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Brief Communication

Enzootics of *Leptospira* Abortions in Danish Sow Herds Practising Loose Housing on Deep Straw Bedding

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For many years the situation about *Leptospira* infections in Danish pig herds remained obscure without confirmed cases (*Mousing et al.* 1995). Recently, suspicion on abortions caused by *Leptospira* has arisen on several occasions and the infection has been demonstrated in 4 herds by immunofluorescent staining of aborted material and by cultivation of *Leptospira* isolates in 3 of the herds. One isolate from 2 herds has been identified as belonging to the serogroup *Pomona,* and the remaining 2 from one herd as serogroup *Tarassovi.* In all 4 herds serological analysis of sows using the Micro Agglutination Test (MAT) revealed positive reactions to various *Leptospira* serogroups, thus further ensuring the diagnosis.

The first diagnosed herd is located on the island of Lolland in the southern part of the country. It was established during the autumn of 1999 by purchase of 600 young breeding pigs from a single healthy multiplying herd in another part of Denmark. The animals were kept indoors with pregnant gilts loose on a bedding with plenty of straw in 3 large groups. In December 1999 aborted material was found in one of the groups and soon after a storm of abortions took place among gilts in late gestation with many dead and mummified foetuses (Table 1). Leptospiras were identified in aborted material by immunofluorescence in January 2000. Serological examination of sows was performed by application of the MAT test and 28 out of 35 samples showed positive reactions with high titres for the serogroup *L. pomona.*

Table 1. Diagnostic data for Leptospira involvement in mass abortions in 4 Danish swine herds.

	Immunofluorescence on foetal organ	placenta	Isolate number	MAT serology on sow blood samples						
				Total number	Number positives for					
	total / pos	total / pos			pom	ict	taras	brat	poi	other
Herd 1	30 / 24	1 / 1	1 L pomona	35	28	6	0	3	2	1
Herd 2	5 / 4	2 / 2	1 L pomona	14	6	0	0	5	0	nd
Herd 3	23 / 17	2 / 0	2 L tarassovi	10	0	0	8	0	0	0
Herd 4	4 / 2	0 / 0		28	19	nd	nd	nd	nd	nd

pos = positive; nd = not done; pom, ict, taras, brat, poi = pomona, icterohaemorrhagiae, tarassovi, bratislava, poi.

Some of the samples were further positive for the serogroup *L. icterohaemorrhagiae* or other serogroups in low titres. A single cultivated isolate of *Leptospira* was identified as belonging to serogroup *Pomona.* After some weeks the disease tended to spread to the other flocks. Treatment with streptomycin as well as vaccination seemed to improve the condition. However, about 1700 dead or mummified foetuses were delivered until May 2000 as compared to 2500 liveborn piglets.

In the second herd approximately 15 kilometers away from Herd 1, some 20 abortions were observed in February-March 2000 among sows in late gestation, and leptospires were detected in kidneys by immunofluorescence test. Serological examination of 14 sows showed high titers to *L. pomona* in 6 sows and to *L. bratislava* with low titres in 5 sows. Also in this herd an isolate of *Leptospira* was identified as serogroup *Pomona.* The 500 pregnant sows/gilts were kept indoors in a few large groups on deep straw bedding. Gradually the abortions spread to the whole herd. Treatment with streptomycin and penicillin together with vaccination appeared to be beneficial. During the month of October straw had been collected for bedding in wintertime, and it was found to contain a lot of mice of unknown species.

The third diagnosed herd is situated in the northern part of Denmark. From December 1999 through April 2000 approximately 30 abortions with many mummified foetuses were observed in 2 groups of sows. *Leptospira* was identified in March by immunofluorescence test on material from foetuses and placentas. Serological examination showed positive reactions to the serogroup *L. tarassovi* in 8 of 10 examined samples from sows, and not for any other serogroup. The herd consists of approximately 600 loose sows devided into several groups on deep straw bedding and by June the disease had spread to the whole herd. Two isolated leptospiras have been identified as serogroup *L. tarassovi.* Also in this herd treatment with streptomycin and penicillin together with vaccination appeared to be beneficial.

The fourth herd is also placed on Lolland about 15 kilometers from Herd 2. Many abortions with mummified foetuses started in one of 9 groups in March 2000 and gradually spread to the whole herd by the end of May. Some few cases of positive immunofluorescence test on foetal kidneys were detected as well as positive seroreactions to *L. pomona* in two thirds of examined sows. Again loose housing of pregnant sows (about 600) in large groups on deep straw bedding is used. No isolation of *Leptospira* has been obtained so far. Mouse and rats are sometimes observed among the sows.

The immunofluorescence examination was performed as an indirect test using rabbit anti-*L bratislava* IgG on glass slides smeared with material of internal organs. Initially pooled organ material was used, but later the kidney was taken alone. Likewise smears of placenta were examined. Organ material was homogenised in a *Stomacher 80* and used for smear preparation by squeezing between 2 glass slides. In many cases the fluorescence (Fig. 1) was dominated by small pieces of specifically shining debris with no similarity to elongated forms, but in between some distinct longer forms could also be discriminated, some of them reminiscent of true leptospires. Control preparations stained with anti-*Mycoplasma bovirhinis* IgG always appeared negative. In some cases uneven distribution of the fluorescence was found and this might indicate the disruption of a diminutive infectious focus. From evaluation of the intensity of fluorescence it appears that the kidney is the site of predilection for the infection. Often also the placenta showed intensive staining. The immunofluorescence procedure is specific within the species *Leptospira interrogans.*

Serologic analysis of blood samples was per-

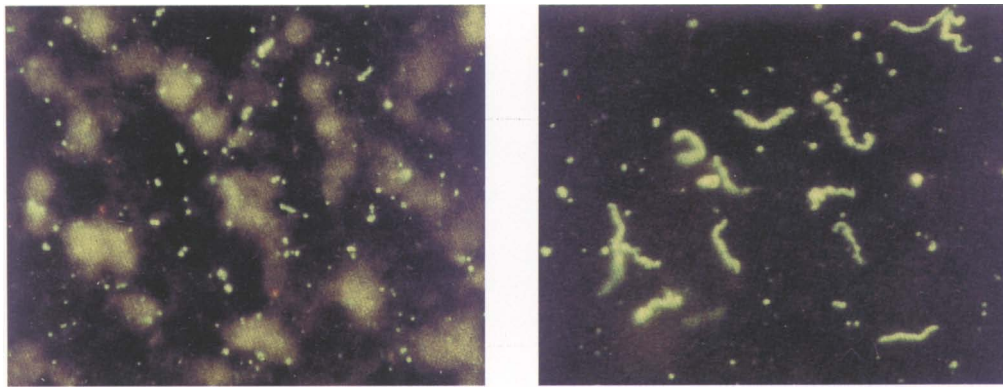

Figure 1. Fluorescent staining of homogenised kidneys of 2 aborted pig foetuses, L60 ("left") and L88 ("right") of Herd 1 visually mummified and normal, resp. "left" dominated by debris and "right" by ordinary leptospira forms. Indirect fluorescent method with rabbit anti-*L bratislava* IgG. L88 yielding *Leptospira pomona* on cultivation. Linear magnification ca 900 ×.

formed by the MAT test, using type cultures of 14 different serogroups potentially occurring in pigs. This test is specific at the serogroup level. The results varied between the herds (Table 1). Thus, in Herd 1 nearly all of the examined sows had antibodies to *L. pomona* with titres up to 1:8,000. A few samples additionally showed antibodies to *L. icterohaemorrhagiae* or other serogroups with low titres. In Herd 2 sows reacted against either *L. pomona* or *L. bratislava* and in Herd 3 reactions were only seen against the serogroup *L. tarassovi.* In Herd 4, 19 out of 28 examined sows were serologically positive for *L. pomona,* the only serogroup examined for.

All four isolates of *Leptospira* obtained from the outbreaks could be propagated in the medium of *Ellis* (1986), but traditionally EMJH medium with 10 per cent rabbit serum or Korthof's medium with 7.5 per cent serum did not support any growth. The isolate from Herd 1 was discovered after 3 weeks of incubation at 36°C and originated from the kidney of a nearly normal looking foetus. The isolates from the other herds derived from kidney of moderately mummified foetuses and were found after 5-6 weeks of cultivation at 28-30°C. The isolates from Herds 1 and 2 reacted in the MAT test above 1:10,000 for *L. pomona* and below 1:100 for other serogroups, while the isolates from Herd 3 reacted at 1:2,000 and 1:1,000 for *L. tarassovi* and below 1:100 for other serogroups. For immunofluorescence and for cultivation the organs were immersed into boiling water for 5-10 sec followed by homogenisation. Cultivation was performed without bacterial inhibitors. Passages were only stable in propagation at temperatures not exceeding 30°C.

The use of IgG against *L. bratislava* (Jez) as diagnostic tool in the immunofluorescence test reflects the traditional thinking that this serovar of serogroup *Australis* would represent a future finding in this country, but this seems not to be the case. Fortunately such an antiserum also reacts against other serogroups of the species *Leptospira interrogans.* Further, the many mummified foetuses should be no characteristic feature in *L. bratislava* infections of swine.

Some decenniums ago *Fennestad & Borg-Petersen* (1972) demonstrated several *Leptospira*

serogroups in Danish wildlife mammals including *L. pomona* in the striped mouse *(Apodemus agrarius)* which has its utmost northern living area on the islands of Lolland, Falster and Møn. In the intervening years the rate of infection among swine is supposed to have been very low. It is noteworthy that 3 of the 4 outbreaks are situated on the island of Lolland and apparently without inter-herd connections, thus indicating that mice are still a source of the infection. Further, it is interesting that all 4 affected herds have used the principle of loose sows in large flocks on plenty of straw bedding (in 3 herds as deep bedding). Obviously this management support spread of the infection; maybe by providing shelter for a mouse population, maybe by keeping a beneficial humid environment for *Leptospira* survival. Further, the free access to eating aborted material as well as urine-contaminated straw might well have facilitated spread of the infection to susceptible sows. Also it cannot be ruled out that an intense epizootic has occurred in the mouse population; because the observation of mice in 2 of the herds investigations in this field are now being carried out.

The origin of the infections is of particular epidemiological and zoonotic interest. Although there is some anamnestic information about the presence of mice in 2 of the herds, it is not yet possible to draw a conclusion from this finding. It would be of special interest to investigate if the infections have been swine maintained or have originated from a wildlife source.

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