infections in sows. Although infusing boar sheaths with antibiotic preparations has been reportedly effective in some situations (Muirhead, 1986; Walton, 1984), it is unlikely that boars would ever be cleared of normal inhabitants of the preputial flora (like *A. suis)* but such preparations may reduce the prevalence of the organism in the preputial sac and reduce its transmission to sows (Dee, 1991; Taylor, 1999). Local medication of the preputial sac with penicillin, penicillin/streptomycin or enrofloxacin, and feed medication with enrofloxacin was unsuccessful to permanently free boars from *A. suis* infection. Bacteria could be detected again in the preputial sac of boars at the latest 18 days after such medications (Wendt et al., 1993). Generally, the response to preputial infusions has been beneficial when used as a treatment program, but it is questionable whether the returns justify the cost and the labor involved when used as a preventative (Dee, 1992). Methods to control bacteria in semen or the genital tract of boars include washing the body and prepuce, surgical removal of preputial diverticulum, use of the gloved hand semen collection technique, using sterile AI equipment and

reagents (Thacker et al., 1984). Broad spectrum or combinations of antibiotics (gentamicin, lincomycin, neomycin, spectinomycin, amikacin, ceftiofur, enrofloxacin, ampicillin, kanamycin, etc.) are routinely included in extender fluids to control bacterial contaminants (Althouse, 1997).

Immunization of sows with a polyvalent human vaccine, containing killed strains of *E. coli*, *Proteus sp*., *Klebsiella pneumoniae*, *Streptococcus faecalis* had little effect in reducing the magnitude of bacteriuria (Berner et al., 1988). This vaccine, however, effectively reduced losses associated with post partum hypogalactia, according to one report (Pejsak et al., 1988). Human medical research indicates that better results may be obtained by oral or local immunization (Wendt, 1998).

1. 5. Non-specific genital tract infections

Genital tract infections in swine can be categorized as **specific**, being caused mainly by obligate pathogenic viruses, bacteria, or other infectious agents, and **non-specific**, caused by opportunistic bacteria when predisposing factors provide favorable conditions for their multiplication. Examples of specific infections are PRRSV-infection, Aujeszky's virus infection, chlamydiosis and brucellosis. Non-specific infections are ascending vaginitis, cervicitis or endometritis caused by members of the fecal flora in certain circumstances.

Inflammation limited in extent to the endometrium is termed **endometritis**; involvement of the entire thickness of the wall is **metritis**; of the serosa, **perimetritis**; and of the suspensory ligaments, **parametritis** (Jubb et al, 1993). The great majority of inflammatory conditions of the uterus begin in the endometrium and are in someway associated with the reproductive process. Inflammation of the endometrial mucosa due to non-specific bacterial infections is in the scope of this discussion.

1. 5. 1. Anatomy and physiology of the tubular genital tract

The female reproductive tract consists of bilateral ovaries and the tubular genital tract: uterine tubes (oviducts), a bicornuate uterus, cervix, vagina, vestibule, vulva and associated glands.

Uterine tubes are bilateral, tortuous structures that extend from the region of the ovary to the uterine horns and convey ova, spermatozoa and zygotes. They have three segments: the infundibulum, a large, funnel shaped portion, the ampulla, a thin walled section extending caudally from the infundibulum and the isthmus, a narrow muscular segment joining the uterus (Priedkalns, 1993). Uterine tubes of sows are 15 to 30 cm long. The infundibulum is large and completely covers the ovary at the time of ovulation. The isthmus is almost straight but has a slight flexure at the uterotubal junction. The tunica mucosa-submucosa of the ampulla is highly folded. The epithelium is simple columnar with both ciliated and nonciliated cells. The mucosa is continuous with the submucosa throughout the female reproductive tract as the lamina muscularis mucosae is absent.

The body of the **uterus** is 3-5 cm long; the uterine horns are extremely long and flexuous. The uterine horns are thick and edematous during estrus, and they became thinner and longer as edema subsides. Length of the uterine horns is about 80 cm in estrous sows and 170 cm in diestrous sows (McEntee, 1990). The uterine wall has three layers: the mucosa-submucosa or endometrium, the tunica muscularis or myometrium and the serosa or perimetrium. The endometrium can be divided in the following parts: surface epithelium, stratum compactum, stratum spongiosum and stratum basale. The surface epithelium is pseudostratified columnar. The height and structure of the epithelial cells depend on the ovarian hormones secreted throughout the cycle. The subepithelial dense layer (str. compactum) consists of a richly vascular, loose connective tissue with many fibroblasts, macrophages and mast cells. Neutrophils, eosinophils, lymphocytes and plasma cells may enter here from the bloodstream.

The str. spongiosum has a loose connective tissue that is less cellular than the str. compactum. Simple coiled, branched tubular glands, lined by ciliated and nonciliated simple columnar epithelium originate from the str. basale and extend through the full thickness of the endometrium and open to the uterine lumen. The myometrium consists of a thick circular inner layer and an outer longitudinal layer of smooth muscle cells that increase in number and size during pregnancy. Between the two layers a vascular layer is present. The perimetrium consists of loose connective tissue covered by the peritoneal mesothelium.

Cyclic changes in the endometrium of sows were described in detail by several authors (Schnurrbusch et al., 1985; Leiser et al., 1988; Priedkalns, 1993).

The uterus of the sow involutes 14 to 21 days post partum, the length of the uterus decrease from 240 cm on day 1 to 120 cm on day 28 (McEntee, 1990). Macroscopic and histological changes in the reproductive tract of the sow during lactation and early postweaning are described in detail by Palmer et al. (1965a, b).

The length of the **cervix** in gilts is reportedly around 17 cm, in sows around 20 cm (McEntee, 1990). The porcine cervix consist of two distinct anatomical parts, the 4-5 cm long uterine and the approx. 10-12 cm long vaginal portion, both being similar in histological appearance (McEntee, 1990). A single layer of columnar cells, with many mucinogenous cells lines the lumen of the cervix. In the sow, more than 90% of the cervix may have a vaginal type of mucosa with stratified squamous epithelium that undergoes cyclic alterations as in the vagina (Priedkalns, 1993). Height of the cervical epithelium reaches its peak during estrus and then undergoes various degrees of reduction in size and secretory activity. The cervical mucus becomes thicker and more adhesive under the influence of progesterone ("cervical seal"), while it is more plentiful and thinner during estrus. The tunica propria is rich in cells and collagenous fibers. This tissue is found mingled with the well-developed circular muscular layer, external to that, there is a thinner longitudinal layer. The tunica serosa consists of a loose connective tissue.

The **vaginal** wall is similar in composition to the rest of the genital tract; it is covered by stratified squamous epithelium that increases in thickness during proestrus and estrus. Lymphatic nodules are present in the propria of the caudal part of the vagina (Priedkalns, 1993). The mucous membrane of the porcine vagina is arranged in high longitudinal folds. Above 2 years of age epithelial invaginations are seen in the tunica mucosa of the vagina as microcysts or cell nests. This is likely to be an age-related, nonpathological change (McEntee, 1990). Remnants of the mesonephric ducts are present at the border of vagina and vestibule; the external urethral orifice is located at this junction. The vestibule extends from the terminal part of the vagina to the labia of the vulva. It is lined by stratified squamous epithelium and contains mucus-producing glands. Histologically it is similar to the vagina, except for an increased number of lymphatic nodules, found especially in the region of the clitoris (Priedkalns, 1993).

1. 5. 2. Normal defence mechanisms

Normal nonpregnant uterus of domestic mammals have **high degree of resistance** to infection, and even in the case of specific genital diseases is incapable of supporting bacterial growth or persistence of bacteria for any extended period (Jubb et al., 1993). The self-limiting nature of most infections has been the basis for recommendation of sexual rest for animals with uterine infection.

The cervix is a very effective protective barrier and often prevents organisms from entering the uterus (McEntee, 1990).

Generally, information on the resistance of endometrium to non-specific infections in sows is quite limited. Sex hormones play an important role in modulating the defence mechanism of the uterus; however, there are considerable individual differences. Uterine resistance varies through the estrus cycle, with susceptibility being the greatest during the luteal phase (Jubb et al., 1993). High estrogen concentrations contribute to a mechanical uterine defense (de Winter et al., 1996). The uterus contracts during proestrus and estrus, resulting in

physical clearance of uterine contents. The increased uterine perfusion, resulting from high estrogen concentrations, is correlated with increased tissue permeability and enhanced leukocyte migration into the uterus (Britt et al., 1999). Bará and Cameron (1994a) found that the number of neutrophil granulocytes was higher in the late luteal phase and through to estrus in uterine lavage fluids. Levels of the bactericidal N-acetyl-β-D-glucosaminidase enzyme and total protein content of uterine flushings were also higher in late luteal phase. These changes coincided with increasing level of oestradiol-17β and decreasing levels of progesterone. These major changes occurring during the late luteal stage suggests that uterine defence mechanism is associated more with changing ratios of estrogen to progesterone rather than with changes in individual hormone concentrations (Bará and Cameron, 1994a). Much of the reduced uterine resistance during diestrus and pregnancy is related to the progesterone-induced secretion into the uterine lumen of imunosuppressants, which are capable of inhibiting lymphocyte proliferation (Jubb et al., 1993).

Immunoglobulin-containing **plasma cells** are present in the lamina propria at all levels of the female reproductive tract. Their number is under hormonal influence and varies in different tissues. Estrus is accompanied by a general increase in the number of plasma cells, while during diestrus cell counts are usually low. There are fewer plasma cells in the oviduct and endometrium than lower down the genital tract - it may be due to the lack of antigenic stimulation in the upper genital tract. IgG is the predominating immunoglobulin found in uterine mucosa, then follows IgA and IgM. The presence of immunoglobulins in the uterine mucosa is influenced by the estrus cycle. IgG is present in the interstitium in high concentrations during estrus, while it can be detected mainly in the glandular and luminal epithelium during the secretory phase. The fact that immunoglobulin synthesis occurs in lymphoid cells within the reproductive tract indicates the presence of a (although relatively inactive) local immune system (Hussein et al, 1983). Immunoglobulins in other species supposedly block the attachment sites of bacteria to the endometrium, agglutinate and opsonize bacteria for subsequent phagocytosis. Also, secretion of nonspecific antimicrobial factors as peroxidase, lysozyme, and complement may increase during the follicular phase (Dial and MacLachlan, 1988a).

Endometritis in most affected gilts resolves after one or more estrous cycles, suggesting that estrogenic stimulation of the endometrium produces an environment less conductive to bacterial growth (MacLachlan and Dial, 1987).

1. 5. 3. Bacterial flora

A wide range of bacteria can be present as part of the normal microflora of the cervical and anterior vaginal areas of healthy sows (1. 4. 4.; Table 1.4.). The cervico-vaginal microflora is a dynamic entity that keeps changing continuously in number due to the cyclic hormonal pattern, immunoglobulins, mucus activity and phagocytic activity of the sow's reproductive tract (Bará et al., 1992). In a study of Bará et al. (1992) the highest number of bacteria could be isolated from the anterior vagina of healthy sows within 24 hours of farrowing and mating. Bacterial load decreased gradually from farrowing to weaning and from mating to three weeks post mating. Higher parity (4 <) sows had higher bacterial contamination in almost all phases. Isolated bacteria mainly belonged to 5 genera: *Streptococcus spp.*, *E. coli*, *Staphylococcus spp.*, *Corynebacterium spp*. and *Micrococcus spp*.

The normal bacterial flora of the uterus is a subject of great controversy. Some reports suggest that bacteria are normally present in the uterus of a substantial portion of pigs, even during pregnancy (Dee, 1992). Others speculate that the uterus does not have a significant resident bacterial flora, and that endometritis can ensue from an abnormal persistence and proliferation of bacteria that have entered during the puerperium or estrus period (Meredith, 1986). Isolation of facultatively pathogenic bacteria from the uterus without macroscopic or microscopic pathological changes is likely to reflect contamination, rather than the presence of a resident flora (McEntee, 1990). The theory of lack of resident bacteria is further supported by the findings of Hussein et al. (1983).

1. 5. 4. Predisposing factors of non-specific endometritis

Generally, several factors predispose sows to non-specific infections of the reproductive tract (Dial and MacLachlan, 1998a). Some of these, like improper facility design, and age are the same as for infections of the urinary tract, and were discussed in detail earlier (1. 4. 5.). Differences in the pathogenesis and time of onset of **post farrowing, post mating and virgin gilt** endometritis justify a separate discussion of their predisposing factors.

In case of **post farrowing endometritis** the **farrowing process** predisposes the reproductive tract to infection. There is a periparturient rise in the number of facultative pathogenic bacteria in the caudal vagina and cervix (Bará et al., 1992). Farrowing is accompanied by contamination of the cranial vagina in nearly all sows and contamination of the cervix and uterus in the majority of sows (Dial and MacLachlan, 1988a). While most bacteria are eliminated within 12 to 32 hours post farrowing, facultative pathogens may overgrow apathogenic microflora and establish persistent infections. This is common in sows with **traumatized and heavily contaminated** uteri, which can occur with **unhygienic manual intervention**. Bará and Cameron (1994b) found that frequent usage of manual intervention during farrowing with inadequate hygiene, failure to wash perineal area of sows before entering to farrowing accommodations and inadequate farrowing crate design resulting in a bildup of feces behind the sow were related to higher incidence of abnormal post farrowing discharges. The same authors further investigated the effect of fecal accumulation in farrowing crates and assisted farrowing on the incidence of post-farrowing discharges and subsequent reproductive performance of sows (Bará and Cameron, 1996a). They found that manual intervention, even under good hygienic conditions increased the incidence of post farrowing discharges (PFD) and endometritis, decreased reproductive performance in the next sexual cycle and increased the number of non-productive days. The experimental group, where manual intervention at farrowing was practiced had the shortest time to onset of PFD. Nevertheless, the group where fecal accumulation was allowed in the farrowing crates did not differ significantly in performance from the control group. In this study, however, the control group had lower average parity than the experimental groups, which might have influenced the results.

In a study of Madec and Leon (1992) sows affected by farrowing disorders (fever, dysgalactia, or vulvar discharge) tended to be **heavier**, **older**, and **had locomotory and urinary problems** with a higher frequency, and **farrowed larger litters**.

The prevalence of uterine infections is increased after **prolonged farrowings** (Madec and Leon, 1992; Bará and Cameron, 1996a).

As sows culled during lactation can have signs of endometritis, it can be speculated that postparturient endometritis can continue throughout lactation and subsequently, can interfere with conception at postweaning mating. **Lack of ovarian cyclicity** is associated with compromised clearing of bacteria from the uterus; the closure of cervix will prevent drainage of uterine contents (Dial and MacLachlan, 1988a).

Because tissue decomposition provides a medium conductive to the growth of a variety of microorganisms, **retention of placenta** and/or dead piglets predispose sows to endometritis and more likely, **metritis**. Thus, the prevalence of vulvar discharges often increases following **abortion** and with epizootics of infectious feticidal or placentatropic agents (Meredith, 1986).

Postcoital urogenital infections are seen more commonly on farms that have **manual manipulation and subsequent contamination of the boar's penis** before mating (Muirhead, 1986). However, endometritis do occur after artificial insemination too, probably due to **contaminated breeding equipment** (Dial and MacLachlan, 1988a). Although definitive evidence is lacking, facultatively pathogenic bacteria might be transmitted from boars to sows at mating. Considering the often heavy contamination of the preputial sac and the less frequent development of post mating endometritis or urocystitis, other predisposing factors (e. q. trauma

at breeding) should play an important role. In a study on **post mating endometritis**, Bará and Cameron (1994c) reported that **unhygienic conditions** of the mating pens, **sows with soiled perineal area**, **boars with soiled prepuce**, **excessive number of services per cycle** and the **high use of cross mating** were the most important predisposing factors to post mating discharges (PMD) in a survey of 36 farms. The same authors further examined the effect of "unhygienic" mating and multiple (two or four) matings on the incidence of post-mating discharges and subsequent reproductive performance. They found that the risk of PMD increased significantly when sows were mated **four times under "unhygienic" conditions**. Mating twice when conditions were "unhygienic" did not affect reproductive performance (Bará and Cameron, 1996b). The exact nature of "unhygienic" breeding conditions was not revealed, moreover, insemination for four times in the same cycle is seldom practiced in swine.

Sows inseminated **late during estrus** are susceptible to discharge problems (de Winter et al., 1992a, b; 1996). There is strong correlation between serum progesterone concentration and the development of endometritis (de Winter et al., 1994, 1996). If animals are serviced as progesterone concentrations begin to rise after ovulation, there is a greater possibility of inducing endometritis. Therefore, some **multiple AI** schemes might contribute to problems with endometritis (Britt et al., 1999). In a study of Carabin et al. (1994), **parity 5 or older sows**, **sows bred three times** in the same cycle, and with a **prolonged weaning to service interval** (>= 6 days) had higher chance to be culled for vaginal discharge. Vaginal discharge appeared more often in **summer months** (May 1st to 31st of August), and was not as restricted to older sows as in winter months (from January to end of April). No cause for this seasonal pattern was proposed.

In a study conducted by Dalin et al. (1997) on histopathological examination of 115 female genital tracts 27% of the animals had endometritis, classified as mild in 50% of them. **Anestral** animals had a higher incidence of endometritis (61%) than animals showing cyclic ovarian activity.

A reduced **lactation length** (< 21 days) is reportedly associated with higher incidences of discharges due to endometritis on some farms. This is supposedly due to the shortened time available for involution (Muirhead, 1984).

Endometritis can be observed in **virgin and recently bred gilts**. Affected animals are those that have been recently commenced cycling after transport or exposure to a boar (Dial and MacLachlan, 1988a). In gilts, vulvar discharge is commonly observed within six days prior to estrus, when animals are in proestrus (MacLachlan and Dial, 1987). It is speculated that onset of estrous cyclicity in gilts, and progestational stimulation of the endometrium in particular, facilitates invasion by opportunistic bacteria. Others suppose that gilts attain puberty while in the finishing houses, infection of the endometrium occurs at this time. Discharge will be seen prior to their second estrus (Britt et al., 1999).

Higher frequency of all types of endometritis can be observed in herds **with high annual turnover rates**, **newly repopulated herds** or in **start-up units** because of the presence of large numbers of immunologically naive animals (MacLachlan and Dial, 1987; Dial and MacLachlan, 1988a; Britt et al, 1999).

1. 5. 5. Pathogenesis of non-specific endometritis

Pathogenesis of most forms of endometritis is based on **persistent bacterial contamination** of the uterine mucosa. Such bacterial persistence could be a consequence of uterine trauma or delayed involution, uterine debris, foreign bodies, intrauterine hemorrhage, and overwhelming bacterial contamination. An additional type of pathogenesis arises when bacteria gain access to the uterus at an **unusual time**, i. e., during luteal phase or during pregnancy. This can occur by hematogenous spread of an extragenital infection, by ascending infection from the vagina and cervix or by mating or inseminating sows in metestrus (Meredith, 1986; de Winter et al, 1996).

Pathogenesis of post mating- and virgin gilt endometritis can be briefly summarized as follows (Dial and MacLachlan, 1988a). Rising levels of estrogen (in concert with other hormones) cause cervical relaxation during proestrus; cervical patency is maintained during estrus and early metestrus. Heavy contamination of the upper genital tract can occur this time. Following ovulation, increasing levels of progesterone in the absence of elevated estrogen levels allow the cervix to close tightly. The progesterone-dominated endometrium is conductive to bacterial growth due to diminished immune responses. Septic exudate can accumulate in the uterine lumen. Pregnancy will not be established in such cases and cyclic activity will continue. In proestrus increasing influence of estrogen will cause the cervix to relax, inflammatory cells infiltrate the endometrium and myometrial activity increases. These will cause expulsion of the accumulated material, resulting in vaginal discharge just before estrus.

In the presence of predisposing factors, bacteria multiplicate on the uterine mucosa and elicit an inflammatory reaction. The intensity of it can vary from a mild superficial endometritis to severe necrotizing or putrid metritis, depending on, among others, the bacterial species involved, number of contaminating bacteria, and integrity of the endometrium. Cyclic activity can clear mild or moderate endometritis in a relatively short term, severe changes (metritis, perimetritis) are likely to result in death or culling of the sow. In some instances chronic inflammation of the uterine mucosa will develop. **Complications** of endometritis, like uterine abscesses, perimetritis and parametritis, and pyemia are relatively uncommon in swine. Purulent salpingitis can follow endometritis with more chance (McEntee, 1990).

Presumably, endometritis at the time of breeding reduces the viability of gametes and/or **interferes with fertilization**. In addition, chronic infections of the genital tract can cause persistent lesions in the oviduct or in the uterus, resulting in the **disruption of gamete or zygote transport**, or **interference with implantation**. Genital tract infections are thus associated primarily with **regular returns to estrus** (Dial et al, 1992). Other authors, however, suggest that early embryonic death is prominent in case of endometritis and thus irregular returns ensue (Tubbs, 1987; Muirhead and Alexander, 1997). Modest reduction in total born litter size occur with urogenital disease, although the reduction is often so subtle as to go undetected (Dial et al., 1992).

1. 5. 6. Diagnosis of non-specific endometritis

1. 5. 6. 1. Record analysis

Herd record analysis should help determining whether a problem in herd fertility is present and if yes, to what extent it can be related to inflammatory diseases of the upper genital tract. **Sporadic** cases of bacterial endometritis occur in all herds of breeding pigs, but the disease can also attain epidemic or endemic proportions (Meredith, 1986). Clinical cases of genital infections most commonly present in the **endemic** form as ongoing problems having persistent effect on fertility. Occasionally, **epidemics** of both vaginitis and endometritis occur (MacLachlan and Dial, 1987; Dial and MacLachlan, 1988a). Interference levels for clinical manifestations of reproductive infections were suggested by several authors and summarized in Table 1.6. (These suggestions are based on conditions not always pertaining to "average" Hungarian herds.)

Table 1. 6.: Suggested interference levels for possible signs of genital tract infections (Dial and MacLachlan, 1988a; Muirhead and Alexander, 1997)

Manifestation	Interference level (% of bred sows)
Vulvar discharge	> 2
Return to estrus 18-24 days after service	> 10
Return to estrus 25 < days after service	> 5
Not-in-pig and fail-to-farrow sows	> 2
Farrowing rate	< 80
Females culled for repeat breeding	> 5

Endometritis can result in **fertilization failure**, early **embryonic death, or rarely, in abortion**. Thus, post mating endometritis can result in regular, or irregular returns to estrus, or in "pseudopregnancy" (Dial et al, 1992; Muirhead and Alexander, 1997; Britt et al, 1999; Straw et al., 1999). **Regular returns with discharge** are highly suggestive of endometritis. Discharges without an observable direct effect on return rates likely to result from vaginitis or urinary tract infections.

Endometritis can be suspected during analysis of production data only when remarks on individual performance of sows are available. Data, which might indicate non-specific infection of the upper genital tract, include increased proportion of regular/irregular returns, especially at higher parities, **without a considerable effect on litter size or preweaning mortality**. Nonspecific infections of the upper genital tract are **not characterized by abortions or elevated number of mummified fetuses**. Sows having post partum endometritis will have a higher chance for repeat breeding in their next cycle. Evaluation of herd records should involve estimating the time period between certain events and appearance of clinical signs (vaginal discharge). Records indicating discharges beyond three days post partum are suggestive of endometritis. Post mating endometritis does not produce discharge until 15-24 days post service (Dial and MacLachlan, 1988b). Value of record analysis in case of urinary tract inflammation was presented earlier (1. 4. 7. 1.).

Herd records can also indicate whether the majority of postcoital infections are associated with a particular **boar**. This chance is optimized when homospermic systems are used (Dial and MacLachlan, 1988b).

A considerable **left shift in the parity distribution** of the herd, especially a high proportion of immunologically naive nulliparous females might come with increased frequency of endometritis on a herd level (Britt et al, 1999).

1. 5. 6. 2. Clinical signs

Relying exclusively on historical data presented by the producer can lead to erroneous conclusions, as the nature and origin of vulvar discharges are many times hard to determine. A thorough inspection of breeding females, boars, facilities, and animal flow through facilities is inevitable when one is to investigate reproductive failure in a herd (1. 3. 2.).

Vulvar discharge is the most frequent sign of endometritis, but in many cases it is very intermittent, and so can be easily missed (Figure 1. 2.). Observing the floor behind sows in gestation or lactation stalls can help detect discharges. Sometimes spreading vulvar labia will reveal small amount of discharge in at-risk females. This technique is advised by Muirhead and Alexander, (1997) to perform in all sows between 18-21 days post mating to detect causes of reproductive failure. A vaginal speculum can be of help to ascertain the site of origin of a discharge (Meredith, 1986). Using a speculum, attention should be paid to the vaginal mucosa, external urethral orifice, sinus vaginalis and external os of the cervix. Increased vascularity of the vaginal epithelium, accumulation of purulent exudate around the cervix or urethra, and erosions of the vaginal epithelium may be present (Dial and MacLachlan, 1988b). Differential

diagnosis of possible vulvar discharges is presented in Table 1. 7. It is important to note that there are "normal" discharges present at every farm throughout different production phases, which should not be mistaken for sign of endometritis (Meredith, 1986; Dee, 1993). In rare cases when **abortion** do occur in connection with non-specific endometritis, aborted fetuses are of the same age and appear almost normal or slightly autolytic with edema; the sow will show no clinical signs (Straw et al., 1999).

Systemic signs are usually lacking in endometritis, in severe cases or in metritis there might be fever and inappetence.

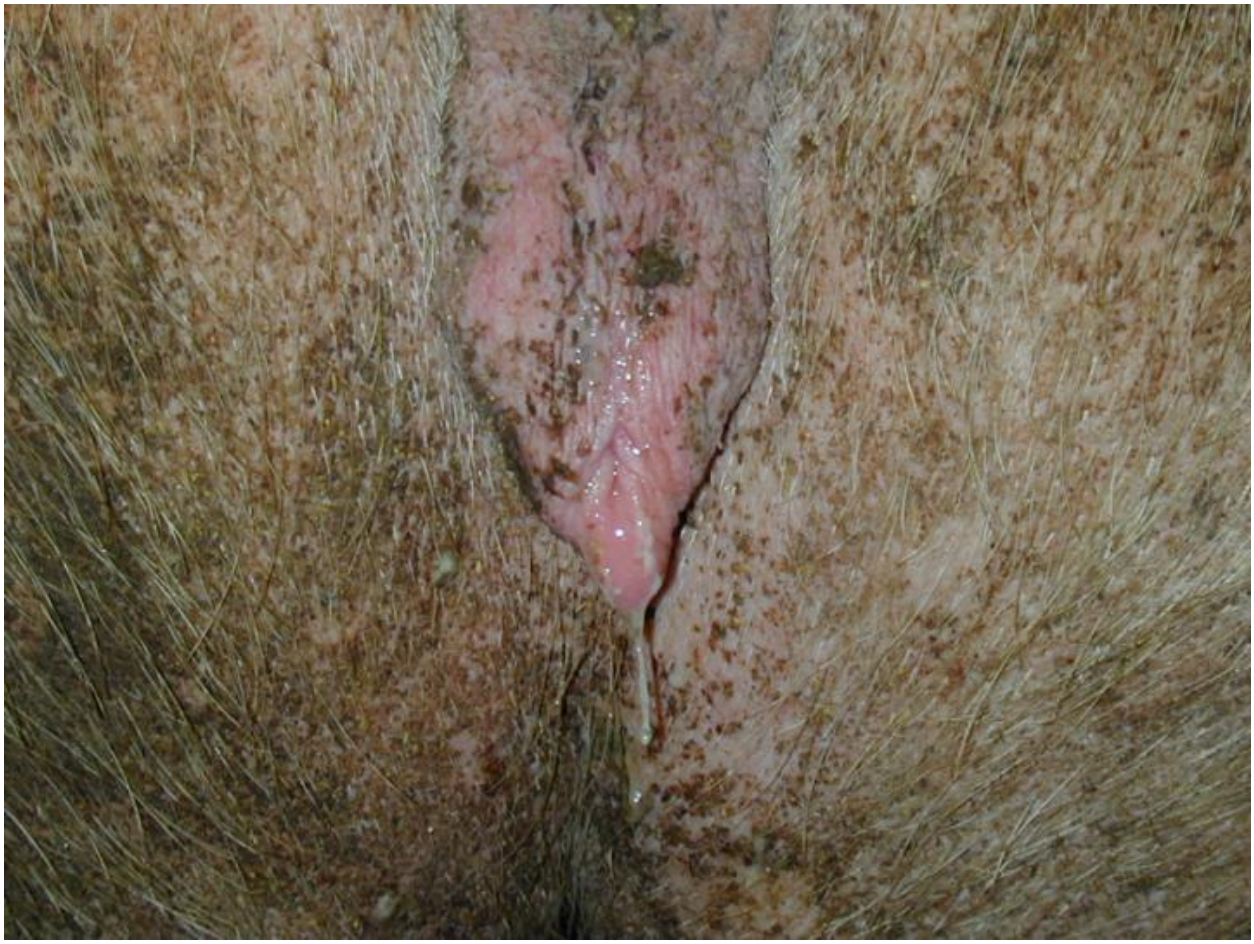

Figure 1. 2.: Mild mucopurulent post mating vaginal discharge

Table 1. 7.: Differential diagnosis of vulvar discharges (Meredith, 1986; Dial and MacLachlan, 1988a; Dee, 1992; Jubb et al., 1993; Bará and Cameron, 1994b, c; Glávits and Ványi, 1995; Britt et al., 1999; Straw et al, 1999)

Type of discharge	Quantity	Consistency	Color, odor	Age and reproductive status of animals affected, time of occurrence
Proestrous, estrous*	small amount	watery, slightly tacky	clear, cloudy or white, nonodorous	all parities, in proestrus, estrus
Seminal*	various	semen components	clear, cloudy or white, nonodorous	all parities, during or shortly after mating or AI
Postmating*	small amount	thick, tenacious	white, gray or yellow, nonodorous	all parities, 8 to 48 hours after service or AI
Late pregnancy*	small amount	thick, tenacious	white, gray or yellow, nonodorous	all parities, at end of pregnancy, probably originates from cervix
Postpartum lochia*	modest amount, 10-50 ml/episode, decreasing	thick	whitish (various), with slightly bad odor	all parities, up to 3 days post partum
Traumatic	small amount	fresh blood	red, odorless	all parities, all stages of the cycle (from vulva biting), or can be associated with mating or AI
Vaginitis	moderate amount, 10-50 ml/episode	thick, tenacious (purulent), can be bloody	white to yellow (reddish), rarely have bad odor	more common in gilts, can be sporadic or epizootic, all phases, not related to cycle
Cervicitis	modest, < 20 ml/episode	thick, nonmucoid (purulent)	white to yellow, rarely have slight bad odor	all parities, more common in cycling females, often occurs with endometritis or vaginitis, not related to cycle
Post mating endometritis	copious amount, often over 100 ml/episode, rapidly decreasing over 1 - 2 days	thick, nonmucoid (purulent)	various, white to yellow, sometimes bloody, often with bad odor	usually high parity sows and gilts, occurs at first mating after weaning or 15-24 days after mating (within 6 days before estrus), associated with sexual cycle
Post partum endometritis	moderate to copious amount, lasts for several days post partum	usually thin, might contain tissue debris, can be thick (purulent)	various, white to yellow, sometimes bloody, often with bad odor	all parities but more frequent in older sows, over three days post partum
Post partum metritis	scant to abundant	thin or thick, might contain tissue debris, can be purulent	dirty yellow to red-black with a fetid odor	all parities, post partum
Estrogenic mycotoxicosis	small amount	thick, (purulent)	white, odorless	mainly virgin gilts, but can occur in mature females
Urolithiasis (crystalluria)	various amount, seen at end of urination	thick, slightly gritty	various, cloudy white or yellow, nonodorous	all parities but more frequent in older sows, all reproductive phases
Cystitis - pyelonephritis	modest amount, ~ 20 ml/episode, seen at end of urination	thick, mucoid, mucopurulent or mucohemorrhagic	various, cloudy white, yellow, or reddish, sometimes with bad odor	all parities but more frequent in older sows, all reproductive phases, at the end of urination

* denotes "normal" discharge

1. 5. 6. 3. Pathology

For diagnostic purposes, complete urogenital tracts can be obtained from sows culled for reproductive reasons (Almond and Richards, 1992; Dalin et al., 1997). However, it is very important to be familiar with **normal cyclical changes** of the porcine endometrium, both in macroscopic diagnosis and histopathology (Schnurrbusch et al., 1985; Leiser et al., 1988; Priedkalns, 1993; 1. 5. 1.). Thorough examination of the genital tract, especially of the ovaries can guide in determining the phase of sexual cycle. Macroscopically, cyclic females culled during diestrus will have markedly congested uterine mucosa, sometimes accompanied with accumulation of small amount of clear watery fluid. These should not be mistaken for signs of inflammation. Endometritis can be suspected mainly on the presence of variable amount of thick purulent material in the uterine lumen (Meredith, 1986). When examining the reproductive tract post mortem, it should be remembered that the endometrium undergoes autolysis very rapidly, resulting in sloughing of the surface epithelium. The sloughed tissue resembles pale tan exudate on gross examination (McEntee, 1990).

Necropsy of discharging animals is frequently unrewarding, as endometritis apparently becomes quiescent during proestrus and estrus. Purulent exudate is most obvious within the lumen of affected uteri during diestrus, but discharge does not occur until the cervix becomes dilated during proestrus allowing inflammatory exudate to drain from the uterus (MacLachlan and Dial, 1987). Therefore, sows discharging for **no longer than 24 (48) hours** should be selected for reproductive examinations (Dee, 1992).

Severe **estrogenic fusariotoxicosis** can prolong the lifespan of corpora lutea, and can induce accumulation of moderate amount of tacky, brownish material, called **uteroferrin** in the uterine lumen, which should not be mistaken for inflammatory exudate (Young and King, 1986; Almond and Richards, 1992). Fusariotoxicosis can induce severe endometrial edema and the accumulation of moderate amount of clear fluid, too (Table 1. 7.). The possibility of concomitant mycotoxicosis should be kept in mind when evaluating the porcine genital tract for the presence of inflammatory lesions.

Developmental anomalies (segmental aplasia of the tubular genital tract) have to be ruled out when a large amount of hazy, white to yellowish, odorless fluid is found in one or both uterine horns at necropsy.

Gross lesions, apart from exudate accumulation might be lacking in a mild form of non-specific endometritis. Widespread necrosis and ulceration of the uterine mucosa are rare and might indicate specific infection (Everitt et al., 1981). Endometrial wall can become rigid in some cases of chronic endometritis, with cystlike structures in the mucosa. In some cases the entire uterus becomes greatly distended with considerable quantities of purulent fluid or partially inspissated necrotic material (Dial and MacLachlan, 1988b).

Histopathology of the uterus is regarded as Gold Standard in diagnosis of its inflammatory conditions. Inflammatory changes in the endometrium may be studied by examination of tissue taken for **biopsy** in the cow and mare; based on such examinations, classification schemes of endometritis were reported in these species (Kenney, 1978; Doig et al., 1981; Bonnett et al., 1991a, b, c; Ricketts and Alonso, 1991). **Endometrial biopsies** are useful in determining cause of infertility and assessing further breeding value of the animals. This technique, however, does not have practical implications in swine medicine, as the commercial value of a sow would hardly justify the expenses and technical difficulties related to such samplings. Nevertheless, information gathered in cows and mares can be useful in assessing the presence of pathological lesions in post mortem uterine specimens of swine. In mares, chronic infiltrative endometritis (mononuclear cell infiltrations) and chronic degenerative endometrial disease (development of gland nests and/or gland cysts and associated periglandular and/or diffuse stromal fibrosis) are the two of the most commonly recognized histopathological features in endometrial biopsy (Ricketts and Alonso, 1991). These regarded as unavoidable sequeale following the repeated natural challenges of aging, coitus, pregnancy, parturition and post parturient uterine involution. Mares up to 9 years of age should not have signs of chronic

degenerative endometrial disease; on the other hand, mares aged 17 years or older are likely to have severe signs (Ricketts and Alonso, 1991). Apart from the increasing probability of endometritis with age, there is no information of such age-group relatedness of chronic endometritis in swine. Endometrial fibrosis is strongly related to infertility in the mare (Kenney, 1978; Doig et al., 1981; Ricketts and Alonso, 1991).

In case of a mild acute endometritis alterations consist of a diffuse but light infiltration of inflammatory cells with slight desquamation of the superficial epithelium and no significant vascular changes (Jubb et al., 1993). Numerous neutrophils are present in the stratum compactum and in the surface epithelium during the early stage of mild endometritis. Subsequently, the inflammation can extend into the lumina of endometrial glands, and the surrounding connective tissue becomes infiltrated with lymphocytes, macrophages, and plasma cells. The best indication of mild endometritis in all species is the **accumulation of plasma cells and lymphocytic foci** in the stroma. Focal accumulation of lymphocytes as well as of neutrophil and eosinophil granulocytes in endometrial stroma, migration of lymphocytes into uterine glands with destruction of glandular epithelium and excessive deposition of hemosiderin (siderocytes) are regarded as pathological changes in porcine endometrium (Schnurrbusch et al., 1985). Unfortunately, interstitial lymphocytic foci can develop in the endometrium in association with numerous specific or non-specific infectious agents; they are inconclusive for the exact cause of inflammation. Severe inflammation can occlude the neck of some endometrial glands, resulting in dilation and subsequent fibrosis of these. Such glands remain cystic after inflammation subsides (McEntee, 1990).

Recovery from the acute phase of infection often results in **chronic endometrial involvement**. The changes depend on the duration and severity of the inflammation, but consist essentially of productive fibrosis and leukocytosis in which lymphocytes and plasma cells predominate. Thickening of the endometrium is by inflammatory tissue; the glands are depleted, atrophic or cystic due to periglandular fibrosis. The mucosa might be intact, denuded in places or might show foci of squamous metaplasia or polypoid hyperplasia. Distrophic calcification of necrotic portions of the endometrium may occur (Jubb et al, 1993).

1. 5. 6. 4. Bacteriology

Numerous agents have been isolated from the uteri of sows and gilts with endometritis and vulvar discharge (1. 4. 4.; Meredith, 1986; Dial and MacLachlan, 1988b; Dee, 1992). Bacterial culture results tend to differ from sow to sow even in the same herd (MacLachlan and Dial, 1987). Uterine cultures often yield a mixed bacterial flora, in that case it is hard or impossible to determine which species initiated the infection - however, simultaneous colonisation is also an option.

Bacteriological examination of discharges or of swabs taken from the genital tract of a living animal is of **limited value** in cases of non-specific inflammatory conditions. Swabs taken with due precaution from the cervical lumen can be occasionally diagnostic when a large number of potentially pathogenic bacteria is isolated; however, negative results are unreliable (Meredith, 1986). In dissection, bacterial isolations from the uterus should be interpreted with caution, particularly if concomitant gross or histopathologic lesions are lacking. Urine can reflux from the bladder to the uterus easily post mortem, which can introduce bacteria to the uterus from the lower urinary or genital tract (Meredith, 1986). Tying of the neck of the bladder and the cervix with a string may lessen contamination of the upper genital tract during transport to the laboratory (Almond and Richards, 1992). Bacterial culture of the vagina is usually meaningless (Dee, 1992).

There is no published information available on the virulence factors of facultatively pathogenic bacteria isolated from porcine endometritis cases. *E. coli* strains from healthy sows and from those having post-mating discharges did not differ in prevalence of virulence factors examined (hemolysin, F and P adhesins, Congo Red binding ability), antibiotic sensitivity of the two group of bacteria did not differ either (Cardoso and Silva, 1988).

Staphylococci can be cultured frequently from cases of endometritis with vaginal discharge. This bacterium is also capable to cause a somewhat specific form of endometritis, as described by Everitt et al. (1981). Fennestad et al. (1955) reported one case where outbreak of staphylococcal endometritis was due to a boar having chronic staphylococcal infection of the accessory glands and urinary bladder. The changes in the endometria of affected sows resembled actinomycosis. **Streptococci** are often isolated from the reproductive tract of sows, including animals that are infertile, have aborted or have postparturient dysgalactia (Swann and Kjar, 1980). ***Arcanobacterium*** (formerly *Corynebacterium*) ***pyogenes***, ***Proteus spp***. are also common isolates from sows with endometritis, most likely they are opportunistic pathogens.

De Winter et al. (1995) experimentally reproduced endometritis and vaginal discharge through inoculating uteri of estrous virgin gilts with *E. coli* or *Staphylococcus hyicus*. Interestingly, similar inoculation of the diestrous uterus with *Streptococcus suis* and *Actinomyces sp.* did not reproduce the syndrome. However, the number of experimental animals was too low to draw conclusions on this.

Some authors speculated that *A. suis* is capable to ascend into the uterus and cause endometritis and infertility (Dee, 1991). Up till now, no clear evidence supports this theory.

1. 5. 7. Therapy of non-specific endometritis

Mild cases of endometritis **can resolve spontaneously** during lactation, proestrus and estrus. It is frequently advised to skip one cycle with discharging females (if economics or animal flow permit it). However, affected animals **should not be kept for further breeding purposes** whenever possible, for permanent uterine damage and infertility may occur (Meredith, 1986).

In severe cases antibiotics have an important role in reducing sow and piglet mortality. Considering the variety of bacterial species possibly involved in endometritis, broad-spectrum antibiotics should be used when treatment is attempted. Sows with endometritis (vulvar discharges) have been treated with antimicrobials by intrauterine, parenteral, and oral routes (Dial and MacLachlan, 1998b; Muirhead and Alexander, 1997). **Intrauterine** application of antibiotics or antiseptic solutions is frequently utilized in the treatment of puerperal endometritis. However, this technique is time consuming, requires practice and can actually introduce bacteria to the endometrium from the lower urogenital tract. After cervical closure intrauterine administration is difficult and likely to result in trauma to the mucosa (Meredith, 1986). Intrauterine therapy might be feasible only in recently farrowed sows before cervical closure, and in some, but not all sows in estrus (Dial and MacLachlan, 1988b). Intrauterine infusion of therapeutic agents in an oil-based vehicle occasionally results in granuloma formation in the uterine wall or in the suspensory ligaments (McEntee, 1990). Intrauterine infusions of irritating preparations can cause widespread surface necrosis of the epithelium. **Parenteral** therapy should be the method of choice in treating genital tract infection in swine. Pharmacologic considerations for the treatment of endometritis and pyometra have been well reviewed, but the medical management of such conditions in swine is understudied (Dial and MacLachlan, 1988b). Information presented earlier on the use of different antimicrobials in urinary tract infections (1. 4. 8.) should pertain to the therapy of genital tract infections, too.

Infusing boar's prepuce with antibiotic preparations is sometimes advised in outbreaks of vaginal discharge problems (Muirhead and Alexander, 1997). The therapeutical value of this intervention have been discussed earlier (1. 4. 8.). Generally, this method would provide only short term (if any) solution.

Muirhead (1998) proposed a new technique to treat post mating discharge in sows. After isolation of the most common bacteria from the anterior vagina of sows and from the prepuce of boars, the resistance pattern of isolates was determined and a suitable antibiotic was selected. That compound was administered into the **cranial vagina** through cattle insemination catheter. All breeding females were treated at least once during the total herd breeding cycle; treatments were carried out 6-12 hours after the last service. In nine out of ten herds a reduction of

discharges and improvement in farrowing rates were seen. However, as the author stated, it was difficult to quantify beneficial effects of the treatment as simultaneous improvements were made to the environment and mating management in all herds and a strict culling policy of affected sows was adopted.

There are few descriptions of the **antibiotic susceptibility** of bacterial pathogens from the genital tract. All *E. coli* strains isolated by Cardoso and Silva (1998) were resistant to ampicillin, most of them were resistant to cephalosporins tested, tetracycline and nitrofurantoin, over 90% of the strains were sensitive to aminoglycosides (gentamicin, neomycin, streptomycin) and quinolones (ciprofloxacin, norfloxacin, ofloxacin, pefloxacin).

In cows, **prostaglandin therapy** has proven to be an alternative to antibiotics in the treatment of endometritis and pyometra. In cattle the corpus luteum is susceptible to prostaglandin-mediated luteolysis. Multiple treatments with prostaglandins can cause successive abbreviated estrous cycles, thus optimizing estrogenic stimulation of the uterus and more rapid elimination of pathogens. Corpora lutea of swine is thought to be unresponsive to prostaglandins until day 11-12 of the estrus cycle (Dial and MacLachlan, 1988b); prostaglandins have their main implication in swine in induction of parturition (Hurtgen, 1986a; Britt et al, 1999). However, Gadsby et al. (1996) demonstrated that Cloprostenol (a long acting prostaglandin $F2_{\alpha}$ analog) given once every 24 or 36 hours between days 6 and 10 of the estrous cycle is effective in causing significant reductions in estrous cycle length. Thus, such compounds might be potentially suitable for estrous cycle manipulation in swine.

Prostaglandins administered after parturition can increase clearance of uterine content and accelerate uterine involution. Kim et al. (1998) reduced the incidence of vaginal discharges at mating by a combined treatment with ceftiofur sodium administered immediately post partum and prostaglandin $F2_{\alpha}$ given 24-48 hours after farrowing. The same treatment did not seem to reduce the incidence of post farrowing discharges, however. Gil et al. (1990) achieved a numerical reduction of returns after the first postweaning mating through the use of prostaglandin $F2_{\alpha}$ given 36-48 hours after parturition.

1. 5. 8. Prevention of non-specific endometritis

Preventive methods can be based on results of epidemiological studies, aiming at finding **risk factors**; however, risk factors are often difficult to consider. There are important "farm effects" (composition and level of feeding, housing, environmental control, herd management, hygiene, etc.), and there are also individual differences in reactions of sows to these factors. For disorders of polyfactorial origin, as urogenital tract problems, all risk factors cannot have the same value in all circumstances (Madec and Leon, 1992).

Generally it is important to **minimize contamination of genital tract** by microorganisms and foreign material, **avoid trauma** to the genital tract, **avoid impairment of innate resistance** to infection.

Overzealous intervention at farrowing should be avoided; the use of (long acting) oxytocin preparations is preferable. Oxytocin treatment may be beneficial with minor uterine lacerations, too (Britt et al, 1999). If there is a need for manual intervention in high percentage of females, the cause should be investigated (obesity, underdeveloped gilts, severe constipation, etc.). Farrowing rooms should be operated **all-in/all-out**, whenever possible. **Frequent cleaning** of farrowing crates and prevention of feces bildup behind sows is important. After weaning, sows showing discharges indicative of endometritis preferably **should not be bred**; they have to be culled or given a rest for the period of one cycle (if economics or animal flow permit it). Too short lactation lengths (< 21 days) should be avoided. **Proper breeding practices** have probably the highest importance in preventing post-mating endometritis. Timing of AI and number of services are important aspects to consider (1. 5. 4.). Cleaning and disinfecting of boar and mating pens and insemination stalls is essential. Insemination stalls should be operated all-in/all-out, whenever possible. Sows showing vaginal discharge on return should also be culled or given a

rest for one cycle length. Clinical reports revealed that when sows failed to discharge at second time and were then mated, their conception rate was similar to those of repeat breeders (Britt et al, 1999). As urinary tract infections might predispose to endometritis, their prevention is also important (1. 4. 9.).

1. 6. Associations between urocystitis and non-specific endometritis

As the previous discussion indicates, urinary tract infections are capable to induce transient sickness or, in case of pyelonephritis, acute death or chronic debilitation of the sow. In acute pyelonephritis, systemic involvement might be responsible for abortion. Apart from this, there are no known mechanisms, which would indicate direct involvement of urinary tract infections in preventing the establishment of pregnancy or causing embryonic or fetal death. However, in recent veterinary literature considerable attention is devoted to urinary tract infections. One reason for it is, that urocystitis-pyelonephritis is a significant cause of sow mortality in some countries, can cause deterioration of the general health of breeding stock and thus can result in increased herd turnover rate (Dial and MacLachlan, 1988a). On the other hand, (subclinical) urocystitis is regarded as a strong predisposing factor to non-specific endometritis and thus, to reproductive failure. This supposed, yet somewhat controversial association is the subject of the last part of this review.

Both et al. (1980) found that bacteriuria (> 10^4 CFU/ml urine) was positively associated with clinical problems as repeat breeding, MMA-syndrome, and vaginal discharge in the postfarrowing period. They concluded that urinary tract infections are important predisposing factors to periparturient diseases. The same authors reported upon the examination of large number of urine samples from 118 herds that bacteriuria (> 10^4 CFU/ml urine) was associated with reproductive failure, because they have found more bacteriuric samples in herds with reproductive problems than in herds without. They have also found that more sows were presented with bacteriuria than gilts in both types of farms (Möller et al., 1981). In these two publications no statistical or epidemiological evaluation of data was performed (or was not reported). This and the known problems with determination of true bacteriuria make their findings somewhat inconclusive.

Thornton et al. (1998) described a case of a 5% drop in farrowing rate in two large herds. Comparison of urinalysis results and production records revealed a relationship between bacteriuria detected by leukocyte strip test and suboptimal farrowing performance of sows. Sows with suspected subclinical urinary tract infection had more mummified and poor viability piglets/litter, and also had more postfarrowing diseases. These were older than the herd average, and had a lower blood total neutrophil count before farrowing. Periodical antibiotic treatments of the two herds diminished the problem. The authors remarked, that the above associations might be confounded by the age of the animals, as older sows have more stillbirths, low viability piglets, lower white cell counts and higher prevalence of urinary tract infections. The principal implication of this study was that chronic urogenital tract disease could be a significant health problem in a herd without being evident clinically.

1. 6. 1. Common predisposing factors and sources of confounding

Madec and Leon (1992) warned, that parity affects a number of physiological and physical characteristics of animals, thus it can act as a **confounder** in studies aiming to find predisposing factors for endometritis. A confounding variable (confounder), by definition, is any factor that is either positively or negatively correlated to with both the disease and hypothesized causal factors that are being considered (Thrusfield, 1995). Rothman (1986) reviewed the concept of confounding in epidemiological studies in more detail. Similarly to parity (age), as it is apparent from the preceding review, there are a number of factors both associated with endometritis and urocystitis in swine. Such factors are "farm hygiene" (accommodations in

gestation and lactation areas, frequency and effectiveness of cleaning and disinfection, animal flow) and "mating hygiene" (use of hygienic mating procedures [AI]). The presence of a confounding factor can distort the relationship between a cause and an effect. Graphical display of the relatedness of proposed causes and disease, called "web of causation", frequently helps in designing observational studies. Such simplified web of causation is presented in Figure 1. 3. for possible associations between certain urogenital tract conditions.

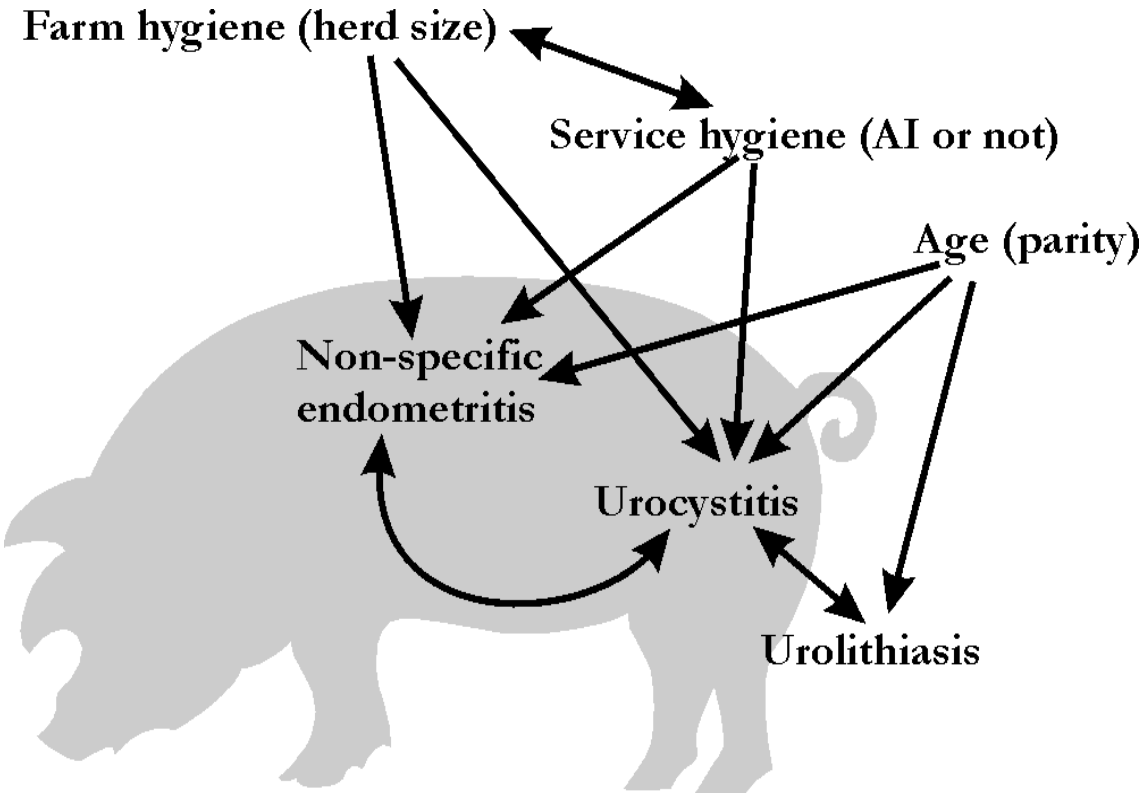

Figure 1. 3.: Simple web of causation for urogenital infections

1. 6. 2. Analysis techniques

In virtually all of the studies related to the scope of this review, statistical associations were not sought for between non-specific endometritis and urocystitis, in some reports even statistical analysis was not performed. **Statistical significance** tests give an indication of the probability that observed differences between groups are due to chance. **Clinical (biological) significance** concerns the relevance of findings to clinical practice. Because statistical significance is partly dependent on sample size, it is possible that small and clinically unimportant differences may become statistically significant. It is also possible that clinically important results may be overlooked because a study's sample size is too small to allow sound conclusions to be drawn. Following is a brief introduction to epidemiological analysis techniques suitable to investigate the proposed association between endometritis and urocystitis in swine.

The concept of biological significance is addressed by epidemiology; epidemiological investigations frequently rely on observational studies. **Observational studies** are used to identify risk factors, and to estimate the quantitative effects of the various component causes that contribute to the occurrence of disease. Such investigations are based on analysis of natural disease occurrence in populations by comparing groups of individuals with respect to disease occurrence and exposure to hypothesized risk factors (Thrusfield, 1995). There are three main types of observational study: cohort, case-control and cross-sectional. Each classifies animals into those with and without disease, and those exposed and unexposed to hypothesized risk factors; the methods of data generation differ between these study types. Each study utilizes 2 x 2 contingency tables (Figure 1. 4.).

	Diseased animals	Non-diseased animals	Total
Risk factor present	a	b	a + b
Risk factor absent	c	d	c + d
Total	a + c	b + d	a + b + c + d = n

Figure 1. 4.: The 2 x 2 contingency table constructed in observational studies

A **cross-sectional study** involves the selection of a sample of n individuals from a larger population, and then the determination for each individual, of the simultaneous presence or absence of disease and hypothesized risk factor. Main advantages of cross-sectional studies are that they are relative easy to conduct, inexpensive, and allow multiple potential causes of disease to be studied simultaneously. This type of study is, however, unsuited to the study of diseases of short duration and usually the temporal sequence of cause and effect cannot be determined.

A hypothesis of association between disease and a factor can be tested using the χ^2 test; however, this cannot be used to measure the degree of association. A more informative measure of the impact of a factor on disease occurrence can be expressed through calculating the ratio of disease occurrence between the two groups. The odds ratio is such measure, it equals to the ratio of the probability of an event occurring to the probability of it not occurring. The odds ratio in a cross-sectional study can be calculated using the notation from Figure 1. 4. as **ad/bc** (Thrusfield, 1995). An approximate 95% confidence interval for the odds ratio can also be calculated. When an odds ratio is significantly greater than 1 at the 95% level, it indicates a **positive association** between the hypothesized cause and effect.

Analysis of epidemiological data can be performed to avoid effect of confounding by several means (Thrusfield, 1995). One method is to adjust for the confounding variable in analysis, like calculating a summary odds ratio (weighted average) from stratum specific odds ratios. In case of non-specific endometritis and urocystitis, the confounding effect of age can be excluded by calculating stratum specific odds ratios (e. q. the risk of having endometritis when urocystitis is present in sows over parity 5 **and** under or at parity 5), and calculating a summary odds ratio, the Mantel-Haenszel odds ratio (MHOR) for the two strata.

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 preputium of boars on some Hungarian farms and its isolation from clinical cases of urocystitis in sows.

2. 1. 1. Animals

Samples were collected in the course of our diagnostic work on reproductive problems (elevated occurrence of vaginal discharges, returns to estrus, etc.) in several swine herds. Three farms were involved in the present examination. On Farm A and B natural mating, on Farm C artificial insemination were practiced.

2. 1. 2. Sampling

On Farm A six, on Farm B 10 preputial swab samples were taken from actively used mature boars (1-4 years old). The animals did not receive any medication for 2 weeks before sampling and showed no clinical signs. Samples were taken with sterile cotton swabs from the preputium while the animals were in standing position. Swabs were transported in Stuart-Ringertz medium at 6-8°C to the laboratory (Stuart, 1959; Ringertz, 1960). 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 Farm C. 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 at 6°C and were processed within 4-5 h.

2. 1. 3. Bacteriological examinations

The preputial swab samples were streaked individually on blood agar plates containing 5% defibrinated sterile horse blood and CCNAM agar plates. They were cultured at 37°C for 6-7 days under anaerobic conditions using anaerobic jars (AnaeroJar, Oxoid). 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 (Barrow and Feltham, 1993). 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.

Colonies having characteristic dry, grayish-white, flattened, opaque surface without haemolysis were subcultured on horse blood agar plates under anaerobic conditions at 37°C for 4-5 days. Morphology, catalase and urease production, hippurate hydrolysis, nitrate reduction, the fermentation of glucose, glycogen, starch, lactose, mannitol and trehalose were tested and the results were compared to the biochemical characteristics of *A. suis* ATCC 33144 (Wegienek and Reddy, 1992; Ludwig et al., 1992).

Oxygen tolerance was examined by inoculating 4 strains of *A. suis* from farm A 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 fixed in 8% formaldehyde solution, embedded in paraffin, stained with haematoxylin-eosin and were examined for the presence of inflammatory reactions.

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

Different antimicrobials are used for the treatment cystitis and pyelonephritis caused by *A. suis*, however, it is usually frustrating, especially in chronic cases (Dee, 1993). The success of attempted therapy largely depends on choosing the appropriate compound, and results of *in vitro* sensitivity studies can guide clinicians in this process. Since testing antibacterial susceptibility of fastidious bacteria like *A. suis* is not routinely carried out and treatment with antibiotics is based on data in the literature, examination of the antibacterial susceptibility of *A. suis* strains including newly introduced antibiotics is of special importance. 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 (Quinn et al., 1994) however, there is no standardized method. Although in most cases the guidelines of NCCLS (National Committee for Clinical Laboratory Standards, Wayne, FL, USA) are followed, valuable results were gained even in the case of obligate anaerobic bacteria, like *Serpulina (Brachyspira) hyodysenteriae* using the disc diffusion method (Molnár, 1997). A special form of the disc diffusion method, the E-test is getting accepted for testing antibacterial susceptibility (Nagy, 1999). Since *A. suis* is reportedly not a true obligate anaerobic bacterium (Biksi et al., 1997), 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.

2. 2. 1. Bacterial strains

Thirteen *A. suis* strains were used in this study. Eight were isolated in a previous investigation (Biksi et al., 1997), two were isolated later from the prepuce of healthy boars, two were cultured from cases of haemorrhagic cystitis of sows, and the type strain DSM 20.639 = ATCC 33144 was previously purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). All of the isolates were identified by biochemical methods and were compared to the type strain (Moore and Holdeman Moore, 1986).

2. 2. 2. Antimicrobials and sensitivity testing methods

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 and 0,5 ml of these was mixed with 19,5 blood agar 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 (prepared using a McFarland No. 1. standard) 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 discs were placed on each agar plate at maximum. Concentration and source of sensitivity discs are included in Table 2. Inhibitory zone diameters were determined with a caliper. 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 using anaerobic jars (AnaeroJar, Oxoid).

2. 2. 3. Statistical procedures

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. Statistical procedures were performed using Minitab for Windows 13.0.

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). Farms were enrolled in this study upon request for reproductive examination. Twenty-one large swine herds from the main pig producing regions of Hungary were included. Some farms were sampled several times during the study period. 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 analysed.

2. 3. 1. Animals and herd characteristics

Animals were selected for culling for reproductive failure by farm managers. Investigators had no influence on animal selection, selection was not made at random. Only animals with clearly indicated reproductive failure in their records were included in the study. Inclusion criteria were: multiple regular or irregular repeats with or without vaginal discharge, anestrus, and "failure to farrow". For each animal parity, reason for culling, and the interval from last weaning or breeding to slaughter were obtained. Due to the lack of computerized herd record system on most farms, data were mainly obtained from handwritten sow registration cards. It was not possible to check herd records for errors; however, in case of any doubt about validity, records of the sow were not processed.

2. 3. 2. Slaughterhouse sampling

Method of slaughterhouse sample collection was similar to that described by several authors (Straw et al., 1986; Almond and Richards, 1992; Tubbs, 1995a, 1995b). Briefly, ~90% of the samples were taken at the same large commercial slaughterhouse with an approximate line speed of 200 sows/hour. The rest of the samples were collected from smaller slaughterhouses. Sows were identified by their eartags at two separate places in the slaughterline. 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.

2. 3. 3. Macroscopic examinations

All urogenital tracts were completely dissected after collecting samples for bacteriology. Lesions were described by the same person in all cases; findings were recorded on microcasette tape. Ovaries were measured, their surface structures were counted, measured and described to determine the stage of sexual cycle and to diagnose ovarian abnormalities (Schnurrbusch et al., 1985; Leiser et al., 1988; Almond and Richards, 1992). Integrity and content of the oviducts were recorded. Diameter and content of uterine horns (amount, physical characteristics, odour), and the state of endometrium (surface integrity, fluid content, vascularization) were checked. **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. The presence of **concretions** in the bladder was also noted, but it did not indicate urocystitis in itself. All visible developmental anomalies of the available urogenital tract and the presence of pregnancy were also noted.

2. 3. 4. Histopathology

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. Samples subsequently were embedded in paraffin, cut at 6 μm, stained with hematoxylin-eosin and were examined for the presence of inflammatory lesions under light microscope by the same person. The examiner was unaware of the history, macroscopic and bacteriological test results of animals from which histological samples were originated. Only one section from each mentioned sampling place was evaluated, sections were evaluated in their entirety.

Stage of reproductive cycle in each animal was estimated using guidelines from the literature (Schnurrbusch et al., 1985; Leiser et al., 1988). If the histological appearance of the endometrium did not compare with the state of ovaries, sows were excluded from further analyses in order to prevent possible misdiagnoses related to estrogenic mycotoxicoses (Ványi et al., 1995; Glávits and Ványi, 1995).

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 (Figure 2. 1.); 2.) when there was more than one focal periglandular or perivascular inflammatory cell accumulation in the stratum spongiosum (Figure 2. 2.); or 3.) when diffuse leucocytic infiltration of the stratum spongiosum was observed (Figure 2. 3.). 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 (Figure 2. 4.).

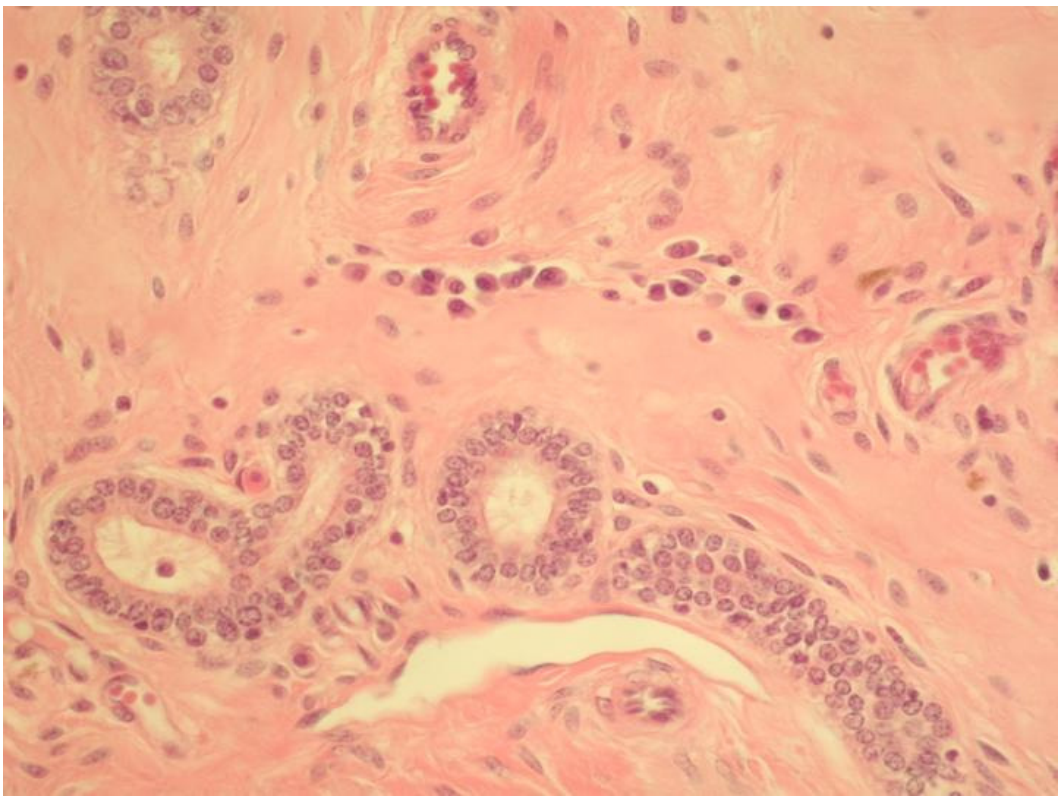

Figure 2. 1.: Accumulation of plasma cells in the stratum spongiosum of the endometrium, endometritis, sow (H-E., 400X)

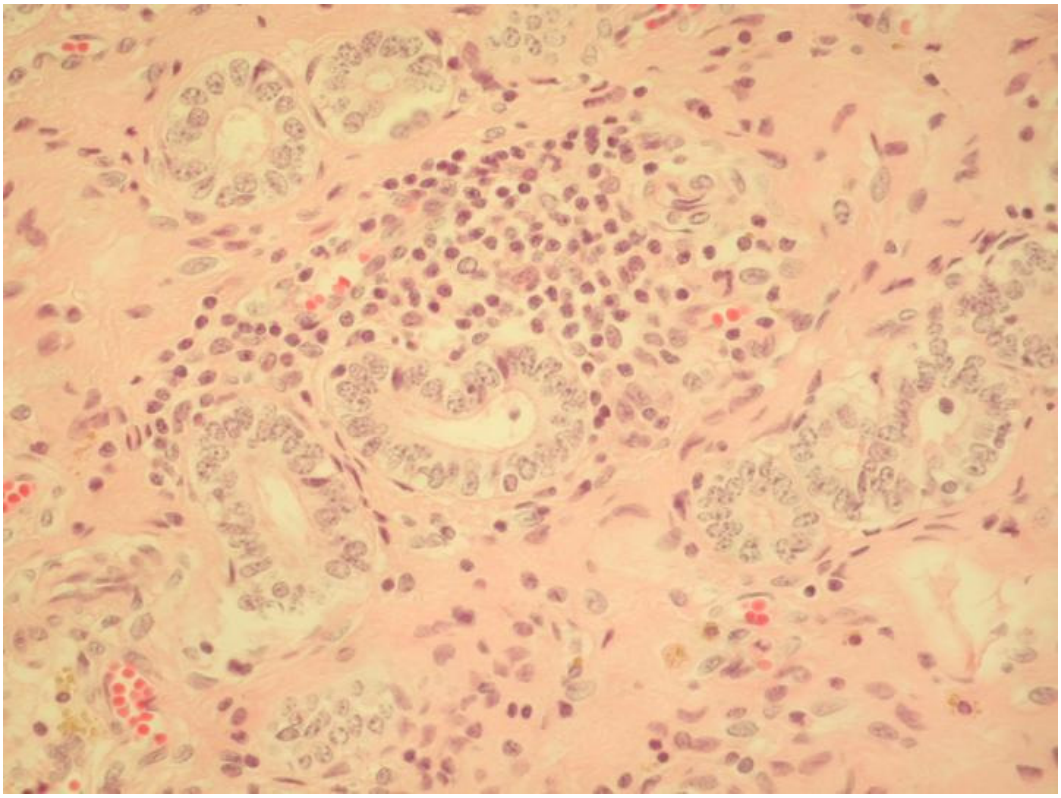

Figure 2. 2.: Periglandular lymphoplasmacytic infiltration in the stratum spongiosum of the endometrium, endometritis, sow (H-E., 400X)

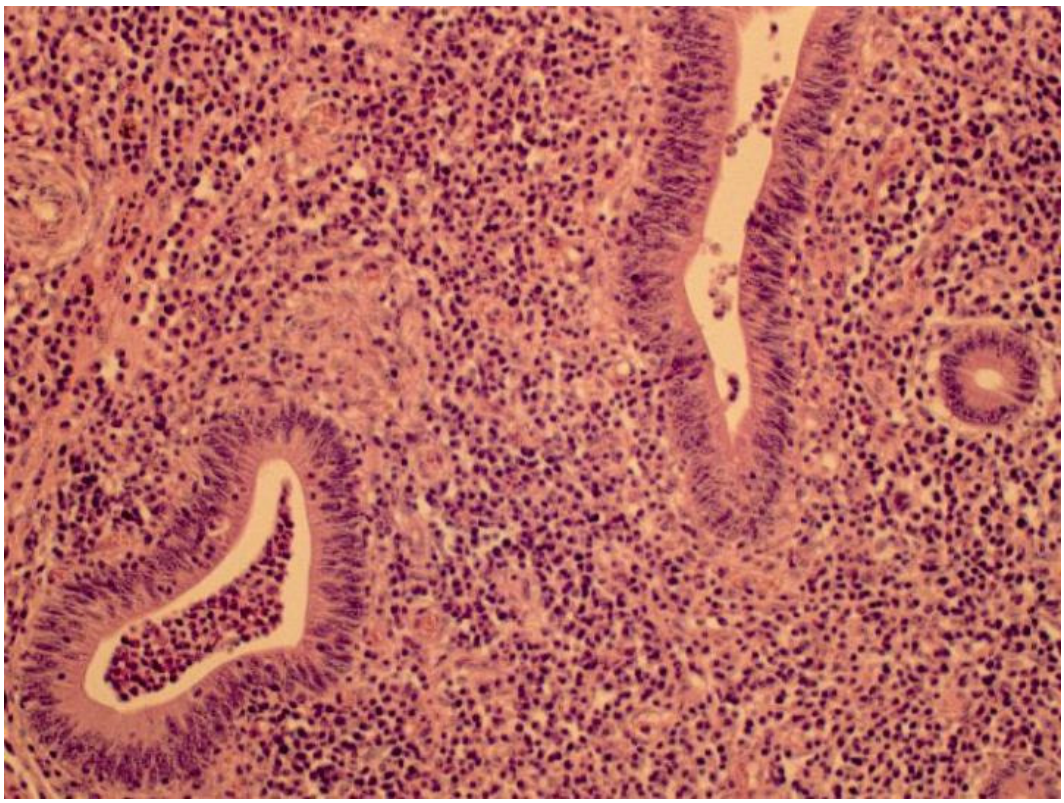

Figure 2. 3.: Severe diffuse mixed leukocytic infiltration in the stratum spongiosum of the endometrium, endometritis, sow (H-E., 400X)

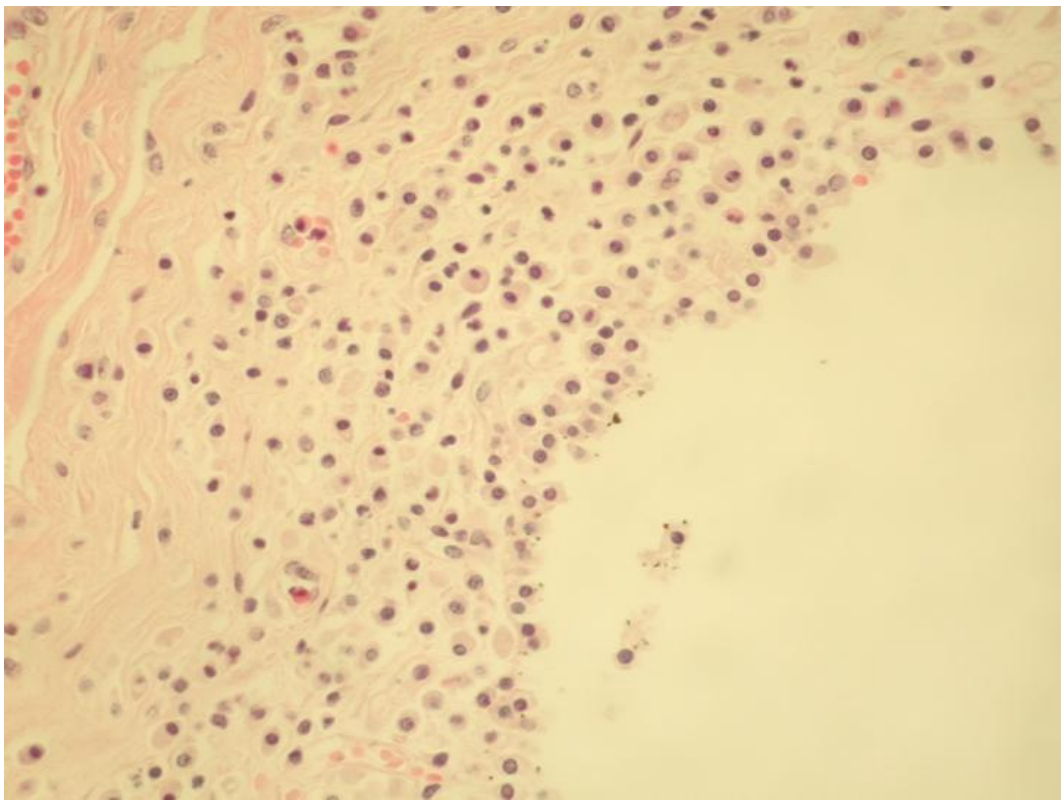

Figure 2. 4.: Severe diffuse lymphoplasmacytic infiltration of the bladder mucosa with epithelial desquamation, urocystitis, sow (H-E., 400X)

2. 3. 5. Bacteriology

All urogenital tracts were examined within 5 hours of collection. Samples from both uterine horns, from the mid-cervix and urinary bladder were collected for bacteriological examinations with sterile cotton tipped swabs. Swabs were streaked on blood-agar plates containing 5% sterile sheep or horse blood and a crystal violet-lactose-bromthymol blue agar plate media for aerobic culture at 37 ºC for 24-48 hours. For the isolation of *Actinobaculum suis*, samples from the urinary bladder were inoculated on agar plates containing 5% sterile horse blood without antibiotics and on colistin-nalidixic acid-metronidazole-supplemented Columbia blood-agar media (CCNAM) and incubated under anaerobic conditions at 37 ºC for 7 days in a commercial anaerobic culture system (AnaeroJar, Oxoid). The same person evaluated all cultures; isolated bacteria were identified using standard methods (Barrow and Feltham, 1993). 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.

2. 3. 6. Statistical analysis

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 was defined as the proportion of true positives that are detected by the given method, specificity was defined as the proportion of true negatives that are detected (Thrusfield, 1995). 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 (Martin, 1977; Martin et al., 1987; Thrusfield, 1995).

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. We have also performed calculations with different parity cut-off points (i. e. 3, 4, 6 or more; data not shown). 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 (Martin et al., 1987). 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 Farm A and three out of 10 samples from Farm B. They were isolated together with saprophytic bacteria. Stained smears of the characteristic, dry, circular, flat, grayish-white colonies with crenated edge (Figure 3. 1.) showed Gram-positive, 2-3 μm long pleiomorphic rods arranged in "Chinese letters" or in palisade forms (Figure 3. 2.).

Biochemical characteristics of the isolated bacteria are presented in Table 3. 1.

Table 3. 1.: Biochemical characteristics of *A. suis* strains isolated in Hungary and of the type strain ATCC 33144

		Own isolates 7 strains	ATCC 33144
Catalase production		**-**	**-**
Urease production		**+**	**+**
Hippurate hydrolysis		**+**	**+**
Nitrate reduction		**-**	**-**
Fermentation of	**glucose**	**-**	**-**
	glycogen	**+**	**+**
	lactose	**-**	**-**
	mannitol	**-**	**-**
	starch	**+**	**+**
	trehalose	**-**	**-**

Growth could not be seen on the aerobically incubated plates inoculated with *A. suis* before the 8th day of incubation. After 10 days of incubation, three out of four *A. suis* strains isolated from Farm A and the reference strain formed pinpoint, shiny, round, non-haemolysing colonies with entire edge. When these bacteria were grown under anaerobic conditions on blood agar at 37°C, 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 (Figure 3. 3.; 3. 4.). The sow was artificially inseminated 4 days before slaughter. From the other four urinary bladder samples no *A. suis* strain but *Escherichia coli* (two cases) and *Enterobacter spp.* could be cultured, one sample was bacteriologically negative.

Figure 3. 1.: Characteristic colonies of *Actinobaculum suis* on CCNAM media after 7 days of anaerobic incubation (8X)

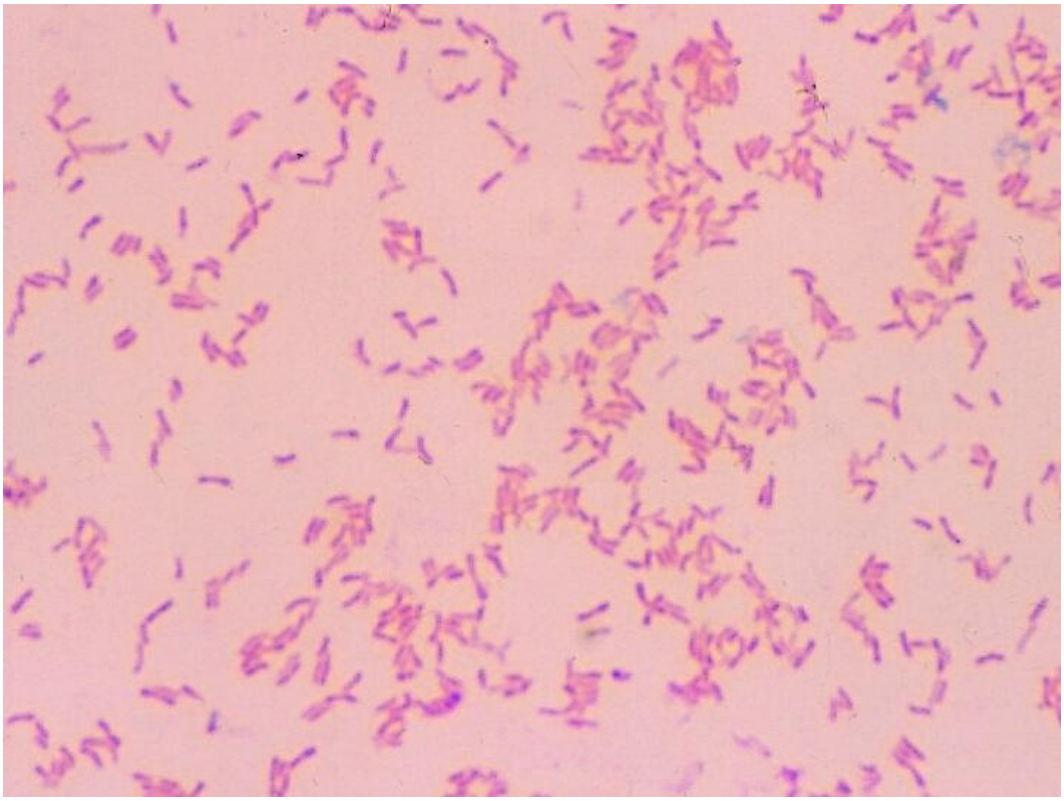

Figure 3. 2.: Gram-stained smear of *Actinobaculum suis* (1000X)

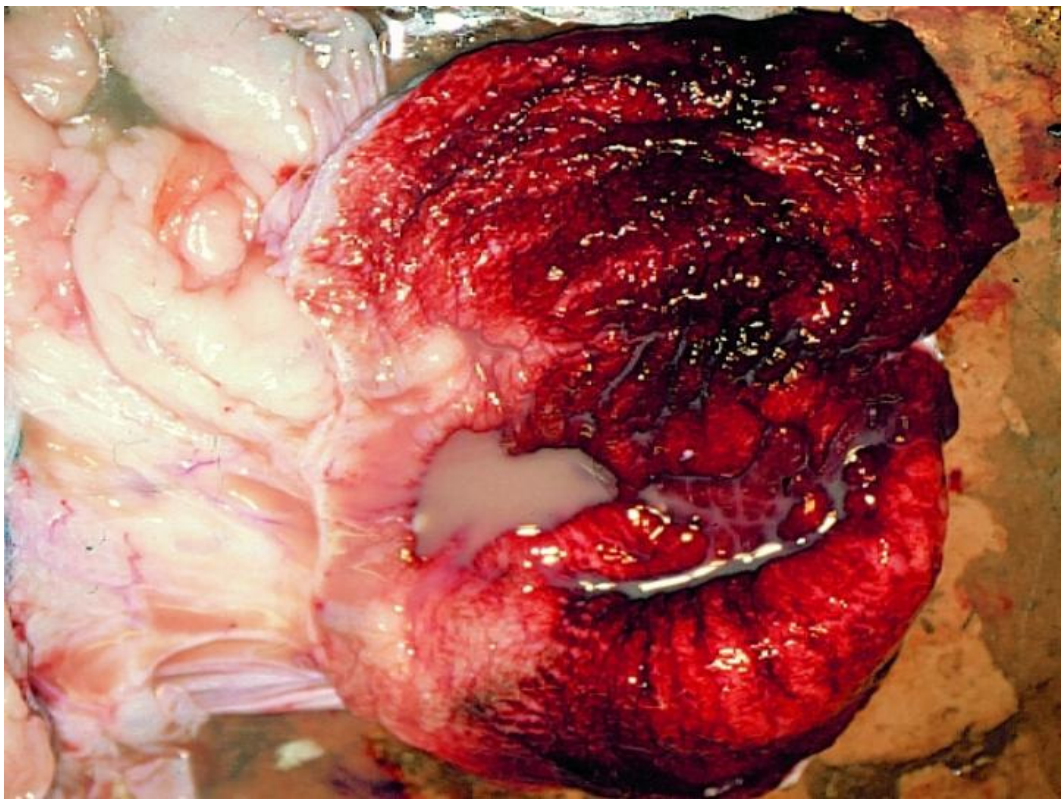

Figure 3. 3.: Severe haemorrhagic-necrotising urocystitis, sow

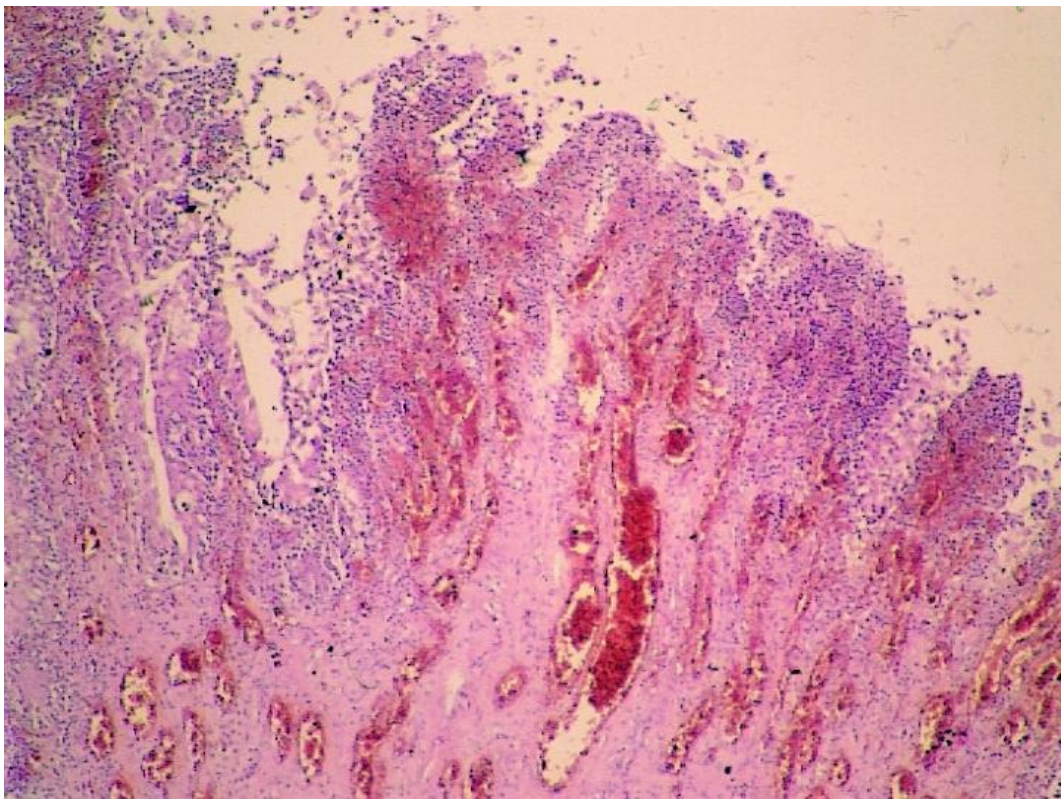

Figure 3. 4.: Severe haemorrhagic-necrotising urocystitis, sow (H-E., 200X)

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

Results are presented in Tables 3. 2. and 3. 3. 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, $p = 0.9$).

Table 3. 2.: Minimal inhibitory concentration of selected antimicrobials for Hungarian *A. suis* isolates and the type strain ATCC 33144 (n = 13)

Antimicrobial (source)	MIC $_{ATCC\ 33144}$ (μg/ml)	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Penicillin (Sigma)	0.2	0.2-12.5	0.2	0.4
Ampicillin (Sigma)	1.6	0.8-25	0.8	2.82
Amoxicillin + clavulanic acid	ND	ND	ND	ND
Cefotaxim	ND	ND	ND	ND
Ceftiofur (Pharmacia)	0.05	0.05-0.1	0.05	0.09
Gentamicin (Sigma)	25	25-50	25	25
Neomycin (Sigma)	100	100	100	100
Streptomycin (Sigma)	50	50-100	50	100
Spectinomycin (Sigma)	6.25	6.25-12.5	6.25	12.5
Oxitetracycline (Sigma)	6.25	6.25-12.5	12.5	12.5
Doxycycline (Sigma)	6.25	1.6-12.5	3.125	6.25
Lincomycin (Sigma)	0.4	0.2-100	0.4	80.625
Tylosin (ELANCO)	0.05	0.05-3.125	0.05	1.44
Erythromycin (Sigma)	0.05	0.05-100	0.05	100
Tiamulin (Novartis AH)	0.1	0.1-0.4	0.1	0.4
Valnemulin (Novartis AH)	0.05	0.05	0.05	0.05
Chloramphenicol (Sigma)	0.8	0.4-0.8	0.4	0.8
Florfenicol (Schering-Plough)	0.4	0.4-0.8	0.4	0.8
Nalidixic acid (Sigma)	100	100	100	100
Flumequine (Sigma)	100	50-100	50	100
Enrofloxacin (Sigma)	3.125	0.8-3.125	1.6	2.82
Marbofloxacin	ND	ND	ND	ND
Ofloxacin (Sigma)	3.125	0.8-3.125	1.6	3.125
Sulfamethoxazole + trimethoprim (Sigma)	100	100	100	100

ND = not done

Table 3. 3.: *In vitro* sensitivity of Hungarian *A. suis* isolates and the type strain ATCC 33144 as determined by the disc diffusion method (n = 13)

Disc (concentration, manufacturer)	Sensitive (%)	Intermediate (%)	Resistant (%)
Penicillin (3 IU, Human)	100	0	0
Ampicillin (20 μg, Human)	92	8	0
Amoxicillin + clavulanic acid (20 + 10 μg, Unipath)	92	8	0
Cefotaxim (30 μg, Human)	100	0	0
Ceftiofur (30 μg, Rosco)	100	0	0
Gentamicin (10 μg, BioMerieux)	0	8	92
Neomycin (30 μg, Human)	0	8	92
Streptomycin (30 μg, Human)	8	15	77
Spectinomycin (100 μg, Sanofi)	69	23	8
Oxitetracycline (30 μg, Human)	92	8	0
Doxycycline (30 IU, Sanofi)	100	0	0
Lincomycin (30 μg, Rosco)	100	0	0
Tylosin (30 μg, Mast Diagnostics)	100	0	0
Erythromycin (10 μg, Human)	85	0	15
Tiamulin (30 μg, Rosco)	100	0	0
Valnemulin (30 μg, Abtek)	100	0	0
Chloramphenicol (30 μg, Human)	100	0	0
Florfenicol (30 μg, BBL)	100	0	0
Nalidixic acid (30 μg, Human)	0	8	92
Flumequine (30 μg, BioMerieux)	15	46	39
Enrofloxacin (5 μg, Unipath)	62	38	0
Marbofloxacin (5 μg, Sanofi)	84	8	8
Ofloxacin (5 μg, Human)	46	46	8
Sulfamethoxazole + trimethoprim (25 μg, Human)	0	15	85

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

3. 3. 1. Prevalence of lesions and bacteriology

The prevalence of different lesions based on macroscopic, bacteriological or histopathological diagnoses are presented in Table 3. 4. Briefly, the 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.

Table 3. 4.: Prevalence of macroscopic, bacteriologic and histopathologic findings in culled female breeding swine between 1995-2000 in Hungary

Variables	Total females	Renge per herd	Prevalence %	95% CI
Macroscopic findings				
Number examined	499	4-53		
Cervical developmental anomaly	2	0-2	0.4	0.0 , 1.4
Cervicitis	12	0-3	2	1.2 , 4.0
Concretions	54	0-11	11	8.3 , 13.7
Cystic ovary (all forms)	50	0-10	10	7.4 , 12.6
Endometritis, pyometra	16	0-5	3	1.8 , 5.0
Hydrometra/mucometra	8	0-3	2	0.7 , 3.1
Ovarian atrophy/inactivity	29	0-13	6	3.9 , 8.1
Paraovarian cyst	16	0-5	3	1.8 , 5.0
Pregnancy	15	0-8	3	1.6 , 4.7
Urocystitis	66	0-9	13	10 , 16
Uterine atrophy/inactivity	17	0-12	3	1.9 , 5.2
Uterine developmental anomaly	6	0-5	1	0.4 , 2.6
Miscellaneous oviduct abnormalities	9	0-2	2	0.8 , 3.3
Bacteriological findings				
Number examined	289	1-40		
Endometritis	73	0-14	25	20 , 30
Urocystitis	111	0-15	38	32.4 , 43.6
Histopathological findings				
Number examined	353	1-46		
Endometritis	55	0-9	16	12.2 , 19.8
Urocystitis	96	0-15	27	22.4 , 31.6

3. 3. 2. Statistical analysis

Sensitivity and specificity in case of macroscopic and/or bacteriological diagnosis of urocystitis and endometritis, compared to histopathologic diagnosis as a "Gold Standard" are presented in Table 3. 5. Briefly, sensitivity of macroscopic observation and bacteriology alone in the case of endometritis 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.

Table 3. 5.: Sensitivity and specificity estimates for macroscopic and bacteriological diagnosis of endometritis and urocystitis as compared to histopathology in culled female breeding swine between 1995-2000 in Hungary

Disease	N tested	Diagnostic method	Sensitivity		Specificity	
			%	95% CI	%	95% CI
Endometritis	353	Macroscopic	18.1	14.1 , 22.1	96.6	94.7 , 98.5
	289	Bacteriologic	31.8	26.4 , 34.2	75.9	71.0 , 80.8
	213	Macroscopic plus bacteriologic in parallel	18.7	13.5 , 23.9	98.9	97.5 , 100
Urocystitis	353	Macroscopic	47.9	42.7 , 53.1	88.3	84.9 , 97.1
	289	Bacteriologic	63.0	57.4 , 68.6	71.0	57.4 , 68.6
	182	Macroscopic plus bacteriologic in parallel	58.0	50.8 , 65.2	96.2	50.8 , 65.2

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 (from parity 0 to 6 and more, data not shown).

Table 3. 6.: Results of stratum specific analyses in the urocystitis – endometritis relationship in culled female breeding swine between 1995-2000 in Hungary

Parity	Urocystitis	Endometritis positive	Endometritis negative
≥ 5	positive	12	24
≥ 5	negative	7	56
< 5	positive	16	44
< 5	negative	20	174

Breslow-Day statistic for the similarity of stratum specific odds ratios (ORs) $\chi^2 = 0.13$, $p = 0.72$
Mantel-Haenszel odds ratio (MHOR) = 3.44
95% confidence interval (CI) for MHOR = 2.85 , 4.13

4. Discussion

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. It is of interest that the colony morphology completely differed from what can be seen after anaerobic incubation.

A. suis strains could be isolated from mature boars showing no clinical signs of urinary tract infection. The importance of asymptomatic carriers has been emphasized by several authors (Jones and Dagnall, 1984).

One *A. suis* strain was isolated together with *Proteus mirabilis* from the urinary bladder of a sow without noticeable involvement of the upper urinary tract. *Proteus spp.* were reportedly isolated from the lower and upper urinary tracts of sows with cystitis-pyelonephritis (Carr and Walton, 1993), but not much is known about its possible interaction with *A. suis* in the development of urinary tract infections. The subacute haemorrhagic-necrotizing cystitis occurred 4 days after artificial insemination of the sow. Since *A. suis* can survive at 20°C for 24 h when mixed with semen (Dee et al., 1993), the possibility of transmission of *A. suis* with diluted boar semen or with contaminated artificial insemination equipment cannot be disclosed.

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 (Carr and Walton, 1993), treatment of these conditions requires the use of broad spectrum antimicrobials or antimicrobial combinations (Dee, 1992). Also, among others, the availability of the active compound at the site of infection and its activity at high pH have to be considered (Wendt, 1998). 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. Unfortunately, data on the concentration of different antimicrobials in the porcine urogenital tract are quite limited (Dee, 1992). 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. We have no ready explanation for the difference in chloramphenicol and erythromycin MIC values of the type strain ATCC 33144 between our results and those reported by Moore and Holdeman Moore (1986). Repeated passage of the type strain might have contributed to this difference in susceptibility.

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

4. 3. 1. Prevalence of lesions

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 (McEntee, 1990; Dalin et al., 1997; Heinonen et al., 1998; Kjelvik et al., 2000). Apart from ovarian cysts, urocystitis and concretions accounted for the majority of lesions. The prevalence of urocystitis was higher than the prevalence of endometritis in all examinations. However, large proportion of examined animals did not show any of the registered lesions. Bacteria isolated from the urinary bladder and endometrium represented members of the normal fecal flora, *Eubacterium suis* has been detected only in three instances. **Our data indicates that urocystitis occurs frequently** in Hungarian sows culled for reproductive reasons.

4. 3. 2. Statistical analysis

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.**

The low predictive value of a positive bacteriological culture in case of both organs probably reflects the effect of bacterial contamination. Bacterial contamination of the upper urinary and genital tracts during slaughterhouse sampling is an important problem (Meredith, 1986; Almond and Richards, 1992), which, as shown here, might influence negatively the validity of a diagnosis. Sensitivity and specificity figures could have been influenced by the method of macroscopic and bacteriological diagnosis of the mentioned conditions, as certain level of subjectivity is unavoidable in such assessments. Nevertheless, 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. Histopathologic diagnosis of urocystitis and endometritis were done somewhat subjectively, as there are no generally accepted diagnostic criteria for such lesions in swine. Unfortunately, virus induced endometrial lesions in swine are practically indistinguishable from certain forms of bacterial endometritis (McEntee, 1990). However, we believe that histopathology still should be the "Gold Standard" in such evaluations.

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.

Actinobaculum suis, a special swine urinary pathogen is present in Hungarian swine, both in the prepuce of healthy boars and as a cause of haemorrhagic-necrotising urocystitis of sows. Hungarian isolates have similar MIC values than the type strain, although some strains might differ in susceptibility to certain antimicrobials. When treatment of urinary tract infections with the possible involvement of *A. suis* is attempted, the use of broad spectrum antimicrobials as semisynthetic penicillines, ceftiofur, doxycycline, florfenicol, marbofloxacin and enrofloxacin should be considered. Non specific bacterial infections of the urinary bladder are relatively frequent findings in culled Hungarian breeding sows. More emphasis should be given to the role of urogenital tract infections in reproductive problem solving in Hungary. Considering the diagnosis of inflammatory conditions of the urogenital tract, macroscopic or bacteriological examination of the urinary bladder or the uterus alone is likely to be not sufficient to arrive at a

correct diagnosis of urocystitis or endometritis. Histology should be utilized whenever possible for establishing such a diagnosis. Urocystitis and endometritis are strongly associated, which is not confounded by the parity of the animal. Animals with urocystitis had an approximately 3.5 times higher chance to simultaneously have endometritis than animals without this condition. Although temporal and causal relationships were not possible to reveal in this study, it might well be possible that prevention of urinary tract disorders can alleviate genital tract problems and decrease losses associated with reproductive failure.

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6. Related publication list of author

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

6. 1. 1. In domestic journals, in foreign languages

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.

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.

6. 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.

6. 1. 3. In domestic journals, in Hungarian

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

6. 2. 1. Non-refereed papers

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

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