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Antimicrobial susceptibility of *Actinobacillus pleuropneumoniae*, *Escherichia coli*, and *Salmonella choleraesuis* recovered from Taiwanese swine

Chao-Fu Chang, Lin-Chu Chang, Yung-Fu Chang, Michael Chen, Tai-Sheng Chiang

Abstract. Minimum inhibition concentrations (MICs) were determined for ampicillin, ceftiofur, cephalothin, chloramphenicol, enrofloxacin, gentamicin, lincomycin, lincospectin (lincomycin/spectinomycin), neomycin, premafloxacin, spectinomycin, sulfamethoxazole/trimethoprim, and tetracycline against a total of 180 isolates of *Actinobacillus pleuropneumoniae*, *Escherichia coli*, and *Salmonella choleraesuis* (60 each) clinically isolated from pigs on farms in Taiwan from 1994 to 1996. No more than 3 isolates per farm were used. Ceftiofur had the highest activity in vitro against isolates of *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis*, with MIC₉₀ values of 0.03, 2, and 1 µg/ml, respectively. Premafloxacin was highly active against isolates of *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis*, with MIC₉₀ values of 2, 8, and 0.5 µg/ml, respectively, which were lower than those with enrofloxacin (MIC₉₀ 8, 32, and 2 µg/ml, respectively). Neomycin was moderately active against *A. pleuropneumoniae* and *E. coli*, with MIC₉₀ values of 8 and 64 µg/ml, respectively, but was inactive with *S. choleraesuis*. Gentamicin showed high activity against *A. pleuropneumoniae* (MIC₉₀ of 2 µg/ml) but was only moderately active with *E. coli* and *S. choleraesuis* (MIC₉₀ of 64 and 32 µg/ml). Cephalothin was highly active against isolates of *A. pleuropneumoniae* (MIC₉₀ of 1 µg/ml) but was inactive with *E. coli* (MIC₉₀ of 128 µg/ml). Lincomycin had moderate activity (MIC₉₀ of 32 µg/ml) against *A. pleuropneumoniae*. Chloramphenicol, lincomycin, and tetracycline were inactive with *E. coli* and *S. choleraesuis* (MIC₉₀ > 128 µg/ml). In conclusion, ceftiofur and premafloxacin were highly active against isolates of *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis*; enrofloxacin and gentamicin were highly to moderately active; cephalothin was highly active against *A. pleuropneumoniae* and moderately active against *S. choleraesuis*; chloramphenicol, lincomycin, and tetracycline were active only with *A. pleuropneumoniae*; neomycin was moderately active against *A. pleuropneumoniae* and *E. coli*. The other antimicrobials tested were inactive.

During the 1990s, pig respiratory disease complex (PRDC) emerged in Taiwan as the most important disease of swine. Porcine respiratory and reproductive syndrome virus, swine influenza virus, hog cholera virus, pseudorabies virus, *Actinobacillus pleuropneumoniae*, *Salmonella choleraesuis*, *Escherichia coli*, *Pasteurella multocida*, *Streptococcus suis* type II, and *Mycoplasma hyopneumoniae* were the most commonly isolated viral and bacterial agents from clinical PRDC cases in Taiwan.^{2–4,9} Although sound management, sanitation, nutrition, and vaccination are vital to successful prevention and control of PRDC on pig farms, appropriate selection and use of antimicrobials for the treatment and control of bacterial infections is the key to avoiding economic loss.^{2–4,9}

Among these bacterial pathogens, *A. pleuropneumoniae*, *S. choleraesuis*, and *E. coli* are particularly troublesome since they can induce pneumonia and acute septicemia with high mortality and can acquire resistance to antimicrobials. *Escherichia coli* predominated in suckling pigs, *S. choleraesuis* was found in

nursery and grower pigs, and *A. pleuropneumoniae* was encountered most frequently in grower to finisher pigs.

The purpose of this study was to investigate the susceptibility of *A. pleuropneumoniae*, *S. choleraesuis*, and *E. coli* to ampicillin, ceftiofur, cephalothin, chloramphenicol, enrofloxacin, gentamicin, lincomycin, lincospectin (lincomycin/spectinomycin), neomycin, premafloxacin, spectinomycin, sulfamethoxazole/trimethoprim, and tetracycline. All except ceftiofur^a were purchased from a single source.^b

A total of 180 porcine isolates were tested in the study. Among them were 60 isolates of each of *A. pleuropneumoniae*, *S. choleraesuis*, and *E. coli* from clinical cases on pig farms in Taiwan from 1994 to 1996. No more than 3 isolates per farm were used, and they were identified by conventional methods.¹² National Committee for Clinical Laboratory Standards (NCCLS)-recommended American Type Culture Collection (ATCC) isolates, *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *A. pleuropneumoniae* ATCC 27090, and *Pseudomonas aeruginosa* ATCC 27853, were used as reference strains for quality control.^{8,10}

Agar-dilution minimum inhibition concentration (MIC) testing was performed as previously described.^{8,10} Mueller–Hinton agar^c was used for *E. coli*

From the Department of Veterinary Medicine, National Taiwan University, Taipei 106, Taiwan (C-F Chang, L-C Chang, Chiang), the Department of Population Medicine and Diagnostic Science, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 (Y-F Chang), and the Animal Health Division, Pharmacia and Upjohn Market Co. Ltd., Taipei 104, Taiwan (Chen).

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and *S. choleraesuis* and veterinary fastidious agar¹⁰ was used for *A. pleuropneumoniae*. Inoculum was dispensed with a replicator^d with 3-mm pins according to the manufacturer's instructions.

The MICs of various antimicrobials to swine bacterial pathogens have been compared in several countries.^{1,13} Because antimicrobial resistance is also a serious problem for the swine industry in Taiwan, the MIC test was performed using 3 bacterial species, *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis* isolated from pigs. The in vitro activity of various antimicrobials to Taiwanese swine isolates of *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis* are listed in Tables 1, 2, and 3, respectively. The MICs for the isolates tested are summarized in Table 4. Ceftiofur had the highest activity in vitro against isolates of *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis*, with MIC₉₀ values of 0.03, 2, and 1 µg/ml, respectively. Ceftiofur was highly active against cattle and swine respiratory-disease pathogens.^{13,15} Minimum inhibition concentrations of 2 µg/ml were obtained with *E. coli*, which is, apparently, higher than previous reports.^{13,15} Whether the difference is due to emerging resistance secondary to heavy use of this antimicrobial in Taiwan is unclear.

The fluoroquinolones generally exhibit activity against Gram-negative bacteria.¹¹ Of these, premarloxacin was highly active with *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis*, with MIC₉₀ values of 2, 8, and 0.5 µg/ml, respectively. Enrofloxacin (8, 32, and 2 µg/ml, respectively) showed weaker activities than did premarloxacin. Enrofloxacin is an efficacious agent for treatment of swine bacterial disease in the United States and Europe.^{1,7,13,14} However, antimicrobial resistance has been well documented since enrofloxacin was approved for use in animals in Taiwan.⁴ Again, this may be due to heavy use of enrofloxacin in the swine industry in Taiwan.

Neomycin was moderately active against *A. pleuropneumoniae* and *E. coli*, with MIC₉₀ values of 8 and 64 µg/ml, respectively, but was inactive with *S. choleraesuis*. Gentamicin showed significant activity with *A. pleuropneumoniae* (MIC₉₀ of 2 µg/ml) but was only moderately active with *E. coli* and *S. choleraesuis* (64 and 32 µg/ml, respectively). Aminoglycosides are poorly absorbed following oral administration, bind to a low extent to plasma proteins (less than 25%), and show low efficiency in penetrating cellular barriers and entering cells. These antibiotics can produce varying degrees of vestibular damage (streptomycin, gentamicin) or cochlear damage (amikacin, kanamycin, neomycin). In patients with prolonged therapy and excessively high serum concentrations, aminoglycosides (especially neomycin and gentamicin) can also cause acute renal tubular necrosis.¹¹ Therefore, these antibi-

Table 1. In vitro activities of various antimicrobial agents against Taiwanese swine isolates of *A. pleuropneumoniae*.

Antimicrobial agents	Cumulative (%) MIC in µg/ml													
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Ampicillin						17 (28)		21 (35)	27 (45)	30 (50)	39 (65)	41 (68)	60 (100)	
Ceftiofur	56 (93)	58 (97)	60 (100)	7 (12)	11 (18)	17 (28)	58 (97)	59 (98)	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)	
Cephalothin	4 (7)	5 (8)	13 (22)	24 (40)	49 (82)	56 (93)	58 (97)	59 (98)	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)	
Chloramphenicol	4 (7)				24 (40)	35 (58)		36 (60)	46 (77)	57 (95)	58 (97)	60 (100)	60 (100)	
Enrofloxacin	3 (5)	9 (15)	14 (23)	25 (42)	33 (55)	39 (65)	56 (93)	52 (87)	58 (97)	60 (100)				
Gentamicin	1 (2)	12 (20)	14 (23)	15 (25)	19 (32)	38 (63)		60 (100)						
Licomycin								3 (5)	5 (8)	32 (53)	54 (90)	60 (100)	60 (100)	
Licospectin											35 (58)	51 (85)	60 (100)	
Neomycin						43 (72)	57 (95)	59 (98)	60 (100)	58 (97)	60 (100)			
Premarloxacin	12 (20)	22 (37)		32 (53)	40 (67)		2 (3)	19 (32)	57 (95)					
Spectinomycin														
Sulfa/trimethoprim					8 (13)	10 (17)	12 (20)	12 (20)	16 (27)	20 (33)	3 (5)	15 (25)	18 (30)	60 (100)
Tetracycline				1 (2)	7 (12)	9 (15)	12 (20)	13 (22)	48 (80)	56 (93)	59 (98)	60 (100)	60 (100)	

Table 2. *In vitro* activities of various antimicrobial agents against Taiwanese swine isolates of *E. coli*.

Antimicrobial agents	Cumulative (%) MIC in µg/ml													
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Ampicillin				19 (32)	32 (53)	47 (78)	3 (5)	12 (20)	16 (27)	17 (28)		18 (30)	28 (47)	60 (100)
Ceftiofur							54 (90)	60 (100)						
Cephalothin									10 (17)	22 (37)	31 (52)	45 (75)	60 (100)	
Chloramphenicol							1 (2)	2 (3)	6 (10)	7 (12)	12 (20)	17 (28)	27 (45)	60 (100)
Enrofloxacin	5 (8)	8 (13)	10 (17)	17 (28)	27 (45)	31 (52)	35 (58)	36 (60)	42 (70)	51 (85)	59 (98)		60 (100)	
Gentamicin				6 (10)	11 (18)				16 (27)	41 (68)	51 (85)	56 (93)	60 (100)	
Licomycin											1 (2)	2 (3)		60 (100)
Licospectin										5 (8)	7 (12)		13 (22)	60 (100)
Neomycin				1 (2)	2 (3)	3 (5)	9 (15)	12 (20)		21 (35)	45 (75)	55 (92)	60 (100)	
Premafloxacin	4 (7)	12 (20)	20 (33)	31 (52)	32 (53)	36 (60)	44 (73)	48 (80)	60 (100)					
Spectinomycin								1 (2)		5 (8)	9 (15)	10 (17)	17 (28)	60 (100)
Sulfa/trimethoprim						1 (2)	4 (7)	6 (10)		14 (23)	23 (38)	24 (40)		60 (100)
Tetracycline								9 (15)	13 (22)	15 (25)	16 (27)	18 (30)	30 (50)	60 (100)

Table 3. *In vitro* activities of various antimicrobial agents against Taiwanese swine isolates of *S. choleraesuis*.

Antimicrobial agents	Cumulative (%) MIC in µg/ml													
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Ampicillin						8 (13)	15 (25)	19 (32)	20 (33)			22 (37)		60 (100)
Ceftiofur		1 (2)	5 (8)	8 (13)	25 (42)	56 (93)	60 (100)							
Cephalothin					1 (2)	6 (10)	21 (35)	33 (55)	40 (67)	49 (82)	60 (100)			
Chloramphenicol						1 (2)	6 (10)	16 (27)	18 (30)	21 (35)	22 (37)	33 (55)	36 (60)	60 (100)
Enrofloxacin	7 (12)	16 (27)	27 (45)	32 (53)	38 (63)	51 (85)	59 (98)	60 (100)						
Gentamicin				17 (28)	22 (37)			25 (42)	31 (52)	41 (68)	60 (100)			
Licomycin												2 (3)	7 (12)	60 (100)
Licospectin											12 (20)	21 (35)	29 (48)	60 (100)
Neomycin				3 (5)	6 (10)	19 (32)	25 (42)	31 (52)	33 (55)			38 (63)	51 (85)	60 (100)
Premafloxacin	9 (15)	18 (30)	37 (62)	49 (82)	60 (100)									
Spectinomycin									1 (2)	6 (10)	14 (23)	19 (32)	28 (47)	60 (100)
Sulfa/trimethoprim							4 (7)	13 (22)	26 (43)	32 (53)	35 (58)			60 (100)
Tetracycline								13 (22)	14 (23)	15 (25)	21 (35)		53 (88)	60 (100)

Table 4. Summary of minimum inhibitory concentrations (MICs) for *A. pleuropneumoniae*, *E. coli*, and *E. choleraesuis**

	Organism (n = 60)		
	<i>A. pleuropneumoniae</i>	<i>E. coli</i>	<i>S. choleraesuis</i>
Ampicillin	0.25–128, 16, 128	2–>128, >128, >128	1–28, >128, >128
Ceftiofur	0.03–0.12, 0.03, 0.03	0.25–4, 0.5, 2	0.06–2, 1, 1
Cephalothin	0.03–8, 0.5, 1	8–12, 32, 128	0.5–32, 4, 32
Chloramphenicol	0.03–64, 1, 16	2–>128, >128, >128	1–>128, 64, >128
Enrofloxacin	0.03–16, 0.5, 8	0.03–128, 1, 32	0.03–4, 0.25, 2
Gentamicin	0.03–4, 1, 2	0.25–128, 16, 64	0.25–32, 8, 32
Licomycin	4–64, 16, 32	32–>128, >128, >128	64–128, >128, >128
Licospectin	32–128, 32, 128	16–>128, >128, >128	32–>128, >128, >128
Neomycin	2–32, 8, 8	0.25–128, 32, 64	0.25–>128, 4, >128
Premafloxacin	0.03–8, 0.25, 2	0.03–8, 0.25, 8	0.03–0.5, 0.12, 0.5
Spectinomycin	32–>128, >128, >128	4–>128, >128, >128	8–>128, >128, >128
Sulfamethoxazole/trimethoprim	0.5–128, 32, 128	1–>128, >128, >128	2–>128, 16, >128
Tetracycline	0.25–64, 8, 16	1–>128, 128, >128	4–>128, 128, >128

* Results are expressed as minimum inhibitory concentration in micrograms per ml as a range, MIC₅₀, and MIC₉₀, respectively, for each antimicrobial agent.

otics have certain limitations to their use in the treatment of bacterial infections.

Cephalothin, a first-generation cephalosporin,¹¹ was highly active against isolates of *A. pleuropneumoniae* (MIC₉₀ of 1 µg/ml), moderately active with *S. choleraesuis* (MIC₉₀ of 32 µg/ml), but inactive with *E. coli* (MIC₉₀ of 128 µg/ml). The lincomycin–spectinomycin combination creates synergetic effects if the 2 antibiotics are present in the proper ratio and the appropriate type of salts of both antibiotics are employed.^{5,13} Lincomycin was moderately active (MIC₉₀ of 32 µg/ml) with *A. pleuropneumoniae* but inactive against *E. coli* and *S. choleraesuis* (MIC₉₀ >128 µg/ml). These data are similar to the results of others that indicated lincomycin and spectinomycin are not very efficacious against *A. pleuropneumoniae* and *S. choleraesuis*.⁶

Chloramphenicol and tetracycline were highly active against *A. pleuropneumoniae* (MIC₉₀ of 15 and 14 µg/ml, respectively) but inactive against *S. choleraesuis* and *E. coli*.

Ampicillin was inactive against *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis* (MIC₉₀ of 108, >128, and >128 µg/ml, respectively). Sulfamethoxazole/trimethoprim, widely used in the swine industry in Taiwan, had relatively weak activity against *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis* (MIC₉₀ of 85, >128, and >128 µg/ml, respectively); these MIC₉₀ values are higher than those reported previously.^{6,8,13}

In conclusion, ceftiofur and premafloxacin were highly active; enrofloxacin and gentamicin were highly to moderately active against the isolates of *A. pleuropneumoniae*, *E. coli*, and *S. choleraesuis*; cephalothin lincomycin, licospectin, chloramphenicol, and tetracycline were highly to moderately active against *A. pleuropneumoniae* only; neomycin had moderate activity with *A. pleuropneumoniae* and *E. coli*; the oth-

er antibacterial agents tested had no activity against these 3 swine pathogens.

Sources and Manufacturers

- Pharmacia and Upjohn, Kalamazoo, MI.
- Sigma, St. Louis, MO.
- Difco, Detroit, MI.
- Cathra, Automad, MN.

References

- Aarestrup FM, Jensen NE: 1999, Susceptibility testing of *Actinobacillus pleuropneumoniae* in Denmark. Evaluation of three different media of MIC-determinations and tablet diffusion tests. *Vet Microbiol* 64:299–305.
- Chang CC, Chung WB, Lin MW, et al.: 1993, Porcine reproductive and respiratory syndrome (PRRS) in Taiwan I. Viral isolation. *J Chin Soc Vet Sci* 19:268–276.
- Chang SC, Chang HJ, Hsiao ML: 1998, Antibiotic usage in public hospitals in Taiwan. *J Microbiol Immunol Infect* 31:125–132.
- Chiu YT, Lu YP, Chang IC, et al.: 1998, Pathologic studies and antimicrobial susceptibility of *Salmonella choleraesuis* pneumonia in pigs. *J Chin Soc Vet Sci* 24:99–108.
- Eaves LE, Blackwell PJ, Fegan M: 1989, Characterization and antimicrobial sensitivity of haemophilii isolated from pigs. *Aust Vet J* 66:1–4.
- Fales WH, Morehouse LG, Mittal KR, et al.: 1989, Antimicrobial susceptibility and serotypes of *Actinobacillus (Haemophilus) pleuropneumoniae* recovered from Missouri swine. *J Vet Diagn Invest* 1:16–19.
- Gutierrez CB, Piriz S, Vadillo S, et al.: 1993, In vitro susceptibility of *Actinobacillus pleuropneumoniae* isolates to 42 antimicrobial agents. *Am J Vet Res* 54:546–550.
- Jorgensen JH, Turnidge JD, Washington JA: 1999, Antibacterial susceptibility tests: dilution and agar diffusion methods. In: *Manual of clinical microbiology*, ed. Murray PR, Baron EJ, Pfaller MA, et al., 7th ed., pp. 1526–1543. ASM Press, Washington, DC.
- Lu YP, Chen TH, Cheng IC, et al.: 1998, Sensitivity of chloramphenicol against porcine pathogenic bacteria in Taiwan. *J Chin Soc Vet Sci* 24:92–97.

10. National Committee for Clinical Laboratory Standards (NCCLS): 1999, Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, approved standard. NCCLS document M31-A. NCCLS, Wayne, PA.
11. Prescott JF, Baggot JD, Walker RD: 2000, Antimicrobial therapy in veterinary medicine, 3rd ed. Iowa State University Press, Ames, IA.
12. Quinn PJ, Carter ME, Markey BK, Carter GR: 1994, Clinical veterinary microbiology. Wolfe Publishing, London.
13. Salmon SA, Watts JL, Case CA, et al.: 1995, Comparison of MICs of ceftiofur and other antimicrobial agents against bacterial pathogens of swine from the United States, Canada and Denmark. *J Clin Microbiol* 33:2435–2444.
14. Smith IM, Mackie A, Lida J: 1991, Effect of giving enrofloxacin in the diet to pigs experimentally infected with *Actinobacillus pleuropneumoniae*. *Vet Rec* 129:25–29.
15. Watts J, Yancey RJ Jr, Salmon SA, et al.: 1994, A 4-year survey of antimicrobial susceptibility trends for isolates from cattle with bovine respiratory disease in North America. *J Clin Microbiol* 32:725–731.

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Placentitis, fetal pneumonia, and abortion due to *Rhodococcus equi* infection in a Thoroughbred

Janet C. Patterson-Kane, J. Mike Donahue, Lenn R. Harrison

Abstract. *Rhodococcus equi* is a rare cause of equine abortion. This report describes pyogranulomatous placentitis and fetal pneumonia in a case of abortion from a Thoroughbred mare. Numerous Gram-positive coccobacilli were noted histologically within macrophages in placental and pulmonary lesions. *Rhodococcus equi* was isolated in pure culture from the placenta, lung, liver, kidney, and stomach content. This is the first description of placentitis due to *Rhodococcus equi* infection in a horse.

Infection by *Rhodococcus equi* organisms carrying plasmid-encoded 15–17 kDa virulence antigens⁷ is an important cause of pneumonia, colitis, and tracheobronchial or colonic lymphadenitis in foals up to 6 months of age. Infection usually occurs by inhalation of contaminated dust,⁷ with intestinal infection in some foals following ingestion of bacteria expectorated from pulmonary lesions. The bacteria survive and replicate within macrophages by preventing phagosome–lysosome fusion.⁸ Few cases of abortion associated with isolation of *R. equi* from the equine fetus have been reported; in one case, pneumonia was described, but placental tissue was not available for examination.^{2,3,6,9} To the authors' knowledge, this is the first description of rhodococcal fetal pneumonia and placentitis in a horse.

A late-term aborted Thoroughbred fetus and placenta were submitted to the Department of Veterinary Sciences, Livestock Disease Diagnostic Center, University of Kentucky, Lexington, Kentucky, for postmortem examination. The fetus was within the chorioallantois, and a focally extensive region of the chorionic surface surrounding the cervical star was red. A small amount of meconium was noted on the dorsum of the neck of the fetus. The lungs were pale and consolidated, and specimens failed to float in 10% neutral buffered formalin. *Rhodococcus equi* was isolated in pure

culture from the placenta, lungs, liver, kidney, and stomach content. Direct fluorescent antibody tests conducted on kidney and placenta did not detect *Leptospira* spp. No antibodies to any of 6 serovars of *Leptospira* were detected in fetal pericardial fluid. Fluorescent antibody tests conducted on frozen sections of lung did not detect equine herpesvirus antigen. Routine tissue specimens were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin wax. Five-micrometer sections were mounted on glass slides and stained with hematoxylin and eosin (HE). Further sections of placenta and lung were stained with Brown and Brenn Gram stain (BB).

Significant histologic lesions were confined to the placenta and lungs. Inflammatory changes were noted in all examined sections from both horns and the body of the chorioallantois. The chorioallantoic stroma was expanded by moderate perivascular and interstitial infiltrates of macrophages, with smaller numbers of neutrophils and lymphocytes (Fig. 1); walls of some blood vessels were infiltrated by small numbers of the inflammatory cells. Many macrophages had an abundant cytoplasm containing numerous intracytoplasmic, Gram-positive coccobacilli (Fig. 1). The inflammatory cell infiltrates were most severe toward the allantoic surface of the membrane. The suballantoic stroma was expanded by small clear spaces with degeneration of collagen bundles; there was multifocal hypertrophy and hyperplasia of overlying allantoic epithelial cells. Small numbers of neutrophils and macrophages were loosely scattered within the amnionic membrane. Bronchioles contained small numbers of clustered, degenerate granulocytes. Alveolar spaces contained

From the Livestock Disease Diagnostic Center, 1429 Newtown Pike, Lexington, KY 40511-1280. Current address (Patterson-Kane): Department of Pathology and Infectious Diseases, The Royal Veterinary College, Hawkshead Lane, Hatfield, North Mymms, Hertfordshire AL9 7TA, UK.

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