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Renal Infarction and Immune-mediated Glomerulonephritis in Sheep (*Ovis aries*) Chronically Implanted with Indwelling Catheters

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Microbial infections are common sequelae in humans and animals implanted with long-term intravascular catheters. Understanding the pathophysiology of infectious morbidity is critical to improving quality of care in catheterized subjects. Here, we describe findings in 6 clinically healthy, male sheep implanted with indwelling aortic or cardiac catheters for 6 to 10 mo. We isolated multiple bacterial species including *Serratia* spp., *Enterobacter agglomerans*, *Escherichia coli*, *Klebsiella oxytoca*, and *K. pneumoniae* in aerobic cultures from catheter tips. Although sheep were clinically asymptomatic, 1 or both kidneys from all animals contained wedge-shaped infarcts of varying size and number. Microscopic examination revealed (a) marked fibrosis with mild inflammatory cell infiltrate consistent with chronic foreign body reaction around catheters; (b) moderate to severe, diffuse, subacute to chronic membranoproliferative glomerulonephritis and mild, multifocal chronic interstitial nephritis; and (c) mesangial immune-complex deposition as demonstrated by direct immunofluorescence technique. The finding of bacterial colonization of catheters together with chronic glomerulonephritis and immune-complex deposits in kidneys in clinically asymptomatic sheep underscores the need for close microbiologic monitoring of catheter implants and assessment of kidney function in animals instrumented for long-term vascular access.

Abbreviations: Ig, immunoglobulin; TRITC, tetramethyl rodamine isothiocyanate

Intravascular catheter use is integral to studying cardiovascular physiology and monitoring hemodynamics. Intravascular catheters are implanted routinely in patients being treated for cardiovascular diseases. Unfortunately, there has been a steady increase in the incidence of catheter-associated infections in recent years.[3,15] Secondary complications due to intravascular catheter-associated infections pose a serious health risk in newborn infants[7] and immunosuppressed adults.[19] However, the pathophysiology of catheter infections in humans is not fully elucidated due to the complex nature of the prior events leading to surgery and the varied health status of subjects involved.[3] Lack of widespread surveillance and timely diagnosis of catheter-associated infections further contribute to high morbidity and mortality in human subjects.[5,14]

Catheters become infected in many ways. Microbes gain entry into the catheter lumen transcutaneously along the tract outside of the catheter during surgery or postoperatively when contaminated infusates are administered.[1] Subsequent to infection, catheter patency may be compromised due to formation of emboli.[16,18] Sheep are commonly instrumented with catheters in the aortic arch, atria, or ventricles for the purpose of studying cardiovascular regulatory mechanisms, system dynamics,[10] and drug evaluation experiments.[11] Nevertheless, the long-term in vivo effects of indwelling catheters in sheep have not been documented. Development of catheter-associated infections may adversely affect animal health and potentially jeopardize the value of experimental data. Here, we describe clinicopathologic findings in 6 clinically normal Dorset sheep catheterized 6 to 10 mo earlier for vascular access and hemodynamic monitoring.

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Materials and Methods

Animals and surgery. Clinically healthy 18- to 24-mo-old, male intact Dorset sheep (n = 6) weighing 20 to 30 kg (Parsons Farms, South Hadley, MA) negative for enteric pathogens, gastrointestinal parasites, and lungworms were housed in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. Animals were housed singly in stainless steel pens with slotted steel flooring and fed a pelleted feed for ruminants (Rumilab, PMI Nutrition International, Labdiet, Brentwood, MO) with mixed grass hay and water ad libitum. Temperature (68 to 74 °F) and humidity (40% to 60%) were controlled in the holding room; there were 12 complete air changes per hour. All experiments involving these sheep were approved by the Massachusetts Institute of Technology Institutional Animal Care and Use Committee.

The sheep were catheterized 6 to 10 mo prior to necropsy, as part of a cardiovascular physiology study. Briefly, after induction of anesthesia with ketamine (22 mg/kg intravenously) and diazepam (0.055 mg/kg intravenously), animals were intubated and anesthesia was maintained with 1% to 4% isoflurane in oxygen. Intermittent positive pressure ventilation was used, and a left lateral thoracotomy was performed following aseptic technique. Polyvinyl chloride catheters were inserted in the right atrial appendage and in the thoracic descending aorta. Each

Table 1. Hematologic parameters of sheep catheterized for 6 to 10 months

	Sheep ID					
	874	654	708	824	Reference range[a]	
White blood cell count	4.3	3.0↓	2.6↓	5.7	4–12	10^3/ml
Red blood cell count	10.52	9.16	10.21	10.41	9–15	10^6/ml
Hemoglobin	12.1	9.4	10.3	11.8	9–15	g/dl
Hematocrit	33.9	26.4↓	27.4	33	27–45	%
Mean corpuscular volume	32	29	27↓	32	28–40	fl
Mean corpuscular hemoglobin	11.5	10.3	10.1	11.3	8–12	pg
Neutrophils (segmented)	36	86↑	26	15	18–50	%
Lymphocyte	61	13↓	74	84↑	50–75	%
Monocyte	1	1	0	1	0–6	%
Eosinphil	1	0	0	0		
Basophil	1	0	0	0		
Platelets	Adequate	Adequate	250	Adequate		
Absolute counts						
Neutrophil (segmented)	1548	2580	676↓	855	700–6000	/ml
Lymphocyte	2623	390↓	1924↓	4788	2000–9000	/ml
Monocyte	43	30	0	57	0–750	/ml
Eosinphil	43	0	0	0		
Basophil	43	0	0	0		

↑, higher than reference range; ↓, lower than reference range.
[a]As reported by Idexx Veterinary Services, North Grafton, MA.

catheter's intravascular tip was fashioned by cutting the end at an approximately 45° angle; there was no further modification of the tip. Catheters also were equipped with cuffs made of polyvinylchloride of appropriate size to provide suture stays. Cuffs were placed at the junction of the catheter's exit site from either the atrial appendage or aorta and at a subcutaneous site close to the catheter's exteriorization from the skin. Catheters were sutured in place by means of swaged silk on taper needles.

To effect atrial pacing and subsequent electrocardiographic monitoring, 1 pair of epicardial electrodes was attached to the right atrial appendage, and another pair was attached to the right ventricular surface. To measure cardiac output, an ultrasonic flow probe was placed around the ascending aorta. All catheters and wires were tunneled subcutaneously to a dorsal interscapular position and exteriorized. The exit sites were dressed with nitrofurantoin ointment, and all implants were protected by use of a vest or chest wrap. Animals were administered analgesics (flunixin meglumine, 1.1 mg/kg intravenously intraoperatively once; buprenorphine, 0.008 mg/kg twice daily for 72 h) and antibiotics (enrofloxacin, 5 mg/kg intramuscularly once daily for 10 d or amoxicillin 20 mg/kg subcutaneously once daily for 7 d) postoperatively. At 6 to 10 mo later, sheep were tranquilized by use of xylazine (0.5 mg/kg intramuscularly), and blood was collected for aerobic and anaerobic culture, complete blood counts, and serum chemistry analysis. Animals were euthanized by intravenous injection of pentobarbital sodium (120 mg/kg).

Bacteriologic and histopathologic examination. After euthanasia, the catheter site was sterilized cutaneously, and the left hemithorax was accessed via a standard thoracotomy approach. Catheters were recovered aseptically and sampled at sequential intervals from their distal tip in the atrium or aorta to the site of insertion at skin level. We collected samples (length, 1 cm) from catheter tips, subcutaneous mid portions and site of emergence from both aortic and intra-atrial catheters and immediately transported them to the laboratory on ice. Samples were placed individually in trypticase soy broth for aerobic culture. In addition, samples from the tips were cultured anaerobically in thioglycollate broth. Bacterial isolation and characterization were performed according to the manufacturer's instructions (API Systems, bioMerieux Vitek, Hazelwood, MO).

Catheter segments with surrounding connective tissue and samples from all major organs including kidneys were collected, fixed in 10% neutral-buffered formalin, routinely processed and embedded, sectioned at 4 µm, and stained with hematoxylin and eosin. Selected sections also were stained with periodic acid Schiff, Congo Red, or Brown–Hopps modified Gram stain.[2]

Immunofluorescent antibody staining. To determine whether affected kidney sections had immunoglobulin complex deposits, we used standard direct immunofluorescence techniques. Briefly, formalin-fixed, paraffin-embedded kidney sections were deparaffinized and rehydrated. Antigen retrieval was performed for 20 min at room temperature with 0.1% trypsin (Sigma, St Louis, MO), 0.1% $CaCl_2$ in Tris-buffered saline. Sections were incubated with tetramethyl rodamine isothiocyanate (TRITC)-conjugated rabbit anti-sheep immunoglobulin (Ig) G (Cappel–ICN, Aurora, OH) for 45 min at 37 °C. Kidney sections from age-matched, noncatheterized healthy sheep were immunolabeled in parallel and served as controls. All sections were mounted (Vectashield, Vector Laboratories, Burlingame, CA), coverslipped, and examined under an epifluorescence microscope (Eclipse i600, Nikon, Melville, NY).

Results

Catheterized sheep showed no noteworthy changes in hemogram and serum analytes. Blood samples were collected for complete blood counts and serum chemistry from the anesthetized sheep prior to euthanasia. Hematology data were available for 4 of the 6 animals (Table 1). Despite changes in some hematologic parameters (white blood cell count, lymphocyte count, hematocrit, and mean corpuscular volume), sheep remained clinically asymptomatic throughout the catheterized

Table 2. Serum chemistry of catheterized sheep

	Sheep ID						Reference range[a]	
	874	654	708	824	912	850		
Alanine aminotransferase	12↓	5↓	10↓	11↓	11↓	10↓	22–38	IU/l
Aspartate aminotransaminase	60	22↓	59↓	57↓	71	58	60–280	IU/l
Albumin	3.9↑	2↓	3.7↑	3.6↑	3.1↑	3.8↑	2.4–3.0	g/dl
Blood urea nitrogen	17	14	11	23↑	15	12	8–20	mg/dl
Glucose	115↑	107↑	142↑	111↑	121↑	113↑	50–80	mg/dl
Calcium	10.3↓	8.6↓	9.6↓	8.6↓	8.1↓	10.1	11.5–12.8	mg/dl
Phosphorous	9↑	4.8↓	6.9	5.7	5.2	7.1	5–7.3	mg/dl
Albumin:globulin ratio	1.2↑	0.3↓	1.3↑	1.2↑	1.3↑	1.5↑	0.4–0.8	

↑, higher than reference range; ↓, lower than reference range.
[a]As reported by Idexx Veterinary Services, North Grafton, MA.

Table 3. Bacterial species isolated from intravascular catheter implants

		Aerobic culture					
		Aortic arch			Intra-atrial		
Sheep ID	Anaerobic culture	Intravascular	Subcutaneous	Cutaneous	Intravascular	Subcutaneous	Cutaneous
912	No growth	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*
850	No growth	*E. agglomerans*	*E. agglomerans*	*K. pneumoniae*	*E. agglomerans*	*E. agglomerans*	*E. agglomerans*
874	No growth	*E. coli*	*K. oxytoca* *E. agglomerans*	*E. coli*	*K. oxytoca* *E. agglomerans*	*E. coli* *K. pneumoniae*	*E. coli*
708	No growth	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*K. oxytoca*
824	No growth	*E. agglomerans*	*E. agglomerans*	*E. agglomerans*	Not determined	*E. coli* *E. agglomerans* *E. sakazakii* *Serratia* spp.	*K. oxytoca* *E. agglomerans*

E. agglomerans, *Enterobacter agglomerans*; *E. sakazakii*, *Enterobacter sakazakii*; *E. coli*, *Escherichia coli; K. pneumoniae*, *Klebsiella pneumoniae*; *K. oxytoca*, *Klebsiella oxytoca*.

period. Serum chemistry was performed on all sheep (Table 2). Mild hyperglycemia was probably related to the known effects of xylazine in this species.[12] Other common findings included hypocalcemia, decreased serum transaminase levels, and increased albumin-to-globulin ratios (Table 2).

Catheter implants were colonized with multiple bacterial species. Anaerobic cultures of catheter implants and antemortem whole-blood samples were negative for all 5 sheep sampled. However, aerobic cultures of all segments from aortic arch and intra-atrial catheters yielded 1 to 3 distinct bacterial species (Table 3). One animal had a monoinfection with *Escherichia coli*, whereas all other sheep had more than 1 species isolated from their catheters. Among the isolates, *E. coli* was the most frequently isolated organism followed by *Enterobacter agglomerans*, *Klebsiella oxytoca*, *K. pneumoniae*, *Enterobacter sakazakii*, and *Serratia* spp. Longitudinal and transverse sections of catheter tips from atrial and intra-aortic locations were examined directly for evidence of bacterial colonization. Catheter tips from intra-aortic and atrial locations revealed fibrin plugs and aggregates of bacteria attached intraluminally to the catheter walls (Figure 1 A, B).

Renal infarction and immune-mediated glomerulonephritis in catheterized animals. Consistent with their 'clinically healthy' condition, catheterized sheep did not have any remarkable changes in major internal organs including liver, gall bladder, spleen, heart, lung, gastrointestinal tract, and mesenteric lymph nodes. Interestingly, however, kidneys from all sheep presented several gray-white pitted foci, which were as large as 1.5 cm in diameter on the capsular surface. Upon sectioning, the foci corresponded to the bases of characteristic wedge-shaped, renal infarcts.

Kidneys from all sheep exhibited a similar spectrum of histopathologic changes of variable severity. The multifocal wedge-shaped infarcts (Figure 2 A) were characterized by fibrosis, loss of parenchyma, and a chronic inflammatory cell infiltrate (lymphocytes, plasmacytes, and macrophages). Gram staining of kidney sections demonstrated aggregates of gram-negative bacteria occasionally in the lumen of the arcuate artery, consistent with septic embolic nephritis (Figure 2 B). Multifocally, Bowman capsules were thickened and Bowman spaces dilated by amorphous to granular eosinophilic proteinaceous material. Many glomerular tufts were adhered to Bowman capsules (synechiae), and small crescents were occasionally present. Some glomeruli were condensed and shrunken, lesions that are consistent with glomerulosclerosis and multifocal periglomerular fibrosis. The majority of glomeruli were enlarged and hypercellular, with widened mesangial spaces. Hyperplasia of resident endothelial and mesangial cells coupled with infiltrating inflammatory cells (primarily mononuclear cells with occasional neutrophils) contributed to glomerular hypercellularity (Figure 2 C). In the renal interstitium, multifocal areas of mild to moderate fibrosis and inflammatory cell infiltrates comprised primarily of mononuclear cells (chronic interstitial nephritis) were noted. Periodic acid Schiff staining revealed thickening of the basement membranes of both glomerular capillaries and capsular epithelium. Occasionally, there were thickened capillary walls with double contours due to splitting of the glomerular basement membrane (Figure 2 D). Our overall morphologic diagnoses for the kidneys included chronic infarction, interstitial nephritis, and membranoproliferative glomerulonephritis.

To further characterize the glomerular lesions, sections from

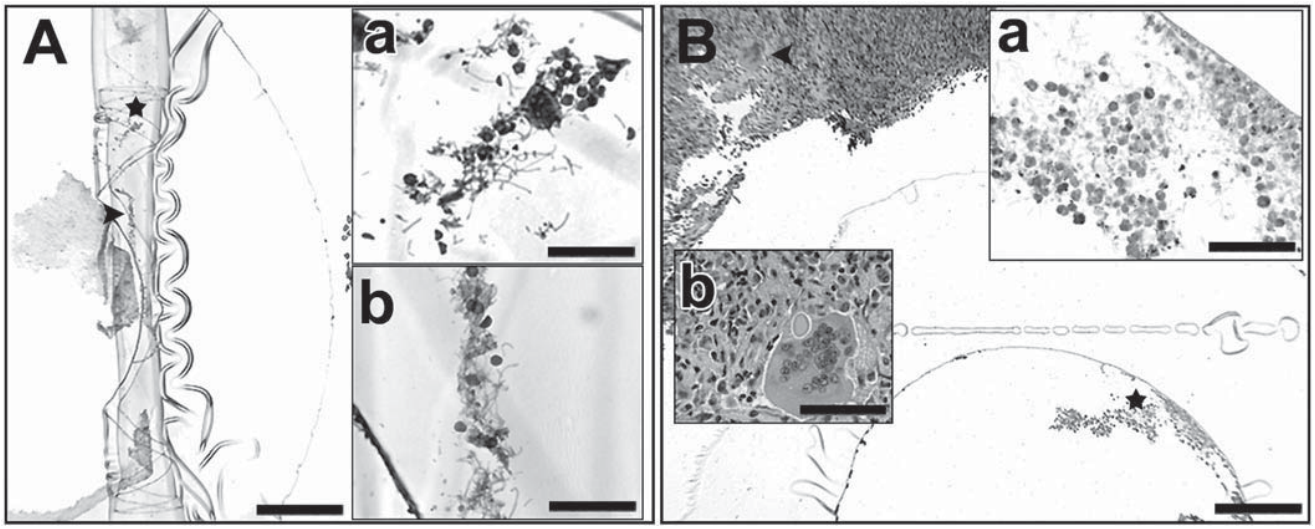

Figure 1. Histopathologic findings in catheter samples. (A) Intra-aortic part (longitudinal section); (B) intra-atrial part (transverse section). The areas marked are shown in higher magnification insets (asterisks, A.a and B.a; arrowheads, A.b and B.b). Aggregates of bacteria in the catheter lumen (A.a and A.b). Fibrin plug attached to the luminal wall of the catheter and clusters of bacteria intermingled with necrotic inflammatory cells (B.a). Macrophages and foreign body giant cell in the connective tissue sheath surrounding the catheter (B.b). (A) and (B) bar, 250 µm; insets: bar, 25 µm. Hematoxylin and eosin stain.

catheterized and noncatheterized (control) sheep were stained in parallel with TRITC-conjugated rabbit anti-sheep IgG. Glomeruli from catheterized sheep (n = 6) demonstrated segmental, irregular, coarsely granular to confluent ('lumpy bumpy') capillary wall and mesangial deposits of IgG (Figure 3 A). Kidney sections from noncatheterized control sheep (n = 4) showed no deposits (Figure 3 B).

Discussion

To study cardiovascular physiology, sheep are commonly catheterized for long periods. However, to our knowledge no study to date has examined the status of implanted catheters in sheep nor have the sequelae of long-term catheterization in sheep been documented. In the current study we found that catheterized sheep remained clinically asymptomatic despite their infection due to contaminated catheters and kidney infarcts. Kidneys from all 6 animals were affected showing membranoproliferative glomerulonephritis and chronic interstitial nephritis to various degrees. Direct immunofluorescence staining with anti-sheep IgG antibody demonstrated segmental, irregular, coarsely granular to confluent (lumpy bumpy) capillary wall and mesangial deposits of IgG in all animals. The observation that the majority of the intravascular catheters were colonized with more than 1 potentially pathogenic gram-negative organism supports the need for periodic evaluation of the microbial status of indwelling catheters. The profile of bacterial species isolated from a given catheter site was not unique and was widely variable between sites on the same animal and among animals.

Despite lack of overt clinical signs of infection, all sheep had gross and microscopic renal damage including infarcts and membranoproliferative glomerulonephritis. Similar findings were noted in a study of catheterized baboons (*Papio cynocephalus*) in which Leary and others[9] reported immune complex-type membranoglomerulonephritis and hepatic dysfunction in baboons with long-term, indwelling, intravascular catheters; *Staphylococcus aureus* was cultured from those animals. Although the mode of entry of microorganisms into the indwelling catheters and their subsequent colonization is a matter of debate,[3] conceivably the skin microbial flora of the host animals constitute a key determinant in the process. In a recent study by Foley and others,[6] rats remained clinically asymptomatic despite infection with *Staphylococcus intermedius* and *S. aureus* and formation of thrombi in their jugular vein catheters.

Our study indicates that bacterial colonization of catheters in sheep itself may not lead to any overt clinical manifestations, at least in the short term. However, once infected, the indwelling catheters triggered the development of immune-mediated kidney lesions, documented by deposits of IgG in glomeruli of these animals. Glomerulonephritis occurred in a patient with chronic *Staphylococcus epidermidis* infection associated with long-term central venous catheterization.[13] Heidel and others[8] documented the presence of complement components 3 and 4 in addition to granular deposits of IgG, IgM, and IgA in the glomeruli of 27 of 60 catheterized baboons that showed signs of renal disease. Although some of the complement components are likely present along with IgG complexes in the affected glomeruli, our efforts to demonstrate sheep C4 using a rabbit antibody specific to human C4c (likely to cross-react with sheep C4c) were unsuccessful.

Catheter infection occurred despite the use of aseptic technique whenever accessing the catheters. Unlike our findings of multiple species of gram-negative bacteria colonizing catheter tips, catheter infections in humans and primates were predominantly associated with gram-positive cocci.[3,17] Perhaps the enteric microflora present in the housing environment and on the skin and wool coat of our sheep is responsible for the observed differences in bacteria species colonizing their catheters. We did not examine the antibiotic sensitivity of our bacterial isolates. Because these animals had been treated prophylactically with antibiotics after surgery and their catheters were sealed with saline containing a broad-spectrum antibiotic, some of the bacteria isolated in this study could have had altered antibiotic resistance profiles. In a recent study involving intravenously catheterized rhesus macaques (*Macaca mulatta*), it was demonstrated that approximately 48% of the infections involved methicillin-resistant gram-positive bacteria.[17]

Catheter design and maintenance are additional potential factors that might have influenced the prevalence of complica-

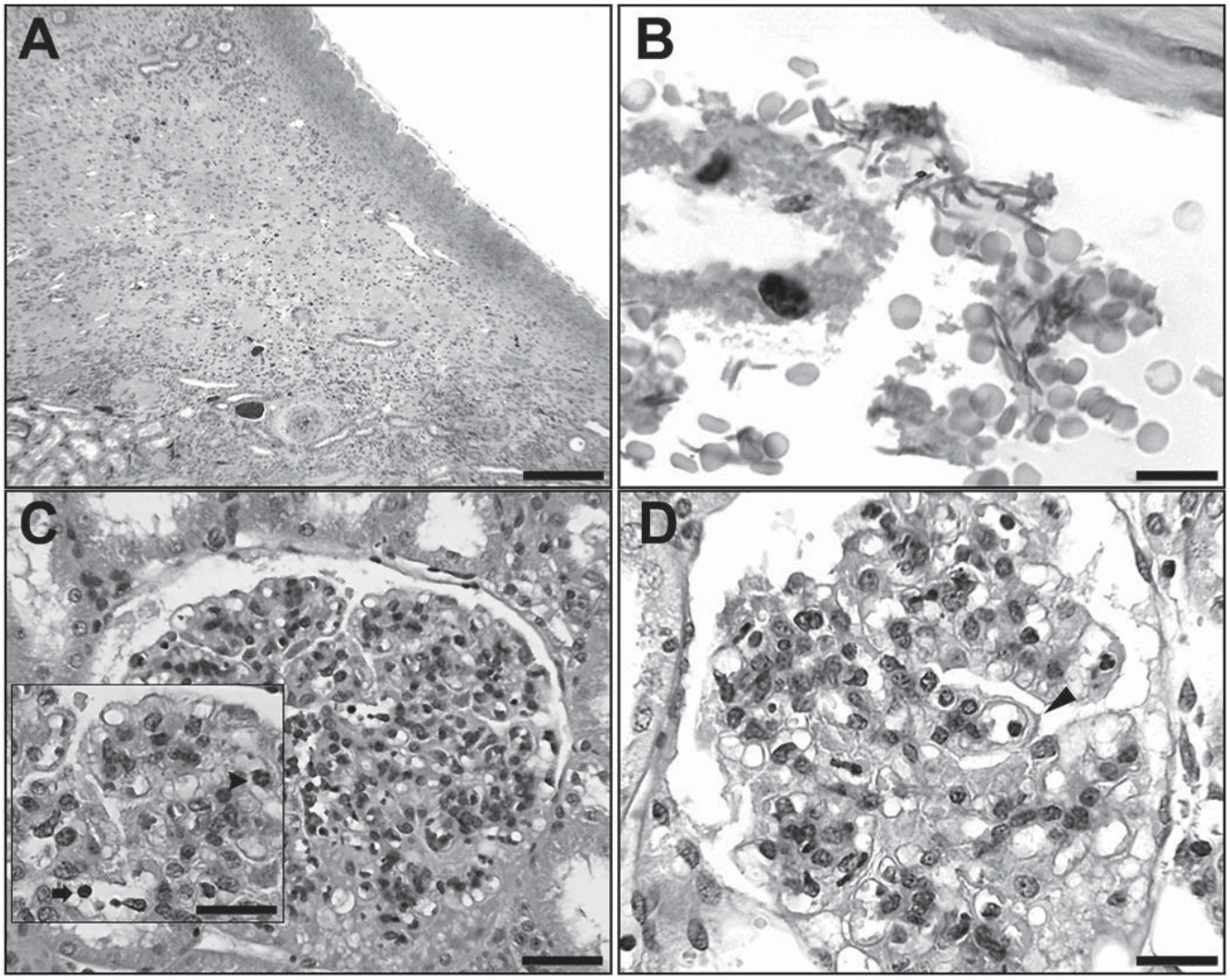

Figure 2. Renal histopathology of catheterized sheep. (A) Renal infarct. Loss of renal parenchyma, fibrosis and hemorrhage in the margins of the lesion. (B) Aggregates of gram-negative bacteria in the lumen of arcuate artery. (C) Membranoproliferative glomerulonephritis. Enlarged glomerulus, increased cellularity and widened mesangial regions. Inset is higher magnification of part of C showing in detail the thickened capillary walls due to mesangial interposition and the inflammatory cell infiltration (arrow, lymphocyte; arrowhead, neutrophil). (D) Thickening of the basement membranes, increased mesangial matrix and double contours (arrowhead) of the walls of the capillary loops. (A) bar, 250 µm; (B) bar, 10 µm; (C) bar, 50 µm; (C inset) and (D) bar, 25 µm. (A) and (C), hematoxylin and eosin stain; (B) Gram stain; (D) periodic acid Schiff stain.

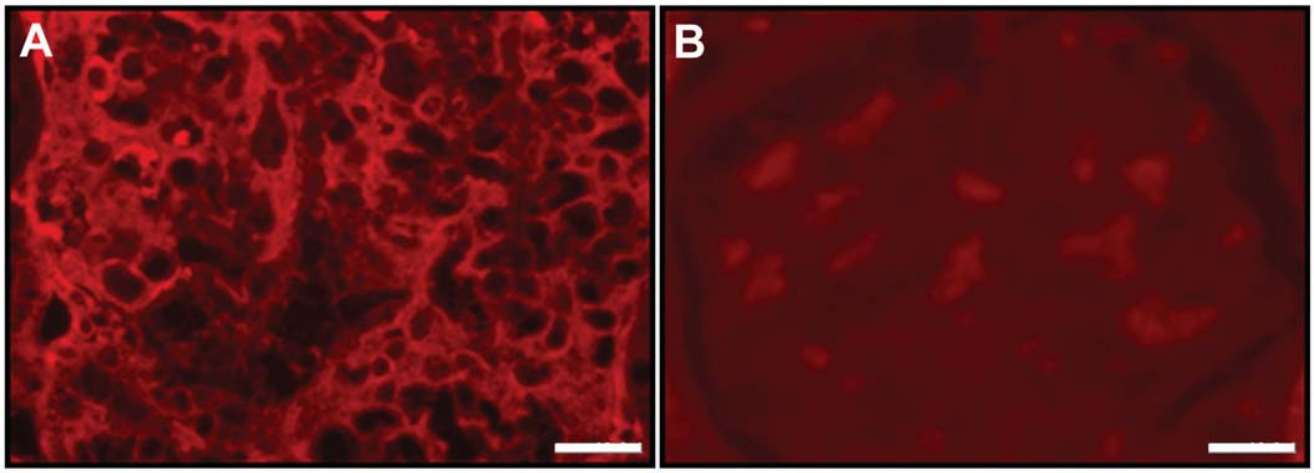

Figure 3. Demonstration of immune-mediated IgG-complex deposits in kidney sections by direct immunofluorescent antibody technique using rabbit anti-sheep IgG conjugated with tetramethyl rodamine isothiocyanate. (A) Kidney from catheterized sheep showing segmental, irregular, coarsely granular to confluent capillary wall and mesangial deposits of IgG complex. (B) Control kidney from noncatheterized animals lacking such staining pattern. Bars, 25 µm; tetramethyl rodamine isothiocyanate stain.

tions. Catheter cuffs of the type used in this study are unlikely to impede the migration of bacteria from the skin adjacent to the catheter exit site because ingrowth of fibroblasts into the cuff itself does not occur. Independent of contamination by skin microflora, vascular catheters also may be convenient adherence sites for bacteria that have made their way intravascularly by other mechanisms. In this regard, catheter tip morphology might have predisposed to thrombus formation or bacterial adherence.[4]

In general, catheter-associated infection is an underdiagnosed event and often results in prolonged hospitalization, increased treatment costs, and increased morbidity and mortality in humans.[3,15] In animals, infections associated with long-term catheterization are potentially detrimental not only to their well-being but are likely to cause variation in the experimental data obtained from such animals. In the study reported here, data were collected early in the postsurgical course, minimizing the potential for adverse effect on research objectives. Catheterized sheep continued to be housed pending data analysis, in case additional studies were warranted. In conclusion, our study suggests that routine microbiologic monitoring of catheter implants and assessment of kidney function need to be considered in subjects instrumented for long-term vascular access.

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