Leptospira Infection Among Pigs in Southern Vietnam

Aspects on epidemiology, clinical affection and bacteriology

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Abstract

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Leptospirosis is a bacterial disease that in pigs primarily causes reproductive disturbances. The disease is a zoonosis, i.e. it can be transmitted between animals and humans. Leptospirosis is spread worldwide, although it is of most importance in tropical regions where animal management and climate favour transmission and survival of the bacteria in the environment, such as the tropical Mekong delta (MD) in southern Vietnam. In the MD, reproduction disturbances in pigs due to infectious agents are of concern. This thesis investigates *Leptospira* infection among pigs in the MD with aspects on epidemiology, clinical affection and bacteriology. Such information is of importance if preventive measures are to be implemented.

This study showed that leptospiral seroprevalences among sows were high and that a larger proportion of sows on small-scale farms compared with large-scale farms were seropositive. Few risk factors were found that could explain seropositivity in the sows. It was also found that the seroprevalences for some serovars were higher during the dry period compared with the wet period. Furthermore, some serovars were associated with impaired reproductive performance of the sows, such as an increased number of piglets born dead per litter and a longer weaning to service interval. Also, seroprevalences among fattening pigs at slaughter were high, and in these animals leptospires were demonstrated in a large number of kidneys with macro- and microscopic kidney lesions. One leptospiral serovar was isolated from a kidney.

Taken together, *Leptospira* infection, indicated by seropositivity, is common among pigs in the MD, which may be explained by a favourable environment rather than certain risk factors. Small-scale farms are in closer contact with the surrounding environment than large-scale farms, which may explain the differences between the farming systems. Even in regions with high leptospiral seroprevalences, infection, as indicated by seropositivity, has a negative impact on the reproductive performance of sows. Furthermore, a large proportion of fatteners with macroscopic renal lesions carry the bacteria, which constitutes a health hazard for personnel at abattoirs and persons exposed elsewhere.

Keywords: *Leptospira*, pig, Vietnam, leptospiral seroprevalence, leptospiral seasonality, reproductive performance, kidney lesions

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Appendix

Papers I – IV

The thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Boqvist, S., Chau, B.L., Gunnarsson, A., Olsson Engvall, E., Vågsholm, I. & Magnusson, U. 2002. Animal- and herd-level risk factors for leptospiral seropositivity among sows in the Mekong delta, Vietnam. *Preventive Veterinary Medicine* 53, 233-245.

II. Boqvist, S., Ho Thi, V.T. & Magnusson, U. Seasonal variation in leptospiral seroprevalence among sows in the Mekong delta in southern Vietnam. Veterinary Microbiology. (submitted for publication).

III. Boqvist, S., Ho Thi, V.T., Vågsholm, I. & Magnusson, U. 2002. The impact of *Leptospira* seropositivity on the reproductive performance in sows in southern Vietnam. Theriogenology. (in press).

IV. Boqvist, S., Montgomery, J.M., Hurst, M., Ho Thi, V.T., Olsson Engvall, E., Gunnarsson, A. & Magnusson, U. *Leptospira* in slaughtered fattening pigs in southern Vietnam: Seropositivity, presence of the bacteria in the kidneys and morphological findings. (submitted for publication).

Introduction

Leptospirosis is caused by any of the pathogenic serovars included within the *Leptospira* genus. It is a bacterial disease that in animals is characterised by reproductive failure, such as abortions, stillbirths and birth of weak offsprings. Other clinical signs that may be seen are fever, jaundice and hemoglobinuria. Following introduction and bacteremia, leptospires can localise in the kidneys where they may persist for long periods and are shed in the urine. This is important in the transmission of *Leptospira* infection (Ellis, 1999). Leptospirosis is also an important zoonosis, which means that it is a disease that is transmitted between animals and humans.

Animal and human leptospirosis is spread worldwide, although it is of most concern in tropical regions. Such areas often present suitable conditions for survival and transmission of the leptospires, such as regular flooding, presence of several animal species that may maintain leptospires, suitable climate for survival of the bacteria in the environment and socio-economic conditions that favour transmission (Faine, 1982). The tropical Mekong delta in the south of Vietnam is such a region. In this area, animal husbandry co-exists with water-rice farming and the former accounts for approximately 25% of total value of agricultural output (Le, 1996). Pig production is considered to be the most important animal industry in the country. This is emphasised by the fact that the pig population has increased from 10 million animals in 1980 to 16 million in 1995 (Hai and Nguyen, 1997). However, diseases are a major constraint in animal husbandry and the control of pig reproductive problems caused by infectious agents, such as *Leptospira*, have been identified as a research area of importance (Chau et al., 1996). Leptospirosis is rarely diagnosed among animals in Vietnam due to limited laboratory facilities, and therefore the incidence, prevalence and economic importance of *Leptospira* infection need to be further investigated (Nguyen, 1996).

This thesis focuses on the epidemiology of *Leptospira* infection among pigs in southern Vietnam. Such data will provide important information in order to reduce infection in tropical regions that are likely to have higher incidence of leptospiral infection in animals and humans compared with temperate regions. Tropical areas usually show suitable conditions for survival and transmission of leptospires in the environment.

Background

Leptospirosis in general

Historical aspects

Weil published the first description of leptospirosis in man in 1886 when he described a disease that caused jaundice and renal failure but, differed from other known infectious diseases. He named the bacterium *Leptospira* ("thin spirals") *icterohaemorrhagiae* (Weil, 1886), although the human form of leptospirosis was given the name Weil's disease. In 1907, Stimson demonstrated the spirochete that caused Weil's disease from a patient having died of "yellow fever" (Stimson, 1907). He proposed the name *Spirochaete interrogans,* as the organism with its hooked ends resembled a question mark. It took almost 30 years after Weil's report until Inada and others isolated the organism in 1914 (Inada et al., 1916). A few years later, Noguchi proposed the new genus *Leptospira* as he saw that this particular organism differed from other spirochetes (Noguchi, 1918). At this time, the rat was identified as a carrier of leptospires (Ido et al., 1917). Leptospirosis in animals was described clinically around 1850, although its etiology was unknown (Torten, 1979). However, leptospirosis was not identified as a disease in domestic animals until the next century, at first primarily in dogs (Uhlenhuth and Fromme, 1919). Leptospirosis was not described as a veterinary problem in food animal production until the late 1930s (Terskich, 1940).

The bacterium

Leptospires are gram-negative tightly helically coiled spirochetes with a length of 6–20μm and a width of 0.1–0.15μm (Fig. 1) (Levett, 2001). They have characteristic hooked ends, which are clearly visible by the spinning motility of the bacterium that is caused by a periplasmic flagellum inserted at each end (Faine, 1982).

Leptospires are obligate aerobes that grow well in semi-solid medium containing 0.2% agar. Growth characteristics for *Leptospira* differ from those of many other bacteria, for example, the optimal temperature for growth in vitro is 28–30°C, even though they are pathogenic for mammals with higher body temperatures (Faine et al., 1999). Another example is the long generation interval of 6–16 hours, requiring 7–10 days for a normal culture to become dense (Prescott and Zuerner, 1993). After this time period, growth in semi-solid media is visualised as one or more layers of heavy growth below the surface of the media.

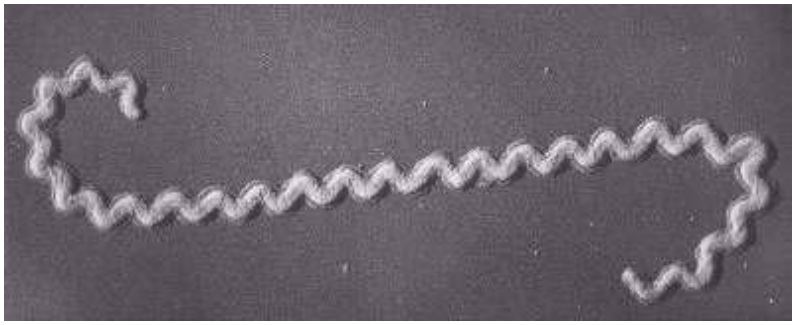

Fig. 1. Electron microscopic illustration of the *Leptospira* bacteria. Image by courtesy of Department of Microbiology, Monash University, Australia. Photomicrograph by Dr Annabella Chang.

Taxonomy and classification

The genus *Leptospira* belongs to the family *Leptospiraceae* in the order *Spirochaetales*. Other genera within the same family are *Leptonema* and *Turneria* (Kaufmann and Weyant, 1995).

During recent years the taxonomy of leptospires has undergone a state of transition from an antigenic to a genetic classification. Previously, the genus *Leptospira* was divided into two species: the pathogenic *L. interrogans* that was found in animals and humans, and the saprophytic *L. biflexa* that was found in the environment. The two species were differentiated by growth characteristics, such as growth of *L. biflexa* at 13°C and in the presence of 8-azaguanine and by the failure of *L. biflexa* to form spherical cells in 1M NaCl. In the antigenic classification, leptospires were classified by serological typing on the basis of agglutination to serovar level, which was the least divisible recognised type. For practical reasons, such as diagnosis and epidemiology, serovars with partially common antigenic structure were grouped together in serogroups (Faine, 1982). There are about 230 recognised pathogenic serovars (Faine et al., 1999) and new ones are being added as discovered. Although the antigenic classification has been replaced by a genetic one it is still accepted for practical reasons that the serovar is the basis of taxonomy at the subspecies level (Ellis, 1995).

The genetic classification is based on DNA-DNA hybridisation, which has revealed considerable heterogeneity in the pathogenic *L. interrogans*. This method has been the basis of the division of leptospires into genomspecies (Brenner et al., 1999; Perolat et al., 1998; Ramadass et al., 1990; Ramadass et al., 1992; Yasuda et al., 1987). The pathogenic genomspecies have been identified as *L. interrogans*, *L. borgpetersenii*, *L. kirschneri*, *L. noguchii*, *L. weilii*, *L. alexanderi*, *L. santarosai*, and, *L. meyeri* (Ellis, 1995). More recently an intermediate group, between the pathogenic and saprophytic groups, has been identified that includes *L. inadai* and *L. fainei* (Letocart et al., 1999). The

saprophytic genomspecies have been identified as *L. wolbachii* and *L. biflexa*, while the former *L. parva* has been placed in the new genus *Turneria* (Levett, 2001). Fortunately, the majority of serologically defined groups correspond with genomspecies, although there are a number of serovars that may be found within different genomspecies.

Pathogenesis and pathology

In animals and in humans, leptospires enter the body through mucous membranes or through small cuts and abrasions in the skin. This results in immediate leptospiremia that lasts up to 7 days and ends with the appearance of specific antibodies. Thus, resistance to infection is mediated by antibodies whose specificity is related to the same agglutinating antigens as those that determine serological specificity. The antibodies attach to the surface of the leptospires and opsonise them for phagocytosis by reticuloendothelial cells (Faine et al., 1999). If a sufficient number of leptospires accumulates in the circulation, tissue damage occurs as a result of cytotoxic effects, which have been reported to be due to direct effect of leptospires on tissue cells and immune complex formation (van den Ingh and Hartman, 1986). This damages the endothelium of small blood vessels, which in turn causes ischemia in organs and subsequent renal tubular necrosis, hepatocellular damage, meningitis, myositis and placentitis. In more severe cases, haemorrhages, jaundice and platelet deficiency occur (Faine, 1982; van den Ingh and Hartman, 1986). It has been reported that pathogenicity may vary between different serovars and even between different strains of the same serovar (Fennestad and Borg-Petersen, 1966; Nagy, 1993). This variation in pathogenicity has been suggested to be one explanation of why low-virulent isolates failed to remain attached to microvilli of proximal renal tubules (Cheville et al., 1980; Nagy, 1993).

In pigs, leptospires invade the foetus during the acute stage of disease, although abortions usually do not occur until 1 to 3 weeks following the death of the foetus (Fennestad and Borg-Petersen, 1966; Hanson, 1982). It has been suggested that the interval between fetal infection and death would be approximately 14 days (Fennestad and Borg-Petersen, 1966). Abortions are usually recorded in the last trimester in pigs and probably result from toxic products that the dead infected foetuses release (Ellis, 1999). It has been reported that birth of weak or dead piglets, due to leptospiral infection, may coincide with birth of normal piglets (Fennestad and Borg-Petersen, 1966; Kemenes, 1984).

Most pathological findings have been recorded after chronic infection and, in pigs, are mainly confined to the kidneys. The leptospires enter the kidneys haematogenously, migrate through vascular endothelium and persist in the interstitial space to finally migrate into the tubular lumina from where they are shed in the urine (Cheville et al., 1980; Marshall, 1976). Lesions in the kidney have been described as multifocal interstitial nephritis, characterised by varying degrees of fibrosis and interstitial cellular infiltration (Baker et al., 1989; Hunter

et al., 1987; Scanziani et al., 1989). Such lesions are often macroscopically visible as grey-white foci of lesions in the renal cortex, so called white-spots

Epidemiology of *Leptospira* infection

Maintenance and incidental hosts

Within the epidemiology of *Leptospira* infection, animals may be classified as maintenance or incidental hosts. Maintenance hosts are animals that carry leptospires in their renal tubules, where the bacteria multiply and are shed in the urine for periods varying from months to more than a year (Ellis, 1999; Hathaway, 1985). These animals are essential as sources of infection for other animals or humans. It has been reported that the infecting serovar may be of lower pathogenicity and may cause chronic rather than acute disease in maintenance hosts compared with incidental hosts. For example, *L. interrogans* serovar (sv) bratislava, *L. interrogans* sv pomona and *L. borgpetersenii* sv tarassovi are maintained by pigs and cause reproductive disturbances in the chronic phase of infection in the same animal species. On the other hand, incidental infections tend to cause acute, severe disease, which is followed by a rapid elimination of leptospires from the kidneys (Prescott and Zuerner, 1993). For example, infection with *L. interrogans* sv icterohaemorrhagiae may cause acute disease in young pigs with symptoms of jaundice, haemoglobinuria and fever (Hathaway, 1985). The same animal species may be the maintenance host for some *Leptospira* serovars and an incidental host for others (Levett, 2001). Furthermore, leptospires have been isolated from most mammals and also from amphibians, arthropods, birds and reptiles (Thiermann, 1984). The extent to which leptospires are transmitted within an animal herd depends on factors such as climate, population density, and degree of contact between maintenance and incidental hosts. Another factor of importance for occurrence of clinical disease is the immune status of the herd, because clinical signs in areas with endemic infection are restricted to a smaller proportion of susceptible, non-immune females (Dial et al., 1992; Ellis, 1999).

Direct and indirect transmission

Among pigs housed together, direct transmission may occur through contaminated urine, although venereal transmission and transmission through infected milk also are important routes of infection (Faine et al., 1999) (Fig. 2). On the other hand, indirect transmission implies that infection is acquired from an environment that is contaminated with leptospires originating from urinary shedding, which may include contaminated effluent, feed, water or soil (Kingscote, 1986; Michna, 1970).

Figure 2. Illustration of direct and indirect transmission of *Leptospira* infection in pigs (illustration by the author).

Climate and ecology

Despite the large number of serovars that have been found there are usually only a small number that are present in a particular region. Most often they are associated with one or more animals that inhabit the area and act as maintenance hosts (Levett, 2001). In contrast, incidental hosts are less important in transmission of infection as they rapidly eliminate leptospires from the kidneys (Prescott and Zuerner, 1993). However, transmission may occur between incidental hosts if the surrounding environment favours survival of leptospires outside the host (Thiermann, 1984). Under optimal conditions, such as a warm and wet environment with neutral to slightly alkaline water, leptospires may survive for weeks in the environment. In tropical areas there are usually a wider variety of *Leptospira* serovars compared with temperate regions and explanations of this are a combination of a suitable climate and generally a large number of animals that may act as maintenance hosts (Faine, 1982).

Epidemiology of Leptospira *infection in a pig herd*

Leptospira infection may be introduced to a herd when new animals that carry leptospires are brought to the farm, through a contaminated environment or through contact with other infected animal species (Hathaway et al., 1983a). The

consequences depend on current leptospiral infectious status in the pig herd, for example, in a herd with waning immunity or in a previously uninfected herd, clinical signs may be seen in all age categories and cause substantial reproductive losses (Ellis, 1999). In subsequent *Leptospira* infection, symptoms are restricted to non-immune gilts or sows introduced from uninfected herds, as previous infection results in immunity (Faine et al., 1999). Pigs that recover from infection acquire a long-lasting serovar specific immunity which prevents subsequent reproductive disturbances against the infecting serovar (Chappel et al., 1992a; Fennestad and Borg-Petersen, 1966; Kemenes and Suveges, 1976).

Prevention and control

In order to reduce infection a combination of measures based on management, treatment and vaccination can be implemented (Ellis, 1999). On farm level, management procedures may, for example, include measures to reduce the rodent population and contacts between domestic and wild animals, to keep different animal species and newly introduced stock separate from the rest of the herd.

To reduce urinary shedding, or eventually eliminate renal leptospirosis, animals may be treated with streptomycin, oxytetracycline, tylosin or erythromycin (Alt and Bolin, 1996). Furthermore, antimicrobial agents in the feed, such as oxytetracycline or chlortetracycline, may reduce clinical signs (but will not eliminate carriers) (Faine et al., 1999)

Vaccination may reduce clinical signs and leptospiruria in a herd, but will not completely eliminate urinary shedding (Ellis et al., 1989; Shibley et al., 1973; Whyte et al., 1982). Experimental studies have shown that immunity to infection lasts around 3 months, although immunity to clinical leptospirosis may last longer (Ellis et al., 1989; Kemenes and Suveges, 1976). These studies also reported that vaccination gives protection against leptospiruria during a limited period of less than six months. As immunity is serovar specific, a vaccine has to include the infecting serovars within the region

***Leptospira* infection in pigs**

Clinical symptoms

Leptospira infection among pigs proceeds most commonly without clinical signs. This subclinical infection is commonly seen in growing pigs which may constitute a health hazard for more susceptible piglets and pregnant sows (Michna, 1970).

Symptoms of anorexia, diarrhoea, jaundice, haemoglobinuria and weakness are seen in the acute stage of leptospirosis (Michna, 1970). This stage of disease coincides with the presence of leptospires in tissues (van den Ingh and Hartman, 1986). It has also been reported that if disease is caused by a strain of low virulence, or if the herd is infected endemically, clinical signs may be mild and overlooked (Ellis, 1999; Ferguson and Powers, 1956; Nagy, 1993).

Reproductive disorders, such as abortions, stillbirths and birth of weak piglets, are associated with the chronic form of leptospirosis (Fennestad and Borg-Petersen, 1966; Hathaway, 1985; Michna, 1970). There are several reports of various serovars that may cause reproductive disturbances, for example *L. interrogans* sv bratislava (Bolin et al., 1991; Bolin and Cassells, 1990; Ellis et al., 1986c; Ellis et al., 1986b; Ellis and Thiermann, 1986), *L. interrogans* sv pomona (Edwards and Daines, 1979; Gummow et al., 1999), *L. borgpetersenii* sv tarassovi (Kemenes, 1984), and *L. interrogans* sv canicola (Paz-Soldan et al., 1991). It has also been reported that *Leptospira* infection, indicated by positive serology or through isolation of the organism, may cause infertility, reduced litter size and reduced farrowing rate (Frantz et al., 1989; Hanson et al., 1971; Hathaway and Little, 1981; Mousing et al., 1995; Neto et al., 1997; Van Til and Dohoo, 1991). Reproductive disturbances may be accompanied by fever, reduced milk production, haemoglobinuria and jaundice (Michna, 1970). However, it has been reported that the reproductive performance in sows and gilts return to normal after clinical leptospirosis with reproductive disturbances (Chappel et al., 1992a; Fennestad and Borg-Petersen, 1966; Kemenes and Suveges, 1976).

Diagnosis

A laboratory diagnosis is necessary as the clinical picture is non-pathognomonic for leptospirosis. Clinical symptoms may be mild and there are other infections that may confuse clinical diagnosis. The laboratory methods used are either serological tests or demonstration of leptospires in clinical and autopsy samples.

Serological tests

The most widely used serologic test is the Microscopic Agglutination Test (MAT). This test is specific for the infecting serovar, although cross-reactions may be recorded against other serovars within the same serogroup. Therefore, local *Leptospira* isolates should be included as antigens in the MAT, or serovars that are known to infect pigs elsewhere (Faine et al., 1999). The MAT has been considered as confirmative for a positive diagnosis in individual animals if a rising titre is recorded in paired samples taken 5-10 days apart or if the initial titre is ≥1:400 (André-Fontaine and Ganière, 1992; Faine et al., 1999; Pritchard et al., 1985). However, the MAT is of limited value in chronic infections as low titres may remain for years after infection in individual animals and, therefore, the test may be considered primarily as a herd test (Ellis, 1999).

Leptospiral antibodies have been shown to appear in the circulation approximately one week after infection and the highest titres have been recorded ten days to three weeks after infection (Farina et al., 1977; Fennestad and Borg-Petersen, 1966; Ferguson and Powers, 1956). However, it has also been shown that titre against infecting serovar was not always detected in serum within two weeks after abortion and that some sows that aborted showed titres below the usually accepted titre 1:100 (Ellis et al., 1986c; Kirkbride and McAdaragh, 1978). Also, the highest titre is not always recorded for the infecting serovar (see section of “Interpretations of titres”) (Faine et al., 1999).

Another serologic test that has been described to be used in pigs is enzyme-linked immuno sorbent assay (ELISA), other tests are less commonly used for testing pigs (Chappel et al., 1992b).

Demonstration of leptospires

Demonstration of leptospires in internal organs or body fluids is confirmative for diagnosis of leptospiral infection. In the acute stage of the disease, organisms can be found in liver, lungs, brain or blood, cerebrospinal-, thoracic-, and peritoneal fluids, and in the chronic stage of infection mainly in kidneys, urine, or in the genital tract (Faine et al., 1999). Different methods to demonstrate leptospires in tissue have been described, such as direct examination by dark-field microscopy and light microscopy after appropriate staining (Faine, 1982), immunofluorescence (Miller et al., 1989; Skilbeck, 1986), immunohistochemistry (Scanziani et al., 1989), culture (Bolin and Cassells, 1990; Ellis and Thiermann, 1986), or the polymerase chain reaction (PCR) (Merien et al., 1992; Wagenaar et al., 2000).

Isolation by culture is effective for epidemiological studies when an infecting serovar needs to be identified, however, it is a time-consuming and fastidious method. Before culture, clinical specimens are homogenised (if tissue), diluted and inoculated in semisolid medium containing antimicrobial agents to reduce contaminating flora, and incubated for up to 26 weeks (Ellis and Thiermann, 1986).

Animal husbandry in the Mekong delta

This study was carried out in the Mekong delta in the southern part of Vietnam. The climate in the region is tropical with a rainy season that lasts from May to October, the rest of the year is dry. Average rainfall during the rainy season is 1400–2000 mm and this, combined with high flow of the Mekong river causes annual flooding of the entire delta region. The warmest months of the year are April–May when temperatures fluctuate between 32–33°C, and the coolest are December–January with temperatures of 23–25°C (Chau et al., 1996).

The Mekong delta is a highly productive region, both in livestock and agricultural production. For example, the region supplies 80% of all exported duck and pork. The pig is considered to be the most important animal, followed by duck, buffalo, chicken and cattle. The production of pigs can be categorised into three major production systems. Large-scale state-owned farms have between 50–600 sows, although they account only for 4–5% of the total production. Second are private commercial pig farms that have between 5–100 sows, 10–500 fatteners and produce around 15% of all pigs. The remaining 80% of the pig production takes place at small-scale family farms with 1–2 sows and less than 10 fatteners (Hai and Nguyen, 1997). Thus, traditional farming practised by rural farmers still comprises the dominant part of pig production in the country.

However, large-scale state farms play an important role in development of pig production. The pig breeds that are used in Vietnam can be classified as high-producing exotic breeds (Yorkshire, Landrace and Duroc), medium-producing breeds, such as improved indigenous breeds (for example, Ba Xuyen and Thuoc Nhieu breeds), that are also crossed with exotic breeds, and, finally, low-producing indigenous breeds (for example, Mong Cai and I breeds) (Thong et al., 1996).

Aims

The present study deals with aspects of leptospiral infection among pigs in the Mekong delta in southern Vietnam. The aims of the study were:

- To assess leptospiral seroprevalences among sows on small- and large-scale farms and differences in seroprevalence between the two farming systems.
- To identify risk factors for leptospiral seropositivity on animal- and herd level among sows on small- and large-scale farms.
- To describe seasonal variation in leptospiral seroprevalence among sows.
- To assess the impact of leptospiral seropositivity on the reproductive performance among sows.
- To demonstrate leptospires and signs of *Leptospira* infection in slaughtered pigs.

Comments on materials and methods

Detailed descriptions of materials and methods used are given separately in each paper (I-IV). In the following sections, the leptospires are mentioned only by their serovar name. For details on species and strains see Table 1.

Selection of farms and animals

In Papers I, II and III, sows from small-scale farms (i.e. small-scale family farms and private commercial farms with less than 20 fatteners) and large-scale state farms were included for sampling for serological screening. The reason for only including sows was that it is possible to record the most common clinical signs of *Leptospira* infection, i.e. reproductive disturbances, which in turn are of utmost importance for pig production. All included animals could be traced back to the village from which they originated or from which large-scale state farm.

Paper I included analyses at animal- and herd level. The epidemiological unit at animal level was the sow and at herd level the small-scale farm. At animal level, sows from both small-scale farms (n=283) and large-scale farms (n=141) were included in order to enable comparisons to be made in leptospiral seroprevalences between the two farm systems. A total number of 424 blood sera were collected. In this analysis, all sows were sampled at each small-scale family farm while the number of sampled sows from each large-scale state farm and larger private farm depended on the size of the herd. At herd level, only small-scale farms (n=151) were included as these farms varied in management and each of them had a few sows, whereas there were only a few large-scale farms (n=7) that varied little in management. All villages and large-scale farms included were chosen because they previously had been visited by the Can Tho University. However, they did not differ from other farms in the region.

In Paper III, only large-scale farms (n=4) were included as these farms kept good reproductive records of their sows. The animals included consisted of lactating or pregnant sows (n=339) in order to ensure that they were reproductively active. The same sows were also used in Paper II and additional ones from one other large-scale farm (n=429). The farms included were visited during the mid-dry season in March, the rainy season in August and the early-dry season in December in order to collect blood samples and data. On each sampling occasion, a certain number of sows from each reproductive stage were included. Each sow was only included once. An advantage with including only large-scale farms was that few persons, usually the manager of the farm, were involved with collecting information, as this needed to be done between the farm visits. Also, collection of the data was facilitated compared with a situation if several small-scale farms had been included.

Animals included in Paper IV were fattening pigs originating from small-scale farms that were slaughtered at the abattoir in the city of Can Tho. From these animals, blood samples were collected (n=143) and their kidneys were examined morphologically and some were collected (n=32). Most fatteners could be traced back to the village which they originated, although in some cases only the province was known.

Serology

Selection of serovars

In Paper I, the collected sera were tested against a panel of 13 live *Leptospira* antigens by use of the MAT (Table 1). These serovars were selected as they were known to cause reproductive disturbances in pigs and/or had been screened for in previous *Leptospira* surveys among pigs in southern Vietnam (Kitaoka et al., 1977; Spinu et al., 1963; Welsh et al., 1972).

Table 1. *Leptospira* serovars used in the Microscopic Agglutination Test (MAT) to investigate leptospiral seroprevalences among sows in southern Vietnam (Paper I)

Species and serovar (sv)	Strain
L. interrogans sv australis	Ballico
L. interrogans sv autumnalis	Akiyama A
L. interrogans sv bataviae	Swart
L. interrogans sv bratislava	Jez
L. interrogans sv canicola	Hond Utrecht IV
L. kirschneri sv grippotyphosa	Duyster
L. interrogans sv hebdomadis	Hebdomadis
L. interrogans sv icterohaemorrhagiae	Kantorowicz
L. borgpetersenii sv javanica	Veldrat Batavia 46
L. interrogans sv pomona	Pomona
L. interrogans sv pyrogenes	Salinem
L. borgpetersenii sv sejroe	M 84
L. borgpetersenii sv tarassovi	Perepelitsin

Out of these 13 serovars, six were included in Papers II and III; they were the serovars autumnalis, bratislava, grippotyphosa, icterohaemorrhagiae, pomona and tarassovi. The serovars bratislava, pomona and tarassovi were included as they have been reported to cause reproductive disturbances in pigs (Ellis, 1999; Hathaway, 1985). Serovar bratislava was also included as it showed a high seroprevalence in Paper I. Furthermore, sv grippotyphosa and sv icterohaemorrhagiae were included as they have been shown to cause clinical disease in pigs (Hanson et al., 1971; Hathaway, 1985). These two serovars have also been reported to be maintained by rodents (Faine et al., 1999; Ido et al., 1917), which were frequently occurring and a sanitation problem according to local farmers and veterinarians. Finally, sv autumnalis was included as it showed a high seroprevalence in Paper I, although there are few reports that have shown any clinical importance of this serovar in pigs. In contrast, there are numerous reports that have shown lack of association between sv autumnalis and impaired reproductive performance in sows (Hathaway, 1985; Inzana and Dawe, 1979; Van Til and Dohoo, 1991). In Paper IV, the same serovars that were used in Papers II and III were included, except for sv autumnalis for the reason mentioned above.

Interpretations of titres

Interpretations of single MAT titres may be less valuable for diagnostic purposes as low titres may remain for long periods after infection (Ellis, 1999). However, in this study single samples were analysed, as the aim was to investigate seroprevalences for different serovars and not to diagnose clinical disease in individual animals. To reduce the risk for subjective variations in reading of the MAT a single person did this (the author).

Table 2. Serological reactions in the Microscopic Agglutination Test (MAT) between antisera and *Leptospira* serovars included in Paper I

	antisera												
antigen	australis	autumnalis	bataviae	bratislava	canicola	grippotyphosa	hebdomadis	icterohaemorrhagiae	javanica	pomona	pyrogenes	sejroe	tarassovi
australis	1:25600			1:100									
autumnalis	1:100	1:51200		1:100		1:100				1:100			
bataviae		1:100	1:25600							1:100			1:100
bratislava	1:6400	1:400		1:25600	1:400		1:200	1:200				1:100	1:100
canicola					1:51200			1:100			1:100		
grippotyphosa		1:100				1:25600							
hebdomadis							1:25600					1:400	
icterohaemorrhagiae		1:400		1:200	1:1600		1:100	1:12800	1:100				
javanica				1:100	1:100				1:25600				
pomona	1:100	1:400		1:100						1:25600			
psyrogenes					1:100					1:100	1:12800		
sejroe							1:400					1:25600	
tarassovi					1:100								1:25600

The MAT is not strictly serovar-specific and may give rise to positive reactions to other serovars within the same serogroup, or even to serovars within other serogroups (Faine, 1982). Also, in humans it has been recorded that during the second or the third week of disease cross-reacting antibodies may appear prior to the appearance of antibodies to the infecting serovar (Faine et al., 1999). In the present study, cross-reactions between antisera and antigens were assessed before screening of collected sera. It was found that cross-reactions mostly were titres 1:100 and 1:200, which were low compared with titres recorded for the specific *Leptospira* serovars (Table 2). However, a high degree of cross-reactivity was recorded between sv bratislava and antisera against sv australis. The explanation of this is likely that these two serovars belong to the same serogroup and share common antigenic structures. As most cross-reactions were of lower titres, they were considered to be possible to adjust for in order to avoid overestimation of seroprevalences and to improve interpretation of the data. This was done according to a method described by Hathaway and Little (1981). By means of this method, titres two or more steps below the highest recorded titre were considered as cross-reactions and were excluded from further analyses. Thus, the highest recorded titre, and titres one step below the highest recorded, were considered as a result of leptospiral infection. It can be suggested that some true titres also were excluded using this method, although, it was considered that the reliability of the data increased. Interpretation of multiple titres within the same sera is further complicated as it has been shown that different serovars provoke different levels of titres due to differences in antigenicity. For example, it has been reported that sv bratislava may induce lower titres compared with sv pomona (Prescott and Zuerner, 1993). In Paper I, this was taken into consideration and two analyses were performed; one when potential cross-reactions of sv bratislava titres were eliminated and one when all sv bratislava titres remained in the data set.

Study variables

All variables studied were chosen as they were considered to represent information of importance for Papers I, III and IV, based on information from previous studies (see below). In Paper I, variables represented possible risk factors for seropositivity, in Paper III, they represented reproductive parameters that may be influenced by seropositivity, and in Paper IV, macroscopic renal lesions that may be caused by *Leptospira* infection. In Paper I and IV, categorical data were collected, whereas the data in Papers III were categorical and continuous.

In Paper I, information on possible risk factors at animal and herd level was collected. These variables can be divided further into the categories ‘management and housing’, ‘sow related’ and ‘environmental related’. In the category ‘management and housing’ possible risk factors were included at animal and herd level. At animal level, the variables included were if the sow had been introduced to the farm or born there, which might provide information about infectious status on the study farm, and if the sow had direct contact with other sows in the herd,

which also has been considered as a risk factor (Michna, 1970). In this category, other variables at herd level were if a natural breeding regimen was used, as leptospires can be spread venereally (Ellis et al., 1985), and if the walls of the pens were open, i.e. the pigs were able to have contact with the surrounding environment, which also has been considered as an increased risk of exposure of *Leptospira* (Michna, 1970). Furthermore, there were a few variables that were excluded because of lack of variations in the answers. Those excluded variables were if the sows at the farm were free-ranged, as it was shown that the vast majority of pigs were kept in pens, and use of fodder supplemented with antibiotics, as this was not done regularly at any of the farms.

Variables in the category 'sow related' also included possible risk factors at animal and herd level. At animal level, the number of parities and age of the sow, were included, as it has been reported that susceptibility for *Leptospira* infection may vary with age and previous exposure due to immunity (Ellis, 1999). It was found that these variables were correlated and the number of parities was later excluded, as it was believed that age contained the more useful information. At herd level, risk factors included were the number of sows in the herd, as it previously has been shown that herd size may influence *Leptospira* seropositivity (Mousing et al., 1995), and temperate pig breed in the herd, as it has been reported that immune status may vary between different breeds (Nguyen et al., 1998). Another risk factor included was if there had been previous reported reproductive failure in the herd during the last year, as a measure of reproductive health. There were also a few variables that were excluded. One of them was the number of pigs in the herd, as this was correlated with the number of sows in the herd and it was believed that the latter contained the more useful information. Other variables were excluded as they showed little variation in the answers, such as vaccination against *Leptospira* infection and against other diseases, since no sows were vaccinated against *Leptospira*, but the majority were vaccinated against other diseases, e.g. against foot-and-mouth disease, hog cholera, *E. coli* infection, pasteurellosis and salmonellosis.

Possible risk factors in the category 'environmental related' were identified at herd level. They included use of flowing water sources for drinking water and cleaning of pens, as it has been reported that contaminated water might be a source of infection (Faine, 1982; Gummow et al., 1999; Kingscote, 1986), and home-produced fodder, as this usually was kept on the open ground and might become contaminated with leptospires from animal urine (Michna, 1970). Other possible risk factors were the presence of other domestic animals on the farm that could be infected and then constitute sources of infection, and no rodent control, as it has been reported that rodents maintain various *Leptospira* serovars (Faine, 1982; Ido et al., 1917).

In Paper III, information that was collected about the sampled sows included identification number, age, breed, number of parities and whether the sow was sampled during gestation or lactation, as these variables may influence

reproductive performance (Dial et al., 1992). Number of parities was later excluded as it showed correlation with age, which was considered to contain the more useful information. The variable farm was also included, as the reproductive performance of the sows may vary between farm, and season, as this has been reported to influence reproduction (Dial et al., 1992; Pritchard et al., 1985; Tummaruk et al., 2000), even in tropical areas (Tantasuparuk et al., 2000). The reproductive data that were collected were considered to be robust and possible to record in a reliable way. Also, these variables have previously been reported to be affected negatively by leptospiral infection, indicated by positive serology and/or identification of infecting serovar. The variables included were number of days from weaning to service (WSI) (Mousing et al., 1995), abortions (Ellis et al., 1986b; Friis et al., 2000; Gummow et al., 1999; Hathaway et al., 1982; Kemenes and Suveges, 1976), mummified foetuses (Farina et al., 1977; Hathaway et al., 1983b), number of piglets born, number of piglets born dead (Bolin et al., 1991; Hanson et al., 1971; Saravi et al., 1989), and number of piglets born weak (Bolin and Cassells, 1990; Hanson et al., 1971; Kemenes, 1984; Kingscote, 1986; Neto et al., 1997). In this study the number of piglets born weak is defined as number of piglets that died within the first 24 hours after birth. The variables abortions and mummified foetuses were excluded because there was too little variation in the answers.

In Paper IV, data were collected about macroscopical lesions on examined kidneys, as these may be caused by *Leptospira* infection (Baker et al., 1989; Chappel et al., 1992b), and from which village and province the pigs originated.

Questionnaires

Information in Papers I, III and IV was collected by means of questionnaires that were performed at the study visits. However, a part of the questionnaire in Paper III was left to the manager of the farm to be filled in by him/herself and collected at the following visit or by courier.

The questionnaires were designed to collect reliable and relevant information about the variables presented in the section above. However, it cannot be excluded that the answers to some questions may have biased the results because of the risk of misunderstandings and entering of wrong answers in the questionnaire. This could have been a source of misclassification bias. However, this possible misclassification was thought to be non-differential rather than differential, i.e. the misclassification was uncorrelated to certain alternative of answers (Thrusfield, 1995). In this case, a non-differential misclassification would bias the results towards the null hypothesis, but not invalidate the findings in the study. However, in order to reduce the risk of misclassification the questionnaires were translated into Vietnamese. Also, personnel speaking Vietnamese from Can Tho University performed the completions of the questionnaires, supported by the author in order to avoid misunderstanding and misclassifications. In Paper I, two questionnaires were used, one that collected

information about the sampled sows and the other about management factors on herd level. In Paper III, one part of the questionnaire was used to collect information on sampled sows and the other part was left to the farmer to collect information on the reproductive performance of the same sows. In Paper IV, a single questionnaire was used to collect data on examined kidneys from slaughtered fatteners.

Closed questions were used in Papers I and IV, i.e. questions that have a fixed number of options of answers. The advantages of using closed questions are that the questionnaires are easier to conduct and to analyse compared with open questions. A disadvantage may be that information of importance may be missed (Thrusfield, 1995). In Paper III, both closed and open questions were used.

Statistical analyses

Statistical analyses were conducted using SAS software (SAS Institute Inc, 1989). The chi-square test was used to study differences in seroprevalences between the two farm types (Paper I) and between seasons (Paper II). Also, this test was used to investigate associations between seropositivity and macroscopical kidney lesions, and between presence of leptospires and morphological kidney lesions (Paper IV). The chi-square test is used to measure associations between samples (Thrusfield, 1995). Furthermore, the Spearman rank correlation test was used in Papers I and III in order to estimate the correlation between age and number of parities (SAS Institute Inc., 1989a), as those variables were suspected to be associated. Also in Paper I, the Wilcoxon rank sum test was used to compare number of litters per sow between the two farm systems, as this test is used to compare groups of observations (Thrusfield, 1995).

In Paper I, two datasets were created, one at animal level and the other at herd level. The outcome at herd level was the number of *Leptospira* seropositive sows in relation to total number of sows per farm (SAS Institute Inc., 1989b). To investigate the relationships between the possible risk factors and *Leptospira* seropositivity, the logistic regression was used in the group generalised linear models (GLIM). This method may be used when the relationship between binary, or ordinal, response variables and explanatory variables is studied (Hosmer and Lemeshow, 1989). As many sows were sampled from each farm, possible overdispersion was investigated by using Williams' method. Overdispersion may occur when there is dependence among the recorded outcomes, for example diseases that cluster by herd (McDermott et al., 1994). Overdispersion was found for sv bratislava and was corrected for by using Williams' method, which may be used when there are unequal sample sizes or when the outcome is events/trial, as in this case (SAS Institute Inc., 1989b; Williams, 1982). For each dataset one model was created for each serovar analysed, i.e. 26 models in total.

In Paper III, normality of data distribution was assessed using normal probability plot and box plot. It was found that the dependent variables WSI,

number of piglets born weak and number of piglets born dead were improved by log transformation. To investigate associations between reproductive performance and leptospiral seropositivity, the general linear model was used (Afifi and Clark, 1984). In this approach, the method of least square to fit the linear models is used. Furthermore, least square means were compared with Student's t-test. One model was built for each serovar for each dependent variable, i.e. four models for each serovar. The variables farm, season and age were considered to possibly affect reproductive performance and were, therefore, included as potential confounders (Thrusfield, 1995). Apart from *Leptospira* seropositivity and the potential confounders, the selection of variables was carried out by backward selection, i.e. the variable with the highest *P*-value in the model was removed and the model was re-run until remaining variables showed significance ($P \leq 0.10$).

Results and discussion

Leptospiral seroprevalence

In Paper I, 73% (95% confidence interval (CI) 69%–78%) of all sera showed leptospiral titres ≥1:100 and of those 40% had titres ≥1:400 (CI 35%–45%). The highest seroprevalence was recorded for sv bratislava (52%; CI 47%–56%), followed by sv autumnalis (14%; CI 11%–17%) (Fig. 2). Although the highest seroprevalence was recorded for sv bratislava, most titres were in the lower ranges of 1:100 and 1:200. The highest recorded titre for sv bratislava was 1:6400, whereas it was 1:51200 for sv pomona. This is consistent with previous reports that have shown that infection with sv bratislava may cause low titres (Ellis et al., 1986c; Prescott and Zuerner, 1993), while sv pomona usually provoke relatively high titres (Faine et al., 1999; Midwinter et al., 1990). Thus, comparisons of titres between different serovars may be of limited value. High seroprevalences against sv bratislava have been reported in previous studies in temperate areas (Hathaway and Little, 1981; Miller et al., 1990). However, there are few reports of the occurrence of sv bratislava in tropical regions and this serovar has not previously been used in serological studies among pigs in the Mekong delta (Phan, pers. comm., 1999). The high seroprevalence recorded for sv bratislava may indicate that pigs are maintenance hosts for this serovar in the Mekong delta.

In Paper I, one-third (n=99) of the seropositive sera (n=311) showed MAT titres against two or more serovars, despite cross-reactions being corrected for. It may be speculated whether multiple titres in single sera are due to multiple infection or to cross-reactions that are not excluded by the method used (Hathaway and Little, 1981). However, it can not always be assumed, especially early in infection, that the highest recorded titre is provoked by the infecting serovar (Faine et al., 1999).

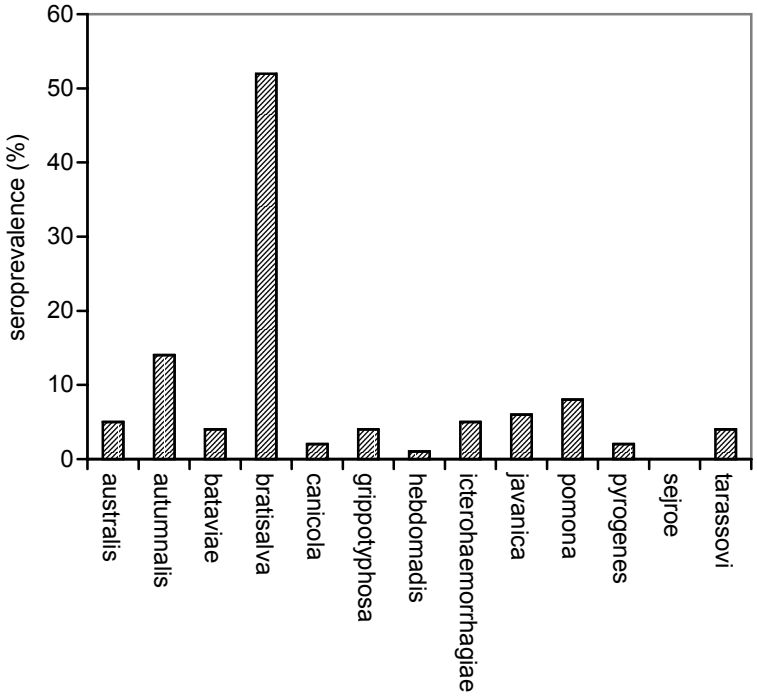

Fig. 2. *Leptospira* seroprevalences, obtained by the Microscopic Agglutination Test, among sows in the Mekong delta, Vietnam. The highest seroprevalence was recorded for sv bratislava.

The overall seroprevalence was found to be higher (P=0.001) on small-scale farms (78%; CI 73%–83%) compared with large-scale farms (64%; CI 54%–74%). This difference was attributed to leptospiral titres ≥1:400, as these titres were more common among sows on small-scale farms (35%; CI 29%–41%) compared with large-scale farms (18%; CI 12%–25%). On the other hand, there was a little difference in seroprevalence between farming systems for lower titres 1:100 to 1:200. This division of titres 1:100–1:200 and ≥1:400 has been considered to differentiate between acute and chronic *Leptospira* infection by numerous reports (André-Fontaine and Ganière, 1992; Faine et al., 1999; Pritchard et al., 1985). Thus, considering management and housing of pigs in the Mekong delta, it is likely that acute *Leptospira* infections are more common on small-scale farms compared with large-scale farms. Explanations of this may be that it is more commonly observed that pigs on small-scale farms are in close contact with the surrounding environment, and that there are more animal species that may act as carriers on these farms compared with large-scale farms.

It was also found that the seroprevalences for sv icterohaemorrhagiae and sv pomona were higher (P=0.04 and P=0.02, respectively) on small-scale farms compared with large-scale farms (Fig. 3). It may be speculated if these differences

are due to management procedures that allow closer contact with the surrounding environment and more frequent contacts with carrier animals on small-scale farms than on large-scale farms.

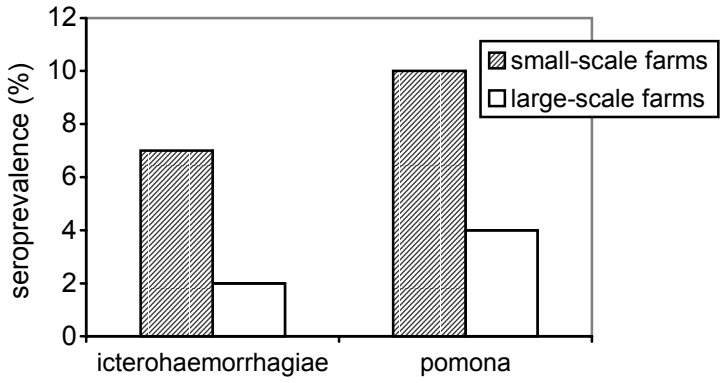

Fig. 3. Differences in leptospiral seroprevalences, obtained by the Microscopic Agglutination Test, for sv icterohaemorrhagiae (*P*=0.04) and sv pomona (*P*=0.02) among sows on small- and large-scale farms, respectively, in the Mekong delta, Vietnam.

Risk factors for seropositivity

At animal level in Paper I, it was found that that sows that had direct contact with other sows were less likely to be seropositive to sv australis (OR=0.3; 95% CI 0.1–0.9) and sv autumnalis (OR=0.4; CI 0.2–0.8). However, the opposite, i.e., direct contact between individuals, has been described to be an important route of transmission of *Leptospira* infection (Faine, 1982; Michna, 1970). Possible explanations of the findings in this study may be that sows in the Mekong delta are exposed to various routes of transmission, apart from direct contact with neighbouring sows, such as contact with contaminated urine from other animal species, open drainage system and use of contaminated utensils. Another explanation may be that sows on large-scale farms often are mixed in different groups over the year. Furthermore, sows that were younger were less likely to be seropositive for sv bratislava (OR=0.1; CI 0.01–0.8) compared with older sows. Reasons for this may be that older sows might have been exposed to leptospires for long periods and that low titres may remain from previous infections (Ellis, 1999). Finally, at animal level it was found that seropositivity for sv icterohaemorrhagiae was associated with sows that had been introduced to the farms as gilts, opposed to having been born on the farms (OR=5.8; CI 1.3–23). It may be suggested that this result reflect a difference in infectious status on different farms.

At herd level in Paper I, it was found that sv javanica was associated with farms that did not take measures to reduce the rodent population (OR=7.8; CI 1.4–140). In contrast, the opposite was found for sv pomona (OR=0.4; CI 0.2–1.0). As farmers in the study area considered rodents to be a problem, it is suggested that both associations may express problems with rodents, either they are considered as a problem and measures to reduce the number of rodents have already been implemented, or no measures have yet been taken. In general, rodents have been reported to be carriers of *Leptospira* infection (Faine, 1982; Ido et al., 1917; Michna, 1970). Finally, it was found that sv pomona was associated with the use of artificial insemination (AI) rather than use of a natural breeding regimen (OR=11.3; CI 1.6–33). Leptospires have previously been isolated from the genital tract of boars, which may result in venereal transmission (Ellis et al., 1986a; Faine et al., 1999). However, to thoroughly evaluate the importance of AI and natural breeding, as means of transmitting *Leptospira* infection, the individual boars should have been included. Unfortunately, no information was available about the specific boars that were used.

As it has been reported that sv bratislava may provoke low titres, despite recent infections (Ellis et al., 1986c; Prescott and Zuerner, 1993), and as the recorded seroprevalence for this serovar was high, a data set was created for sv bratislava without elimination of potential cross-reactions. Apart from the results presented here, it was also found that seropositivity was associated with the use of home-produced fodder, rather than use of commercially produced fodder (OR=3.3; CI 1.2–9.1). An explanation of this may be that home-produced fodder might have been contaminated with infected urine from rodents or other pigs. In this analysis, it was also found that use of flowing water for drinking and cleaning the pens, as opposed to water from a well or tap (OR=2.0; CI 1.1–3.7), constituted a risk factor. Possibly, open water may have been contaminated with *Leptospira* bacteria from the surrounding environment or from other animals.

Seasonal variation

In Paper II, higher seroprevalences were recorded during the mid dry season in March compared with the wet season in August for sv bratislava and sv icterohaemorrhagiae (P=0.07 and P<0.001, respectively) and the early dry season in December (P=0.07 and P<0.001, respectively) (Fig. 4). An earlier report has shown that leptospiral seroprevalences in bovines in a subtropical area were uncorrelated with rainfall (Elder et al., 1986). On the other hand, there have been numerous reports of human cases of leptospirosis associated with a wet environment (Douglin et al., 1997; Levett, 2001; Tangkanakul et al., 2000; Trejevo et al., 1998). It may be suggested that even in regions with a climate that favours survival and transmission of leptospires there are seasonal variations in leptospiral seroprevalences. Explanations of why seroprevalences for some serovars did not show variation over the year may be that rivers and creeks do not dry out completely.

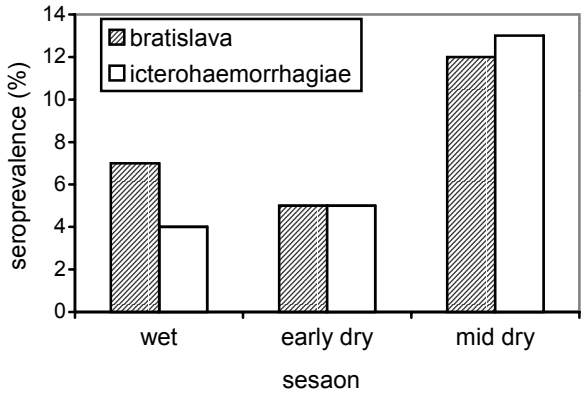

Fig. 4. Seasonal variations in leptospiral seroprevalences, obtained by the Microscopic Agglutination Test, among sows in the Mekong delta, Vietnam. Higher seroprevalences ($P \leq 0.07$) were recorded during the mid-dry season compared with the other seasons.

Reproductive impact of leptospiral seropositivity

Results from Paper III showed that, on average, ten piglets were born per litter, and of those, on average, one was born dead and one out of ten born as weak (defined as the number of piglets that died within the first 24 hours). The majority of the sows had a WSI up to seven days, which is considered normal (Dial et al., 1992). Similar reproductive results have been shown in Thailand, where the climate is similar to that in Vietnam (Tantasuparuk et al., 2000).

In Paper III it was shown that sows that were seropositive against sv grippotyphosa had a one day longer (P=0.06) WSI compared with seronegative sows (8.6 and 7.7 days, respectively). This serovar has previously been isolated from clinical cases of abortions (Hanson et al., 1971; Miller et al., 1990). However, as leptospirosis is endemic in the study area it may be suggested that sv grippotyphosa causes less obvious clinical signs of reproductive disturbances, such as prolonged WSI.

Furthermore, it was found that seropositivity against sv tarassovi resulted in one more piglet born dead per litter (P=0.06) compared with seronegative sows (2.4 and 1.6 piglets, respectively). This serovar is reported to be maintained by pigs, although little information is available on the epidemiology of this serovar (Ellis, 1999). However, a possible explanation of why this serovar showed less obvious clinical signs may be that pigs may act as maintenance hosts for this serovar in the Mekong delta. Furthermore, it has previously been reported that sv tarassovi has been isolated from pigs (Davos, 1977; Hathaway, 1985; Zamora et al., 1988), and experimental infection has been shown to cause abortions (Kemenes, 1984).

In this study, few abortions were recorded among the included sows and it is suggested that sows in the Mekong delta may have developed partial immunity, preventing reproductive disturbances, as management and environmental conditions favour exposure of leptospires in the area. Taken together, these data suggest that clinical signs following *Leptospira* infection in endemic areas may cause less dramatic signs of leptospirosis, compared with regions with sporadical outbreaks.

Leptospires in slaughtered pigs

In Paper IV it was found that the highest seroprevalence among fatteners sampled at an abattoir was recorded for sv bratislava (22%; CI 15%–29%). This is in agreement with earlier results that have been recorded from sows in the same area (Paper I). In Paper IV, macroscopical changes, characteristic of white spots, were recorded in 22% of all kidneys examined, ranging from few, small foci (1–3mm) to large, generalised foci (>3mm). However, no association could be demonstrated between macroscopical findings and leptospiral seropositivity. Thus it seems that serology is not useful to diagnose renal carriers in areas with high leptospiral seroprevalences.

Using a conjugate that reacted against a large number of serovars, leptospires were demonstrated by direct immunofluorescence in 22 of the 32 kidneys examined (69%) (Paper IV). Thus, it can be suggested that pigs are common renal carriers of leptospires in the Mekong delta, even if most kidneys examined were selected according to presence of renal white spots. From one of the sampled kidneys, *L. interrogans* sv bratislava was isolated, which supports previous suggestions that sv bratislava is an infecting serovar among pigs in study area (Paper I).

It has been reported that presence of leptospires in kidneys was associated with renal gross lesions (Baker et al., 1989; Hunter et al., 1987). However, no association could be demonstrated between white spots and presence of leptospires in the kidneys in Paper IV. This is in accordance with previous reports that have shown that white spots not always were present despite presence of leptospires in the kidneys (Chappel et al., 1992b; Jones et al., 1987). One explanation of this may be that the kidneys might have been sampled early in infection before the appearance of white spots (Michna and Campbell, 1969). On the other hand, it has also been suggested that white spots may indicate past infection (Jones et al., 1987). It should also be mentioned that there are other bacteria, than *Leptospira*, that can cause gross renal lesions (Jeffcott et al., 1967; Larsen and Tondering, 1954; Weidlich, 1954), although the lesions described in those studies differed from the ones described in Paper IV. In this study, 24 (75%) of the 32 examined kidneys showed multifocal interstitial nephritis of varying degree. These changes have previously been described in *Leptospira* infected kidneys (Cheville et al., 1980; Scanziani et al., 1989). However, Scanziani et al. (1989) found that leptospires were not always demonstrated in kidneys showing

interstitial nephritis. This agrees with findings in Paper IV, in which no association was found between microscopical lesions and presence of leptospires in the kidneys.

Taken together, as leptospires were demonstrated in a large number of examined kidneys with morphological lesions it may be suggested that *Leptospira* infection is common among fattening pigs in the region. Also, considering the management, housing and slaughter process of the pigs, it may be suggested that they may be considered as health hazards for abattoir workers and meat inspectors.

Concluding remarks and perspectives

- Overall leptospiral seroprevalences were found to be high among pigs in the Mekong delta and the highest seroprevalence was recorded for sv bratislava. Further, it was found that seroprevalences were higher on small-scale farms than on large-scale farms. It is suggested that management and housing that allow close contact with surrounding environment and other pigs are more affected by leptospiral infections. In agreement with findings in sows, high leptospiral seroprevalences were also recorded among fatteners.

- Few risk factors were identified for leptospiral seropositivity. In tropical regions, the close contact between domestic and wild animal carriers and *Leptospira* contaminated water and soil are likely to be more important than single risk factors.

- Higher leptospiral seroprevalences were recorded during the mid-dry season for some serovars, compared with the rainy and early-dry season, respectively. For other serovars, seasonality seems to be less in areas where leptospires may survive in the environment regardless of season, such as the tropical Mekong delta.

- Also in this area of high leptospiral seroprevalences, seropositivity was found to be associated with impaired reproductive performance. However, only weaning to service interval and number of piglets born dead per litter were affected.

- About one-fourth of kidneys macroscopically examined at ordinary slaughter showed gross-lesions typical for leptospirosis. Also, leptospires were detected in the majority of kidneys examined, of which some showed histological changes typical for *Leptospira*. It is suggested that it is common that pigs in the region carry leptospires in the kidneys, which constitutes a health hazard for man.

In this thesis, *Leptospira* infection among pigs in southern Vietnam was studied, with aspects on epidemiology, clinical affection and bacteriology, and new knowledge was provided. This may be used to implement measures in order to reduce infection in the region. For example, by reducing the contact between different groups of pigs, to keep newly introduced animals separate from the rest of the herd and to create a less favourable environment for rodents.

Further studies about leptospiral infection in the Mekong delta may be to assess the economic importance of *Leptospira* infection in pig herds and to investigate if vaccinations against *Leptospira* in pigs would improve the reproductive performance. Also, it would be of interest to investigate other animal species that may act as reservoirs for infection and be sources of infection for animals and humans.

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Svensk sammanfattning

Leptospiros är en sjukdom som kan orsakas av olika bakterier inom *Leptospira* familjen. De mest typiska symptomen hos djur är reproduktionsstörningar, tex aborter, födsel av svaga eller döda kultingar och infertilitet. Under en infektion lokaliseras bakterien i njurarna där de kan förekomma under len längre tid och sporadiskt utsöndras i urinen. Detta är viktigt för överföring av infektionen mellan individer eftersom sjukdomen sprids via infekterad urin. Sjukdomen är också en zoonos, dvs den kan överföras mellan djur och människa. Leptospiros är spritt över hela världen, men är av störst betydelse i tropiska regioner där djurhållningen och klimatet ofta främjar smittspridning mellan individer samt, överlevnad av bakterien i den omgivande miljön. Det tropiska Mekongdeltat i södra Vietnam är ett exempel på ett sådant område. I Mekongdeltat är gris det viktigaste av lantbrukets djur och sjukdomar som orsakar reproduktions-störningar, tex leptospiros, innebär försämrad ekonomisk avkastning och även lidande för djuren. I Vietnam diagnostiseras leptospiros sällan inom veterinärmedicinen pga begränsade laborationsmöjligheter och det finns lite information om epidemiologin (dvs förekomst av sjukdomen, smittvägar och praktiskt betydelse) kring leptospiros i Mekongdeltat.

Syftet med avhandlingen är att undersöka leptospiros hos grisar i Mekongdeltat ur ett epidemiologiskt, kliniskt och bakteriologiskt perspektiv. Sådan information är av betydelse för att man på sikt ska kunna genomföra åtgärder för att minska risken för infektion hos djur och människa.

Fyra delstudier ingår i avhandlingen. I den första undersöktes blodprov från suggor, i vilka förekomster av antikroppar mot ett flertal *Leptospira* varianter (serovarer) analyserades. Både djur från småskaliga familjegårdar och från storskaliga statsägda gårdar inkluderades, detta i syfte att kunna jämföra andel testpositiva suggor på de båda gårdssystemen. Resultaten visade att en övervägande majoritet av suggorna hade exponerats för bakterien och att det var fler grisar på de mindre privata gårdarna som var testpositiva än på de större statligt ägda. Det senare kan tyda på att det är en viss skillnad mellan de två gårdssystemen i fråga om skötsel och inhysning, tex var de större gårdarna ofta mer avskärmade från omgivningen än de mindre och generellt sett hade de större gårdarna även bättre hygien hos grisarna jämfört med de mindre gårdar. Detta är exempel på faktorer som kan ha betydelse för exponering av bakterien. I samma studie undersöktes även om det fanns speciella riskfaktorer som kunde sammankopplas med att suggor testades positiva, tex skillnader i skötsel, typ av foder och boxsystem. Det visade sig att få riskfaktorer direkt kunde sammankopplas med testpositiva djur. Detta kan i sin tur tyda på att bakterien är så vitt spridd i miljön att det finns färre riskfaktorer i Mekongdeltat jämfört med områden där grisarna exponeras mindre av bakterier i den yttre miljön.

I den andra studien undersöktes om andelen suggor som testades positiva för visa serovarer varierade mellan regnperiod, tidig torrperiod och mitt-torrperiod, eftersom kliniska utbrott av leptospiros ofta är sammankopplat med perioder med mycket regn. Tvärtemot detta visade resultaten att det var fler suggor som testade positiva mot vissa serovarer under torrperiod jämfört med regnperiod. Detta tyder troligtvis på att tropiska områden som är vattenrika året runt, tex Mekongdeltat, visar mindre säsongsbundenhet i *Leptospira* infektioner än exempelvis tempererade områden, vilket leder till att det är en jämn risk för infektion året runt i Mekongdeltat.

Huruvida testpositiva suggor hade sämre reproduktionsförmåga än testnegativa suggor undersöktes i den tredje studien. Blodprov undersöktes återigen på förekomst av *Leptospira* antikroppar och resultaten av detta analyserades mot insamlad information om suggornas reproduktionsförmåga, tex kullstorlek, aborter, födsel av svaga eller döda kultingar och infertilitet. Resultatet visade att *Leptospira* positiva suggor fick fler dödfödda kultingar och hade fler dagar mellan förlossning till ny betäckning jämfört med testnegativa suggor. Leptospiros kan sålunda orsaka försämrad reproduktion även i områden med endemisk leptospiros, dvs i områden där sjukdomen är normalt förekommande. Till skillnad mot områden med sporadisk infektion registrerades få aborter bland suggor i Mekongdeltat, vilket kan förklaras av att tidig exponering kan ha gett upphov till viss immunitet, som i sin tur kan förhindra aborter men inte mindre tydliga reproduktionsstörningar.

I den sista studien undersöktes andelen testpositiva slaktsvin där deras njurar analyserades på förekomst av *Leptospira*-typiska förändringar. Dessutom undersöktes förekomsten av slaktsvin som bar *Leptopsira* bakterier i njurarna och odlingsförsök genomfördes för att påvisa infekterande serovarer. Detta är av betydelse eftersom djur som bär bakterien i njurarna kan överföra smitta till andra djur eller människor. Resultat visade att även en stor andel av slaktsvinen var testpositiva och dessutom visade ungefär en fjärdedel av de undersökta njurarna tydliga förändringar som kan härledas till *Leptospira* infektion. Förekomsten av bakterier i njurar med förändringar var vanlig och en serovar isolerades. Det kan förmodas att personal vid slakterier utsätts för risk att smittas av leptospiros varmed de kan utveckla sjukdomen.

Sammanfattningsvis har detta doktorandarbete visat att *Leptospira* infektion är vanligt bland grisar i södra Vietnam och att klimatet i regionen gynnar förekomst av *Leptospira* infektion. Dessutom har visats att såväl en stor andel grisar har exponerats för bakterien som att en stor andel bär också bakterien i njurarna. Därmed kan de utgöra en smittorisk för andra djur och människor. Uppgifterna i avhandlingen kan användas för att på sikt försöka reducera *Leptospira* infektion. Ytterligare studier om leptospiros i området skulle kunna vara att undersöka den ekonomiska betydelsen av reproduktionsstörningar orsakade av *Leptospira* infektion hos grisarna. Dessutom vore det av vikt att undersöka andra djurarter som bärare av *Leptospira* som kan utgöra smittorisker för djur och människor.