Technical Report: A Novel Porcine Model of Acute Pyelonephritis

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Summary
A porcine model of acute *Escherichia coli* pyelonephritis is described. Nine female pigs with a mean weight of 19 kg were included in the study. Following laparotomy and ureterotomy, catheters were placed bilaterally in the renal pelvis. A culture of a porcine *E. coli* strain (10^9 CFU/mL, volume 3.25 mL) was inoculated unilaterally in the renal pelvis. Following bacterial inoculation, the catheter was occluded for 20 min. The inoculation procedure was repeated after two and four hrs. The contralateral kidneys served as controls and were inoculated with sterile saline. Six hrs after the first inoculation pigs were euthanized and samples of blood, abdominal swabs, urine and specimens from kidney, liver and spleen were cultured. The inflammatory response in the kidneys, renal lymph nodes, liver and spleen was evaluated both macroscopically and histologically and *E. coli* was identified by immunohistochemistry. *E. coli* was reisolated from all the inoculated kidneys and was detected immunohistochemically in all inoculated kidneys, in four renal lymph nodes from inoculated sides and in four spleens. Evidence of systemic bacterial spread was observed in five pigs. Gross and histological lesions of acute pyelonephritis comparable to human cases were demonstrated in all but one inoculated kidney. In conclusion, a novel porcine model for evaluating the pathogenesis of acute pyelonephritis was established.

Introduction
Pyelonephritis is a common disease in humans, which can cause serious clinical symptoms and may result in irreversible renal scarring with sequela of hypertension and chronic renal failure. *Escherichia coli* are known to be responsible for more than 80% of acute pyelonephritis cases in women (*Czaja et al., 2007*). Although pyelonephritis caused by *E. coli* has been a subject of intense study, several aspects of the acute pathogenesis including the local host response to infection and the influence of bacterial virulence factors, need further investigation.

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Monkey models with intra-vesical or intra-pelvic inoculation of *E. coli* have been used (*Roberts et al., 1981; 1985*) but partly due to ethical reasons such models are not preferred. Instead rodent models with inoculation of *E. coli* in the urinary bladder, with or without urinary outflow obstruction, have been commonly used for studying pyelonephritis (*Miller and Robinson, 1973; Brooks et al., 1974; Larsson et al., 1980; Hagberg et al., 1983; Johnson et al., 1993*). However, both due to the small size of rodents with the risk of the lack of blood, urine and tissue samples for investigation and due to the dissimilarity in anatomy, physiology and immunology between rodent and human, rodent models are not always favourable for studying human pyelonephritis. One important anatomical difference between rodents and humans influencing the applicability of rodent models for pathogenesis studies is the presence of unipyramidal kidneys in rodents in contrast to the multipyramidal kidneys with compound pa-

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pillae found in humans as compound papillae are known to be predisposed to intra-renal reflux of urine (*Ransley and Risdon, 1975a; 1975b; 1978*).

In contrast to rodents, pigs possess renal anatomy and physiology much more comparable to humans including the presence of multipyramidal kidneys (*Terris, 1986*; *Swindle and Smith, 1998*). Consequently, pigs are well suited for nephrological studies. In addition, lesions of spontaneous pyelonephritis in pigs are comparable to human pyelonephritis and *E. coli* is also known to be an important pathogen in urinary tract infection in pigs (*Carr and Walton, 1993*). In previously used porcine pyelonephritis models, vesicoureteral reflux is induced surgically and followed by inoculation of *E. coli* together with molten paraffin in the urinary bladder (*Ransley and Risdon, 1978*). Although widely used, such amodel has some disadvantages for studying several aspects of the acute pathogeneses of pyelonephritis. First of all the exact time of infection and the bacterial number reaching the kidney are difficult to control. Next the model is time-consuming and close monitoring of the pigs, repeated bacterial inoculation and temporary urinary outflow obstruction are difficult or impossible to perform.

Therefore, the aim of the present study was to establish a reliable, time saving and easily controllable porcine model for evaluation of the acute pathogenesis of pyelonephritis. The present model has been used in a pathological study of the influence of *E. coli* virulence factors on pathology and pathogenesis of porcine pyelonephritis (Isling et al., 2011).

Material and Methods

Animals

Nine female Danish Landrace/Large White crossbred pigs with a mean weight of 19 kg were purchased from a specific-pathogen free (SPF) pig farm (Erik Mølbak, Herlufmagle, Denmark), health monitored according to the standards of SPF-Denmark (www.spf.dk). After acclimatization for one week, the pigs were randomly allocated into three groups (A, B and C). The pigs were housed in groups on solid concrete floors with straw bedding and wood shavings and were observed at least twice daily. The pigs were fed a commercial pelleted dry feed twice daily (Svine Erantis, Brogaarden, Lynge, Denmark) and allowed free access to water.

The pigs used in this study were treated in accordance with the Animal Experimentation Act of Denmark, which is in accordance with The Council of Europe Convention ETS 123. The National Animal Experimentation Board licensed the study according to Danish law.

Surgical procedure

Prior to surgery the pigs were sedated with 0.06 mL/kg/body weight (BW) of "swine mixture" intramuscularly (i.m.) (125 mg tiletamine and 125 mg zolazepam (Zoletil®50, Chemvet, Silkeborg, Denmark) + 125 mg xylazine (Nacoxyl® vet 20 mg/mL, Intervet/Schering-Ploug Animal Health, Skovlunde, Denmark) + 125 mg Ketamine (Ketaminol® vet 100 mg/mL, Intervet/Schering-Ploug Animal Health, Skovlunde, Denmark) + 25 mg butorphanol (Torbugesic vet 10 mg/mL, Scanvet, Fredensborg, Denmark). Anaesthesia was induced with 2-3 mg/kg/BW propofol (Rapinovet® vet 10 mg/mL, Intervet/Schering-Ploug Animal Health, Skovlunde, Denmark) intravenously (i.v.). Subsequently, the pigs were intubated and anaesthesia was maintained with 5-10 mg/kg/BW/hrs propofol (Rapinovet® vet 10 mg/mL, Intervet/Schering-Ploug Animal Health, Skovlunde, Denmark). Four hrs after induction of anaesthesia, 0.2 mg/kg/BW butorphanol (Torbugesic vet 10 mg/mL, Scanvet, Fredensborg. Denmark) and 0.6 mg/kg/BW diazepam (Stesolid® 5 mg/mL, Actavis, Gentofte, Denmark) were given i.m. and i.v., respectively. Furthermore, 0.01 mg/kg/BW atropinsulfat inj. (Atropin 1mg/mL, Pharmacy Services, Faculty of Life Sciences (LIFE), University of Copenhagen (KU), Copenhagen, Denmark) was given i.v. if heart rate dropped below 50. During the entire experiment pigs were anaesthetized and were given 10 mL/kg/BW/hrs Ringers acetate i.v. (Fresenius kabi, Copenhagen, Denmark).

The anaesthetized pig was placed in dorsal recum-

bency. The surgery area was prepared according to standard operating procedures for preparation of a surgical site (Division of Laboratory Animal Science and Welfare, LIFE, KU, Copenhagen, Denmark). A ventral laparotomy (pubic bone to xiphoid cartilage) was performed and the intestines were moved caudally by sterile cotton cloths dipped in isotonic saline (37˚C). The ureter was isolated from the surrounding tissue by blunt dissection and placed over a tissue forceps. Ten cm caudally to the renal pelvis a small incision was made in the ventral part of the ureter. A sterile catheter (8F (2.6mm) x500mm 1.8mm ID RET400/220/080, Portex® Feeding Tube, Infant Female Mount-Red, Smiths, Hørsholm, Denmark) containing three 1-2 mm thick silicone retention beads (silicone glue, RTV 108, V-ADH-2, e-vet, Haderslev, Denmark) on its surface (9-11 cm from the tip of the catheter) was introduced in the abdomen by a small transverse transabdominal incision in the ventral flank region. By means of an introducer the catheter was then placed in the renal pelvis through the ureterotomy. Two ligatures (Ethicon vicryl 2-0) were placed cranially to the ureterotomy and caudally to a silicone retention beads and one ligature was placed caudally to the ureterotomy. The tip of the catheter in the renal pelvis was confirmed by palpation. The same surgical procedure was executed bilaterally. After repositioning of the intestines, a culture of *E. coli* strain LK67, LK76 or LK82 (10^9 CFU/mL, volume 3.25 mL) (*Krag et al., 2009*) resuspended in sterile isotonic saline was inoculated during 1 min unilaterally in the renal pelvis in three pigs (Table 1). The three different *E. coli* strains were isolated from cases of pyelonephritis in Danish slaughtered sows and expressed different combinations of the virulence factors (*Krag et al., 2009*). Following bacterial inoculation the catheter was occluded for 20 min and the ureterotomy was controlled for leak. A simple continuous suture pattern (Ethicon vicryl 2-0) was used to close linea alba. Subcutis and cutis were closed by staples. Following obstruction the catheter was connected to a urine bag. The inoculation procedure was repeated 2 and 4 hrs after the initial inoculation. During each bacterial inoculation the contralateral kidneys received the same amount of sterile isotonic saline following exactly the same procedure as for the inoculation of the test kidneys. Six hrs post inoculation (PI) the laparotomy wound was reopened and a swab was taken from the abdominal cavity for bacteriological investigation. The kidneys were removed by clamping the ureter and vessels with hemostatic forceps. Immediately after nephrectomy the pigs were euthanized by i.v. injection of 200 mg/

Table 1. Caption: Experimental design.

Pig no.	Group	Number of kidneys	Bacterial strain/sterile saline	Inoculation time (hrs)	Obstruction after each inoculation (min)	Euthanasia (hrs) post inoculation
AI, AII, AIII	A	3	LK67	0, 3, 4	20	6
		3	Sterile saline			
BI, BII, BIII	B	3	LK76	0, 3, 4	20	6
		3	Sterile saline			
CI, CII, CIII	C	3	LK82	0, 3, 4	20	6
		3	Sterile saline			

kg/BW pentobarbital (200 mg/mL) (Pharmacy of LIFE, KU, Copenhagen, Denmark).

Blood samples, bacteriology, gross pathology and histopathology

A detailed description of material and methods is given in Isling et al. (2011). In short, blood samples were collected before initiating the surgery and at 3 and 6 hrs PI for total leukocyte and differential counts, clinical chemistry and for measurements of acutephase proteins and pro-inflammatory cytokines. Blood samples, catheter urine and specimens from the cranial and caudal part of the kidney, spleen and liver were collected right after euthanasia for re-isolation of *E. coli*.

A total necropsy was performed and gross lesions recorded. Specimens from kidneys, ureters, urinary bladder, renal lymph nodes, liver and spleen were sampled from predetermined positions. The specimens were fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin. Haematoxylin and eosin (HE) stained sections were used for overall evaluations. *E. coli* and selected inflammatory cells were visualized immunohistochemically in kidneys, renal lymph nodes, liver and spleen.

Results

Detailed results are presented in Isling et al., (2011). Briefly, neutrophilia and monocytopenia were observed in all three groups 6 hrs PI and the concentration of C-reactive protein increased from 3 to 6 hrs PI. *E. coli* was re-isolated in pure culture from kidney tissue and urine from all inoculated kidneys. Furthermore, *E. coli* was isolated in pure culture from the liver, spleen and blood from 56% (5/9), 22% (2/9) and 33% (3/9) of the pigs, respectively. Both kidney tissue and urine from control kidneys and all abdominal swabs were sterile. No gross or histological lesions corresponding to acute pyelonephritis were observed in any of the control kidneys. In all but one kidney (pig BII) inoculated with *E. coli* gross lesions corresponding to acute pyelonephritis were present. The lesions were small variably sized irregularly distributed greyish-white foci that were often confluent and surrounded by a thick hyperaemic and haemorrhagic rim. The pelvic mucosa was in places slightly hyperaemic. In all but one kidney (pig BII) inoculated with *E. coli,* variable degrees of acute pyelonephritis were present histologically. The main findings in both cortex and medulla were multifocal interstitial infiltrations with inflammatory cells, mainly neutrophils and lymphocytes, and variably sized interstitial aggregations of neutrophils. Many tubules dilated with suppurative exudates; widespread tubular destruction and variable degrees of hyperaemia, haemorrhage end oedema were also present. In pelvis intraepithelial and subepithelial infiltrations with neutrophils and mononuclear cells were found. Immunohistochemically stained *E. coli* were seen in all inoculated kidneys. Most severe pyelonephritis lesions were observed in inoculated kidneys in group C. Neutrophil infiltrations were present in the renal lymph nodes corresponding to the *E. coli* inoculated side in 67% (6/9) of the pigs and in all spleens. *E. coli* was detected immunohistochemically in four lymph nodes and four spleens.

Discussion and Conclusions

The present study demonstrated that repeated inoculation of porcine *E. coli* in the renal pelvis of female pigs followed by 20 min of catheter occlusion was able to induce both gross and histological lesions of acute pyelonephritis 6 hrs PI in an otherwise normal urinary tract. Re-isolation of *E. coli* from the kidney tissue and urine was possible post mortem. Additionally, *E. coli* could be detected immunohistochemically in all inoculated kidneys and in some of the corresponding renal lymph nodes and spleens. The observed pathology in the inoculated kidneys corresponds to that seen in acute pyelonephritis in humans both with respect to lesion distribution and the character of the gross and histological lesions (*Jeannett, 2009*). Consequently, the current model is appropriate for studying the acute pathogenesis of pyelonephritis in man. The fact that bacteraemia was detected in 5/9 pigs in the present study suggest that

the model may be suitable for evaluation of urosepsis, a well-known phenomenon in relation to acute pyelonephritis in humans (*Johnson et al., 1987*). To our knowledge bacteraemia has not been observed in previous experimental pyelonephritis porcine models. The current novel porcine pyelonephritis model has several advantages compared to earlier porcine pyelonephritis models. First of all the introduction of bacteria is not dependent on the urine flow rate and the time interval before bladder emptying as in old models with inoculation of *E. coli* together with molten paraffin in the bladder (*Ransley and Risdon, 1978*). Consequently close control of the infection time and the number of bacteria reaching the renal pelvis is possible. Next, repeated bacterial inoculation and temporary obstruction of the urinary flow is possible, which enables study of those aspects of the pathogenesis. The present model also provides the option of closely monitoring the pig during the entire experiment. Lastly, the high incidence of pyelonephritis in the current model (89%) in contrast to previous pig studies (*Ransley and Risdon, 1981; Rushton et al., 1988; Majd, 2001*), which only manage to induce pyelonephritis in about half of the kidneys three to seven days after bladder inoculation of *E. coli*, and the use of internal controls make reduction in the number of experimental pigs needed possible which fulfils the concepts of the 3Rs. Inoculations with porcine instead of human *E. coli* strain can be preferable as it ensurea correlation between possible animal-specific bacterial adhesions and animal specific uroepithelial receptors. However, as pigs are known to express uroepithelial receptors for important *E. coli* adhesions comparable to human receptors, the present model will also be suitable for *in vivo* evaluation of the role played by human *E. coli* virulence factors in acute pyelonephritis (*Källenius et al., 1981*). Two disadvantages of the present porcine model compared to rodent models are the cost of pig models and the fact that the use of inbred animals is not possible. In conclusion, a porcine model suitable for evaluation of acute pyelonephritis has been established.

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