Article

An investigation of exudative epidermitis (greasy pig disease) and antimicrobial resistance patterns of *Staphylococcus hyicus* and *Staphylococcus aureus* isolated from clinical cases

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Abstract – Exudative epidermitis (EE) is a common skin disease of young pigs, caused mainly by *Staphylococcus hyicus*. Increased prevalence of EE and poor response to treatment are reported. Common strategies used by Ontario pork producers to treat pigs with EE were determined using a survey. Injection of penicillin G was reported as the most common parenteral antibiotic choice. Antimicrobial resistance patterns of *S. hyicus* and *Staphylococcus aureus* isolated from clinical cases (30 herds with samples from approximately 6 pigs per farm) showed that 97% of *S. hyicus* isolates were resistant to penicillin G and ampicillin; 71% of these isolates were resistant to ceftiofur. Similar resistance was noted among *S. aureus* isolates. Antimicrobial resistance has become a problem in the treatment of EE in Ontario.

Résumé – Enquête sur l'épidermite exsudative (eczéma séborrhéique du porc) et les tendances d'antibiorésistance de *Staphylococcus hyicus* et de *Staphylococcus aureus* isolés des cas cliniques. L'épidermite exsudative (EE) est une maladie cutanée courante des porcelets qui est causée principalement par *Staphylococcus hyicus*. Une prévalence accrue d'EE et une réponse mitigée au traitement sont signalées. Les stratégies couramment utilisées par les producteurs de porcs pour traiter les porcs atteints d'EE ont été déterminées à l'aide d'un sondage. L'injection de pénicilline G a été signalée comme le choix d'antiobiotique parentéral le plus courant. Les tendances d'antibiorésistance de *S. hyicus* et de *Staphylococcus aureus* isolés de cas cliniques (30 troupeaux avec des échantillons provenant d'environ 6 porcs par ferme) ont montré que 97 % des isolats de *S. hyicus* étaient résistants à la pénicilline G et à l'ampicilline; 71 % de ces isolats étaient résistants au ceftiofur. Une résistance semblable a été signalée pour les isolats de *S. aureus.* L'antibiorésistance est devenue un problème dans le traitement d'EE en Ontario.

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Introduction

E xudative epidermitis (EE), commonly known as "greasy pig disease" is a generalized or localized skin disease of piglets characterized by exfoliation, sebaceous exudation, and formation of a crust that may cover the entire body (1). The disease is most commonly caused by strains of *Staphylococcus hyicus* that produce exfoliative toxins (2,3). Less frequently, it was reported that the disease can also be caused by toxin-producing strains of *Staphylococcus aureus* and *Staphylococcus chromogenes* (4,5) since some of their staphylococcal exfoliative toxins can cleave the target molecule, swine desmoglein1 (6,7). Exfoliative

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Use of this article is limited to a single copy for personal study. Anyone interested in obtaining reprints should contact the CVMA office (hbroughton@cvma-acmv.org) for additional copies or permission to use this material elsewhere. toxin-producing staphylococci may also penetrate the epidermis directly since the exfoliative toxins cleave cell-to-cell adhesion in mammalian skin and then destroy the barrier function of the skin, with subsequent blister formation (7). Further, damage from biting (particularly newborns with unclipped needle teeth), or from scratches from rough bedding or rubbing against projections on pen walls, can expose the dermis and facilitate the entry of staphylococci, which are commonly present on pigs and in the environment, to start infection (1,8).

The disease occurs worldwide and is a sporadic endemic problem on most farms, but occasionally, major outbreaks involve large numbers of piglets. The recent trend by the swine industry to discontinue the practice of cutting the tips of needle teeth at birth, coupled with the trend of increased litter size, may lead to a rise in the prevalence of EE. There have been anecdotal reports that the disease has become more common and more difficult to treat.

The main objectives of this study were to determine what treatments for EE were being used in Ontario swine, to isolate *S. hyicus* and *S. aureus* from cases of EE, and to determine their antimicrobial resistance profiles.

Materials and methods

Animal use was approved by the University of Guelph Animal Care Committee and was in keeping with Canadian Council of Animal Care Guidelines.

Survey

A survey of pork producers (n = 58) was conducted to obtain information regarding treatment of EE. The researcher completed a questionnaire by interviewing pig farmers who attended a regional trade show (28/58), or alternatively, by interviewing pork producers who participated in a cross-sectional study of farms with cases of EE (30/58). Questions were related to herd type, treatments, and perception of the efficacy of medication, as well as questions about other approaches to control the disease, such as improving hygiene, management changes, and autogenous vaccine use. A survey of swine veterinarians (n = 15)was also conducted in order to obtain their opinions regarding recommendations for treatment and prevention, and whether or not they thought the disease was becoming more difficult to control. The questionnaire was distributed at a regional meeting of Ontario swine veterinarians and was completed during the meeting by all swine veterinarians in attendance. The responding swine veterinarians constituted 53.6% of all Ontario swine veterinarians who practiced in the region (15/28).

Cross-sectional study: Bacterial culture and antimicrobial susceptibility test

Thirty pig farms from southwestern Ontario (Canada) were purposively selected for the study. The inclusion criteria for the cross-sectional study included farms that veterinary practitioners identified as having an outbreak of EE, as well as local farms that were conveniently chosen and identified by the researcher as having pigs with clinical signs of EE. One hundred and eighty-six pigs from the 30 farms were included in the study. An average of 6 pigs per farm (range: 4 to 10) was chosen for sampling. Pigs with localized or systemic clinical signs of EE were chosen. Generally, pigs with the most severe lesions were selected over pigs with mild clinical signs. When large numbers of pigs with clinical disease were present, attempts were made to select from different pens and rooms. However, if only a small number of affected pigs were available, then multiple piglets from the same litter or the same pen were sometimes included. Skin sampling from the facial lesions of pigs affected by EE was accomplished with 1 scraping and 1 swab per pig. Skin scabs from pig facial lesions were scraped into a sterile container by using a melon-baller. The melon-baller was cleaned and disinfected with 70% isopropyl alcohol between pigs. Skin swabs were collected using cotton-tipped swabs after application of 1 mL 0.9% sodium chloride to the lesions. Skin scrapings were placed in empty clean tubes and cotton-tipped swabs were placed in liquid Stuart's medium and transported in a container in ambient temperature. All samples were submitted on the day of collection by taking them directly to the Animal Health Laboratory (AHL), University of Guelph, Ontario, Canada. Bacterial culture from skin samples and swabs was done, and isolates were identified as S. hyicus and S. aureus by standard

Table 1. Treatments for exudative epidermitis that farmers (n = 58) and veterinarians (n = 15) stated that they would use or recommend

	Farmers	Veterinarians	
Treatment	(%)	(%)	
Injectable antibiotic only	17.2	6.7	
Topical oil only	12.0	0	
Topical antibiotic + topical oil +			
injectable antibiotic	10.3	13.3	
Antiseptic + injectable antibiotic	8.6	0	
Topical oil + injectable antibiotic	8.6	0	
Topical antibiotic + antiseptic +			
topical oil + injectable antibiotic	5.2	66.7	
Topical antibiotic + topical oil	5.2	0	
Antiseptic + topical oil + injectable			
antibiotic	5.2	6.7	
Antiseptic + topical oil	3.4	0	
Topical antibiotic + antiseptic +			
injectable antibiotic	3.4	0	
Topical antibiotic + antiseptic +			
topical oil	1.7	6.7	
Topical antibiotic + injectable antibiotic	1.7	0	
Leave untreated	17.2	0	

laboratory techniques including colony morphology, hemolysis, Gram-stain, catalase reaction, and coagulase reaction. The recovery rates of the 2 pathogens were determined at the herd and pig levels. Antimicrobial susceptibility to penicillin G, ampicillin, ceftiofur, spectinomycin, sulphonamide, tetracycline, tiamulin, and trimethoprim/sulfamethoxazole was determined by the disk diffusion method (Kirby-Bauer Procedure) defined by the Clinical and Laboratory Standards Institute (9). For the purpose of analysing the data, intermediate level was included with the resistant level (4 of the S. aureus isolates). The antimicrobial resistance patterns of S. hyicus and S. aureus isolates were compared between 2 categories of farms: commercial farms that used antibiotics and "antibiotic-free" farms. The first group were the farms on which antibiotics were generally used routinely in-feed and by injection as needed to prevent or treat disease. The second group of farms ("antibiotic-free") raised pigs for a special market so that pigs from birth-to-market were raised without receiving antibiotics. On these farms, if a pig became sick and needed treatment, it would be identified and removed from the production stream. Seven of the 30 farms in the cross-sectional study were categorized as "antibiotic-free" farms.

Data management and statistical analysis

The survey data from the questionnaires for farmers and swine veterinarians were entered into EpiData Entry v.3 (The EpiData Association, Odense, Denmark) and verified manually for accuracy of entry. Descriptive statistics were carried out using Stata10.1 (Statistics/Data Analysis, Texas, and USA). The results of the antimicrobial susceptibility tests of *S. hyicus* and *S. aureus* isolates were entered into Microsoft Office Excel 2007 and subsequently all data were transferred to Stata10.1 for statisticcal analysis.

A statistical analysis to compare the difference in antimicrobial resistance to tetracycline between *S. hyicus* and *S. aureus* isolates was performed using logistic regression with herd as a random effect on the intercept. The dependent variable was the tetracycline-resistance status of an isolate (yes/no) and the

Table 2. Antimicrobial resistance profiles for *S. hyicus* (n = 142) and *S. aureus* (n = 89) isolates from pigs with clinical signs of exudative epidermitis (all 30 farms)

Antimicrobial	S. hyicus		S. aureus	
	% Resistant	95% CI	% Resistant	95% CI
Penicillin G	97.2	94-100	92.1	86–98
Ampicillin	97.2	94-100	92.1	86-98
Ceftiofur	71.1	64-77	76.4	67-85
Spectinomycin	45.1	37-53	48.3	38-59
Sulfonamide	8.5	4-13	13.5	6-21
Tetracycline	55.6	47-64	87.6	81-91
Tiamulin	31.0	23-39	15.7	8-23
Trimethoprim/sulfa	2.1	0-5	0	N/A

CI — Confidence interval.

N/A — Not available

independent variable was species designation of *Staphylococcus* (i.e., *S. hyicus* or *S. aureus*).

The association of the prevalence of antimicrobial resistance to each of the antimicrobials (penicillin G, ampicillin, ceftiofur, tetracycline, streptomycin, tiamulin, sulphonamide, and trimethoprim/sulfamethoxazole) of *S. hyicus* and *S. aureus* separately between the commercial farms that used antibiotics and the "antibiotic-free" farms was evaluated using 2 sets of univariable Poisson regression models. Number of *S. hyicus* or number of *S. aureus* isolates that were resistant to each antimicrobial was used as a dependent variable in a separate univariable Poisson model. Number of antimicrobial susceptibility tests performed for the corresponding staphylococci was used as an offset, and herd-level categorical variable indicating antimicrobial usage on a farm was used as an independent variable (i.e., "antibiotic-free" farms versus commercial farms that used antibiotics).

Results

Survey for treatment of exudative epidemitis

The most common approach to treatment of EE (41/58 farmers) was topical therapy, including mixtures of topical antibiotics, antiseptics, and/or mineral oil, mostly in the form of a spray (Table 1). The most frequently used topical antibiotic treatment was a mixture of procaine penicillinG and novobiocin (Novodry; Pfizer Canada, Kirkland, Quebec) (69%), penicillin G (18.7%) and cephapirin benzathine (Cefa-dri; Wyeth Animal Health, Guelph, Ontario) plus cloxacillin benzathine (Dry-Clox; Wyeth Animal Health) (6.2%). In addition, 55.2% of respondents (32/58) stated that they used injectable antibiotics and most farmers using this method (93.8%; 30/32) indicated that they preferred to use injectable penicillin G. The other injectable antibiotics chosen by a small number of producers were trimethoprim/sulfadoxine (6.3%; 2/32), ceftiofur (3.1%; 1/32), and streptomycin (3.1%; 1/32). One farmer reported using ivermectin for treatment of clinical cases of EE.

Swine veterinarians commonly recommended novobiocin (66.7%; 10/15) as a topical treatment. In the case of antibiotics for injection, 40% of veterinarians (6/15) recommended penicillin G and 26.7% (4/15) of the veterinarians recommended ceftiofur, followed by 20% (3/15) for trimethoprim/sulfadoxine. Swine veterinarians reported that they also commonly recommended clipping needle teeth (12/15), reducing humidity (6/15), changing ventilation (4/15), and improving hygiene

(14/15). Approximately a quarter of the veterinarians (4/15) recommended autogenous vaccines as an aid to controlling EE, but only 5% of the farmers (3/58) considered vaccination to be an option. Five swine practitioner respondents (33.3%) in surveys expressed some concern that response to treatment was poor.

Cross-sectional study: Bacteriology and antimicrobial susceptibility testing

The recovery rate of *S. hyicus* from skin samples was 76.9% (143/186) and the recovery rate of *S. aureus* was 48.9% (91/186) based on parallel interpretation of the 2 methods of sampling, skin scraping and skin swabs. Both *S. hyicus* and *S. aureus* were cultured from 39.8% of pigs (74/186), whereas *S. hyicus* was cultured alone from 33.9% of pigs (63/186) and *S. aureus* was cultured alone from 6.5% of the pigs (12/186). At the farm level, the recovery rate of *S. hyicus* was 100% (30/30) and the recovery rate of *S. aureus* was 80% (24/30), based on at least 1 positive isolate from a farm.

The overall antimicrobial resistance profiles are presented in Table 2. Antimicrobial susceptibility testing revealed that most *S. hyicus* and *S. aureus* isolates were resistant to the β -lactam antibiotics: penicillin G, ampicillin, and ceftiofur. Over 90% of isolates of *S. hyicus* and *S. aureus* were resistant to penicillin G and ampicillin. Over 70% of isolates of *S. hyicus* and *S. aureus* were resistant to ceftiofur. Antimicrobial resistance of *S. hyicus* (55.6%) and *S. aureus* (87.6%) to tetracycline was also common. Antimicrobial resistance patterns of the 2 pathogens were very similar, except that resistance to tetracycline was higher in *S. aureus* than in *S. hyicus* [odds ratio (OR): 14.29, 95% confidence interval (CI): 4.50–47.62, P < 0.01].

Resistance to 1 or more antimicrobials was detected in 99.3% (142/143) of S. hyicus isolates. Resistance to 5 or more antimicrobials was detected in 40.6% (58/143) of S. hyicus isolates. The most common resistance patterns for S. hyicus isolates were penicillin G-ampicillin-ceftiofur (24.5%, 35/143), penicillin G-ampicillin-ceftiofur-spectinomycin-tetracycline-tiamulin (12.6%, 18/143), penicillin G-ampicillin-spectinomycintetracycline-tiamulin (11.2%, 16/143), and penicillin G-ampicillin-ceftiofur-tetracycline (9.1%, 13/143). Resistance to 1 or more antimicrobials was detected in 98.9% (90/91) of S. aureus isolates. Resistance to 5 or more antimicrobials was detected in 39.6% (36/91) of S. aureus isolates. The most common resistance patterns of S. aureus isolates were penicillin G-ampicillin-ceftiofur-tetracycline (28.6%, 26/91), penicillin G-ampicillin-ceftiofur-spectinomycin-tetracycline (22.0%, 20/91), penicillin G-ampicillin-tetracycline (8.8%, 8/91), and penicillin G-ampicillin-ceftiofur (7.7%, 7/91).

When examined descriptively, the difference in prevalence of resistance of *S. aureus* isolates to penicillin G and ampicillin between commercial farms that used antibiotics and "antibioticfree" farms was 24.9% (Figure 1). Similarly, there was a lower prevalence of resistance of *S. aureus* isolates from "antibioticfree" farms to all other antimicrobials (with the exception of tiamulin) (Figure 1). In contrast, the prevalence of resistance of *S. hyicus* isolates from "antibiotic-free" farms to penicillin G, ampicillin, and ceftiofur was numerically higher than for isolates

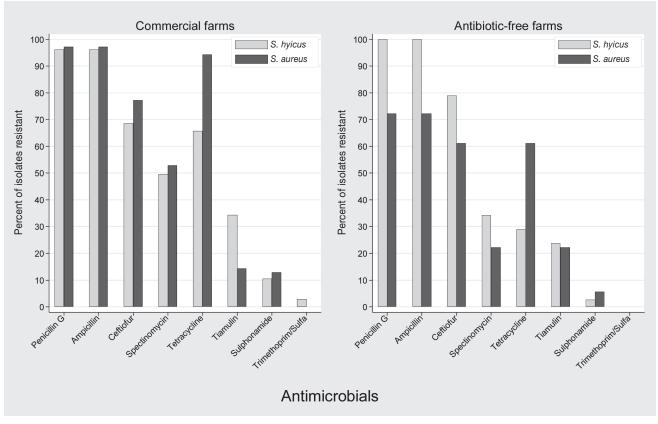


Figure 1. Antimicrobial resistance profiles of *S. hyicus* and *S. aureus* isolates from commercial farms that used antibiotics (n = 23) and isolates from "antibiotic-free" farms (n = 7).

from farms that used antibiotics (Figure 1). However, there was no significant difference in prevalence of antimicrobial resistance of *S. hyicus* isolates (P > 0.328) or *S. aureus* isolates (P > 0.486) to any antimicrobial when "antibiotic-free" farms and commercial farms that used antibiotics were compared.

Discussion

Exudative epidermitis (EE) is a sporadic disease that causes significant problems and economic losses on certain farms, particularly those that are newly populated (1). Mortality and morbidity may be high during an outbreak of EE. However, even mild expressions of the disease can negatively influence the price of feeder pigs, because the readily visible skin lesions make weanling pigs with clinical signs of EE difficult to sell. It is possible that recent trends in the industry such as an increase in litter size and a move to not clip needle teeth at birth may be leading to an increase in EE.

The traditional treatment for EE has been the prompt use of antiseptics for wounds or injection of clinically affected pigs with procaine penicillin G (10). The surveys of pork producers and veterinarians demonstrate that penicillin G is still considered an appropriate drug for treatment of EE, but antimicrobial susceptibility results strongly contradict this idea. Studies from other countries have also demonstrated a high level of resistance to penam penicillins among *S. hyicus* isolates (11–18).

The Danish national monitoring program showed the antimicrobial resistance profiles of bacteria from diagnostic submissions (17). In the report from this program, *S. hyicus* isolates from cases of skin disease (2001 to 2008) showed a moderately high resistance to penicillin G (60% to approximately 80%) (19). The present study of Ontario pigs shows a much higher proportion of resistance with over 90% of isolates from cases of EE resistant not only to penicillin G, but in most cases, to other members of the β -lactam family of antibiotics, including ampicillin and ceftiofur. These results help to explain the poor response to treatment of EE reported by farmers, because penicillin G as seen in the study was the farmers treatment of choice to resolve EE. There should be more timely and regional antimicrobial resistance profiles to provide guidelines for using effective antimicrobials (20,21).

Overall the antimicrobial resistance patterns for *S. hyicus* and *S. aureus* were similar in all isolates from 30 farms; however, tetracycline resistance was more common in *S. aureus* isolates compared with *S. hyicus* isolates with the penicillin G-ampicillin-ceftiofur resistance pattern being the most prevalent in *S. hyicus* isolates and penicillin G-ampicillin-ceftiofurtetracycline resistance pattern being the most prevalent pattern in *S. aureus* isolates. When examined descriptively, frequency of resistance of *S. aureus* to β -lactam antibiotics appeared lower for isolates from "antibiotic-free" farms compared with isolates from farms that used antibiotics. The reverse was true for descriptive examination of *S. hyicus* isolates: the frequency of resistance to β -lactam antibiotics appeared lower for isolates from commercial farms compared with isolates from "antibiotic-free farms." It appeared that reduced antibiotic pressure was associated with a reduction of resistance in the *S. aureus* population but not in the *S. hyicus* population. Selection of *S. aureus* strains with resistance plasmids or chromosomally encoded resistance genes might be an explanation of this phenomenon. Information on how long farms had maintained their antibiotic-free status was not available for the present study and this knowledge might have been useful in interpreting the resistance data. In general, one can conclude that whether or not antimicrobials are being used on the farm, *S. hyicus* and *S. aureus* will likely be resistant to penam penicillins, at least according to *in-vitro* testing.

In the present study, the disk diffusion method (Kirby-Bauer Procedure) was used to test for antimicrobial susceptibility of S. hyicus and S. aureus. The antimicrobial susceptibility test results apply to the population of animals on the farm, but not necessarily to individual animals. The disk diffusion method has some limitations in extrapolation of the data to minimum inhibitory concentration (MIC) (22). Furthermore, when we apply the results of antimicrobial susceptibility testing to clinical cases, to achieve better treatment success, pharmacokineticpharmacodynamic parameters should be considered: the bound versus unbound state of the agent, tissue versus plasma concentrations, drug degradation over time, variations among microorganisms, and factors associated with the specific environment at the infection site (23). Failing to consider these parameters contributes to discrepancies between in-vitro results of antimicrobial susceptibility tests and clinical outcomes following using of the selected antimicrobials (24). Ceftiofur was the injectable antimicrobial second most frequently recommended by veterinarians in the present study. This antimicrobial is resistant to penicillinases so that it would seem more likely to be effective in the treatment of a staphylococcal infection that is resistant to penam penicillins. However, ceftiofur is not a good choice for staphylococcal infection because of its relatively high MIC90 (Minimal Inhibitory Concentration needed to inhibit the growth of 90% of the bacterial population, 1.0 μ g/mL). In addition, the MIC90 of desfuroylceftiofur (a metabolite of ceftiofur in the body) is 4.0 to 8.0 µg/mL, in contrast to that for other organisms such as Pasteurella multocida and Actinobacillus pleuropneumoniae (MIC90, 0.03 µg/mL). Thus, a higher dosage of ceftiofur is required to treat a S. hyicus infection than to treat other bacterial infections (22).

The antimicrobial agents that were tested in the study were limited; for example, the use of novobiocin was reported frequently, but it was not included in our antimicrobial susceptibility test. Information on antimicrobial resistance of *S. hyicus* and *S. aureus* to novobiocin would be useful for farmers and veterinarians.

In conclusion, the likely reason for the poor response to treatment of EE in the southwestern Ontario region in this study was the presence of widespread antimicrobial resistance of *S. hyicus* and *S. aureus* isolates, especially to β -lactam antibiotics. Therefore, pork producers and swine veterinarians would benefit from having bacterial culture and antimicrobial susceptibility tests done prior to treating EE diseased pigs; if that is not feasible trimethoprim-sulfa appears to be a reasonable choice in that almost all staphylococcal isolates examined in this study appeared to be susceptible *in vitro*.

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- ARTICLE
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Answers to Quiz Corner Les réponses du test éclair

- **1. d)** Diazepam is a centrally acting muscle relaxant that counteracts the muscle rigidity caused by ketamine alone.
 - d) Le diazépam est un relaxant musculaire qui agit centralement pour neutraliser la rigidité musculaire causée par l'utilisation de la kétamine seule.
- **2. b)** These breeds are predisposed to subaortic stenosis.
 - b) Ces races de chiens sont prédisposées à la sténose subaortique.
- b) Patients with chronic renal failure are often anemic because of erythropoietin deficiency.
 - b) Les animaux qui souffrent d'insuffisance rénale chronique sont souvent anémiques à cause d'une carence en érythropoïétine.
- **4.** e) Viral infections, such as canine distemper, are less likely causes of epistaxis than are the other conditions listed.
 - e) Les infections virales, comme le distemper canin, sont moins susceptibles de causer de l'épistaxis que les autres affections énumérées.
- b) The insulin requirement decreases with time because the insulin antagonism of glucocorticoids is lessened by reduced production of glucocorticoids.
 - b) Les besoins en insuline diminuent avec le temps parce que l'antagonisme insulinique des glucocorticoïdes est amoindri par la diminution de production des glucocorticoïdes.
- 6. d) E. coli is most likely to be involved with pyometra. The drug with the most efficacy against this bacterium should be chosen. Of the choices available, a second-generation cephalosporin would be most effective.

- d) E. coli est la plus susceptible d'être impliquée dans un pyomètre. Le médicament qui possède la plus grande efficacité contre cette bactérie doit être choisi. De tous les choix disponibles, une céphalosporine de deuxième génération est la plus efficace.
- 7. d) Cystorrhaphy is the surgical term for suturing the bladder.
 d) La cystorraphie est le terme chirurgical pour désigner la suture de la vessie.
- b) Acute and convalescent antibody titers are the most practical method of confirming equine influenza viral infection.
 - b) Les titres d'anticorps aigus et convalescents sont la méthode la plus pratique pour confirmer une infection virale d'influenza équine.
- **9.** e) Plasma fibrinogen concentrations become elevated as a result of a chronic inflammatory stimulus.
 - e) Les concentrations de fibrinogène plasmatique augmentent à la suite d'un stimulus inflammatoire chronique.
- **10.** b) Lymphosarcoma commonly involves the peripheral lymph nodes, right atrium, and spinal cord.
 - b) Le lymphosarcome implique communément les nœuds lymphatiques périphériques, l'oreillette droite et la moelle épinière.