



Newsline

California Association for Medical Laboratory Technology

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CAML Political Update

April 2015

By Public Policy Advocates, LLC

LEGISLATURE

The Legislature is now past the legislative bill introduction deadline of February 27. As of this writing 2,509 bills have been introduced. CAMLT was particularly challenged by the chiropractors and optometrists this last session—second runs at clinical laboratory science for both of these groups. While we have been able to stem and limit their efforts to date regarding clinical laboratory science, we anticipate that optometrists will be back with major scope expansion bills—most likely including incursion into clinical laboratory science without requisite training and education. Optometrists met with us to discuss introducing a bill this year that will expand their scope of practice to order and perform any laboratory test related to any medical condition that manifests in any way in the eye—even if it is not a condition of the eye. Though we have not seen a bill yet, they fully intend to revisit scope expansion into clinical laboratory science and we are expecting to see amendments to an existing bill. We are also seeing legislation sponsored by an equipment manufacturer to water down personnel standards for those testing for protein levels in plasma centers.

What does this all mean for CAMLT? CAMLT must be strong both financially and in membership. CAMLT is the only professional organization that exclusively protects the legislative interests of CLSs, MLTs and other laboratory personnel in the best interest of the patient and in the best interest of the laboratory profession. It is the only professional association representing clinical laboratory science that retains a lobbying firm in Sacramento to be the voice for clinical laboratory personnel in Sacramento before the legislature and state government. If CAMLT does not grow its membership or raise the necessary revenue to support its legislative program, there will be no legislative program to protect the profession and the patients it serves.

Organized labor has been extremely helpful in the past, and indeed, co-sponsored our CLS waived lab director bill. But labor unions also represent chiropractors, optometrists, and pharmacists in addition to CLSs. So Labor has not been in a position to take these other professions head on when they want to expand their scopes into clinical laboratory science. That has fallen to CAMLT. Other laboratory personnel professional associations may provide continuing education, certification, or fellowship—but none have a Sacramento presence or help to underwrite CAMLT's governmental representation on behalf of the entire profession. The chiropractors, optometrists, and pharmacists have strong associations, even though they number far fewer in terms of licensees state wide. They have

strong and expansive political grass roots networks, and they have Political Action Committees that contribute to candidates in the hundreds of thousands of dollars. The clinical laboratory profession must come together to unify and shore up its efforts to support CAMLT's legislative and political agenda if it wants to preserve its profession—not tomorrow, but now.

RECRUIT NEW CAMLT MEMBERS! CONTRIBUTE TO LAB-PAC!

This last legislative session was a very rigorous and active legislative session in terms of bills legislating a frontal attack on the CLS and MLT professions, depleting CAMLT resources. This session will be more of the same. Only you can ensure the growth and vibrancy of a strong, well organized CAMLT. Rise to the challenge. Recruit members to your professional organization — CAMLT. Contribute to your Lab-PAC. Meet with your legislators. Ensure the best possible patient safety in laboratory testing, and preserve your important profession.

LEGISLATION

The following is legislation that we are tracking after the close of the bill introduction deadline. Check the CAMLT website for updates on bill status, Legislator lists, and Committee assignments.

AB 599 (Bonilla) Clinical Laboratories: Cytotechnologists, as introduced 2/24/15 and amended 4/6/15 NEUTRAL/WATCH.

This bill is sponsored by the California Society of Pathologists and would authorize a licensed cytotechnologist to perform all tests and procedures pertaining to cytology, including, but not limited to, microscopic and nonmicroscopic methodologies and tests and procedures that utilize molecular or genetic methodologies. On April 6, the bill was amended to strike methodologies and add methodologies that are performed on cytologic specimens related to infectious disease or cancer diagnosis. Location: Heard April 14 in the Assembly Business and Professions committee.

Continued on page 3

IN THIS ISSUE . . .

POLITICAL UPDATE.....	1,3
MESSAGE FROM THE PRESIDENT	2
NOMINATIONS, CLS OF THE YEAR, NEWSLETTER AWARD	4
REVIEW SEMINARS	4
WISH LIST, E&R WINNERS.....	5
TWO (2) NEW CE COURSES.....	6-27
CE ANSWER FORM, CE ORDER FORM	28-29
DOMINICAN UNIVERSITY.....	30
MEMBERSHIP APPLICATION.....	31
2015 CONTINUING EDUCATION SCHEDULE	BACK COVER

Message From the President



CAMLT held its state-wide continuing education seminars on March 21st and 22nd at Kaiser Regional Laboratory, North Hollywood, CA and April 18th and 19th at John Muir Medical Center, Concord, CA. Eastland and Foothill Chapters held their annual Spring Symposium on March 28th at City of Hope, Duarte, CA. Special thanks to Becky Rosser and Marc Bernaldez, Los Angeles/ Los Valles Chapter, Ilene Dickman, West Side Chapter, Linda Burton, East Bay Chapter and Mary Jeanne Stavish, San Francisco Chapter for site and workforce coordination, on-site registration, and coffee break services. More than 100 registrants enjoyed a wide variety of seminar topics at all three events. Check our online continuing education calendar for the most up-to-date information about both statewide and local chapter offerings.

Knowledge and information are the staples for our professional lives. When was the last time you attended a statewide or local chapter continuing education seminar? Many employers offer “in-house CE” that will address site specific issues such as lab safety or proper operation of in-house equipment. However, this is no substitute for high caliber speakers and up-to-date information found at our Annual Meeting and CE Seminars.

What if you just can't get away from work to attend a CE seminar? Have you considered our on-line distance learning courses? CAMLT offers many courses on a wide variety of topics at minimal cost. See our current listing of distance learning courses in this issue. Full text distance learning courses are always available on our website. For your convenience, this issue includes the full text and answer sheets from our latest courses: 1) The Many Diseases Caused by *Fusobacterium necrophorum* and 2) Ebola Virus Disease. Thanks to our new Webmaster, Lowell Volk, Jr., all courses on our website can be completed on-line. You can register for the course, submit secure payment, take the quiz on-line and receive your graded score in real-time. When you pass, your certificate will be mailed to you from the CAMLT office. Talk about around the clock service!

For over twenty years, the CAMLT Legislative Committee and Board of Directors have been working tirelessly with our legislative advocate, Public Policy Advocates (PPA) on a number of bills impacting clinical laboratories. More information about our current efforts can be found in the CAMLT Political Update section in this issue of Newsline and Legislative Section of our website. Check the CAMLT website for updates on bill status, Legislator lists, and Committee assignments as they happen. Legislative advocacy is an expensive endeavor. Please consider a donation to Lab-PAC.

Summer Pre-Exam Seminars to take place in August and September are now accepting registrations. These review seminars are designed to assist those who will be taking the state approved licensing/certification exams for CLS or MLT. Since program inception in 2004, hundreds of candidates for licensure have benefited from these classes. See schedule of classes published in this issue for dates and locations of these extraordinary sessions and visit our website to download registration forms.

CAMLT would not exist without your assistance. Your membership dues support a team of legislative advocates well schooled in the importance and value of laboratory professionals and their role in patient care. Board members and the CAMLT Governmental Affairs Committee review pending bills, drafts amendments and provide testimony on key legislation that directly impacts our profession. Therefore, it is imperative that we grow our membership and that current members renew their memberships promptly so that resources are available to continue our efforts without interruption. I thank you in advance for your assistance.

Finally, I encourage you to be an active participant in association efforts. There are many opportunities to contribute your talents and time – from simple one time efforts (monitor a workshop, help with a chapter meeting, work as a convention volunteer, etc.) to longer term commitments like running for chapter or state office or serving on a standing committee. Contact the Nominations Committee Chair, Valerie Trenev, Board of Directors or CAMLT office staff if you or someone you know is interested serving. There is no better time to get involved than now.

CAMLT/NEWSLINE

Volume 41, No.2
Dora Goto, President

Editorial correspondence should be addressed to CAMLT/ *Newsline*, PO Box 1814, Fremont, CA 94538.

Scientific articles submitted for publication in the CAMLT/ *Newsline* should be approximately 1,000 words in length or about three pages, double spaced, typed materials. Please include references when appropriate. Since the articles are relatively short, the editors do not feel that an abstract is necessary. All articles should be accompanied by a picture of the author.

Articles describing procedures should include materials and methods as well as results. All graphs and charts should be reducible. Abbreviation (except for those in common scientific usage) should be defined and introduced parenthetically when first used in text.

Advertising correspondence should be addressed to PO Box 1814, Fremont, CA 94538, (510) 792-4441, FAX (510) 792-3045, e-mail: office@camlt.org.

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Best wishes,

Dora Goto
President, CAMLT

AB 757 (Gomez) Healing Arts: Clinical Laboratories, as introduced 2/25/15 and amended 3/26/15 - OPPOSE

AB 757 is sponsored by the laboratory instrument manufacturer of the Reichert Protein analyzer and would let non-qualified personnel do moderate complexity testing with regard to plasma. This bill would declare the intent of the Legislature to enact legislation to identify who, and under which circumstances he or she, may perform a total protein refractometer test analysis in a licensed plasma collection facility in this state. AB 757 would allow lesser trained and educated persons than currently permitted by law to perform a total protein refractometer analysis, categorized as a moderately complex test by the Food and Drug Administration, in a licensed plasma collection facility. If the protein refractometer test and/or calibration is done incorrectly there is potential to cause donor harm. AB 757 was amended on 3/26/15 which would add Section 1246.7 to the Business and Professions Code to read: 1246.7. A medical assistant, as defined in Section 2069, may perform a total protein refractometer test analysis in a licensed plasma collection facility in this state if all of the following conditions are met: (a) He or she has earned a high school diploma or equivalent, as determined by HCFA pursuant to CLIA. (b) He or she performs the total protein refractometer test analysis in a licensed plasma collection facility. (c) He or she has been instructed by a physician and surgeon licensed in this state or by a licensed clinical laboratory director who is in charge of the licensed plasma collection facility in the proper procedure to be employed when performing a total protein refractometer test analysis. (d) He or she performs the total protein refractometer test analysis under the direction and supervision of the physician and surgeon or licensed clinical laboratory director. (e) He or she submits the analysis for interpretation to the physician and surgeon or licensed clinical laboratory director under whose direction and supervision he or she performed the analysis. Location: Heard on April 28 in the Assembly Business and Professions committee.

AB 940 (Ridley-Thomas and Waldron) Clinical Laboratories, as introduced 2/26/15 and amended 3/23/15 -WATCH

According to the sponsor, the California Clinical Laboratory Association, AB 940 is intended to allow for Co-Directors of high complexity laboratories, an applicant for bioanalyst to obtain out of state training, create embryology and biochemical genetics licensure categories, allow the Department of Public Health to issue licenses for clinical embryologist and clinical biochemical geneticist, and allow the Department of Public Health to charge a fee for the licensing of clinical embryologist, clinical biochemical geneticist, clinical cytogeneticist or clinical molecular biologist. On March 23, minor amendments were taken, including adding Assembly Member Waldron as a co-author. Location: Heard on April 21 in the Assembly Business and Professions committee.

HAVE YOU MET WITH YOUR LEGISLATORS? IF NOT, WHY NOT?

For the last twenty years, CAMLT has continued to successfully weather advertent or inadvertent legislative assaults on clinical laboratory testing that would jeopardize patient safety, but these assaults continue. It is critical that CAMLT build its membership and engage with and educate California's elected officials. Legislators are eligible to serve up to 12 years under our new term limit law. It is imperative to build "legislative champions" for clinical laboratory

science and the patients who rely on educated, qualified laboratory personnel for accurate and reliable testing results.

Have you met with your legislators? Make sure to educate your elected officials about clinical laboratory issues! Meet with your legislators in your district, send letters explaining CAMLT's philosophy, invite legislators and their staff to tour your laboratories, and introduce yourself as a constituent. The sponsors of legislation, such as optometrists and chiropractors, to expand their scope of practice into area of clinical laboratory testing are well heeled and well organized. It is imperative that CAMLT members engage in the process that affects the profession.

- Which Legislator represents your home or laboratory? Visit the CAMLT website for a current roster of Legislators and the cities they represent.
- Visit their offices. Make an appointment with your Legislators' District offices.
- EDUCATE! Explain to Legislators and their consultants what it takes to be a laboratory professional; what you do; why it is important to maintain the integrity of the Laboratory Director when other personnel are doing laboratory tests, even if they are waived; why other allied health providers shouldn't be Laboratory Directors; the personnel shortage and what it takes to eliminate it.

Make it a priority to meet with your Legislators. Remember, these interactions are integral components of your grassroots program. For tips, please refer to the CAMLT Grassroots Guide on the website.

STRENGTHEN YOUR VOICE: CONTRIBUTE NOW!

Please donate to the CAMLT Lab-PAC fund. Lab-PAC is a critical means of supporting and electing Legislators to the California Legislature who share a like-minded philosophy with CAMLT and who are open-minded to learning the issues and challenges facing your profession. Encourage the colleagues you work with. Get your chapters and chapter members to contribute. Talk to your vendors. Get involved! Your voice in the political process is much louder as CAMLT than as an individual. Contribute to the collective resources of CAMLT to grow your political clout. Visit the Lab-PAC Page for a donation form. Your gift in any amount will help your profession. Contribute now!

Please mail donations made payable to:

LAB-PAC

PO Box 1814,

Fremont, CA 94538.

Please write a check to LAB-PAC now!

REQUEST FOR NOMINATIONS FOR 2015-2016 STATE OFFICES AND COMMITTEES

Nominations are now being accepted for CAMLT State Officers and Committee Chairpersons. Don't delay – the Nominations Committee needs time to determine eligibility and to contact the prospective candidate. If you are interested in serving in a State office as a member of the Board of Directors, or in a State Committee Chairperson position, or know of someone who is a good candidate, submit your name, address, telephone number, and email address to:

Valerie Trenev
CAMLT Nominations Chair
4623 Marlene Dr.
Santa Maria, CA 93455
Phone: 805-266-2340
vsttrenev@yahoo.com

The following positions are open:

President-Elect (One Year Term)
Secretary (One Year Term)
Treasurer (Two Year Term)
District I Consultant (Two Year Term)
District III Consultant (Two Year Term)
District V Consultant (Two Year Term)
Bylaws Chair (Two Year Term)
Judicial Committee (Two Year Term) – Two positions
Nominations Committee (One Year Term) – Five positions
(Districts I through V)

NOMINATIONS ARE IN ORDER FOR CLS OF THE YEAR!

Now is the time to submit nominations for the prestigious CLS of the Year Award. Contact:

Christine M. Darmanian
CAMLT Awards Chair
6291 E. Alta Ave.
Fresno, CA 93727-5629
Phone: 559-225-6100, ext. 5719
Christine.darmanian@va.gov
or
the CAMLT Office, office@camlt.org,
for details on qualifications and deadlines for submission.

Enter your newsletter for the annual Newsletter Award!

Send copies of your newsletter for judging to:

Christine M. Darmanian
CAMLT Awards Chair
6291 E. Alta Ave.
Fresno, CA 93727-5629
Phone: 559-225-6100, ext. 5719
Christine.darmanian@va.gov

California CLS/MLT Licensure Examination Review Seminars 2015**

This program was initially held in 2004 and repeated in 2005-14. Evaluation of the California CLS and MLT exam results show that participants in our review classes passed the exam at significantly higher rates than historical passing rates. Therefore, CAMLT will be presenting review sessions in 2015. These seminars are directed toward persons preparing for the California CLS or MLT licensing and/or certifying examinations and licensed individuals in need of a comprehensive review.

This review seminar is not eligible for continuing education credit for current licensees.

Dates	Presentation Location	Time	Cost
August 15, 16, 29, 30 & September 12, 13 Sat & Sun	John Muir Medical Center (Concord) 2540 East Street Concord, CA	8:30am - 6pm all days	\$80 per day or \$395 for all 6 days

Additional information, exact locations of seminars, seminar abstract, registration forms, and related educational/course material for purchase are updated regularly at www.camlt.org and click on the Professions and Examination Review Seminars course link.

**Sponsored in part by: John Muir Health
Bay Valley Medical Group, Affiliated with University HealthCare Alliance

CAMLT's Got a Little List, We've Got a Little List...



WHEN YOU WISH UPON A STAR, as they say, dreams can come true. But what if the Star has the wishes? We know you think CAMLT is a stellar organization for laboratory folks. Maybe you feel you have benefited personally from CAMLT's programs – met some great people, attended seminars that helped you grow in your profession, learned about the legislative process and how each of us can become involved. Maybe you would like to return the favor by helping to fund one of CAMLT's excellent programs. Or maybe you would like to fill an immediate, concrete need for the organization. Well, we have just the thing for you...our CAMLT Wish List!

Wish List Donors

We extend our sincere thanks to the individuals who have contributed to CAMLT's Wish List from December 1, 2014 to March 31, 2015.

RIGEL \$500 - \$999
Dora Goto

SPICA \$100 - \$499
Corrine Carroll
Helen Sowers

CASTOR \$20 - \$99
T. Castillo
Noreen Rancourt

Donation categories are named after stars seen from Earth in descending order of brightness.

If you are feeling generous, please contact the CAMLT Executive Office at office@camlt.org or complete the information below and fax to (510) 792-3045 or return to:

<p>CAMLT PO Box 1814 Fremont, CA 94538</p> <p>Name: _____ Address: _____ _____ City/State/Zip: _____ Phone: _____ Email: _____</p>	<p>\$ _____ toward technology/website updates \$ _____ cash donation</p> <p><input type="checkbox"/> My check is enclosed, payable to CAMLT. <input type="checkbox"/> You may charge my donation to my credit card. <input type="checkbox"/> Visa <input type="checkbox"/> Master Card</p> <p>Card # _____ Exp. _____ 3 digit security number on back of card _____</p> <p>Signature _____ Date _____</p>
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For those of you who have given in the past, thank you. Your kindness is appreciated and valued by all!

Please Note - "Contributions or gifts to California Association for Medical Laboratory Technology are NOT tax deductible as charitable contributions for income tax purposes. However, they may be tax deductible as ordinary and necessary business expenses subject to restrictions imposed as a result of association lobbying activities. CAMLT estimates that the non-deductible portion of your contributions - the portion which is allocable to lobbying - is 33%."

**ANNOUNCING CAMLT EDUCATION AND RESEARCH
FOUNDATION WINNERS – 2015!**

Winners of free continuing education scholarships (6.0 hours CE) are chosen from among attendees at CAMLT seminars and the convention.

Winter Seminar South - North Hollywood:
#1: Daisy Santa Maria
#2: Terrance Scroggin

Spring Seminar – Concord:
#1: Mary Swisher
#2: Debbie Lee

THE MANY DISEASES CAUSED BY *FUSOBACTERIUM NECROPHORUM*

Course #DL-012

3.0 CE

Level of Difficulty: Intermediate

James I. Mangels, MA, CLS, MT(ASCP)

Consultant

Microbiology Services

Santa Rosa, CA

OUTLINE

A. Introduction

B. History of *Fusobacterium necrophorum* infection

C. Transmission

D. Illness/Symptoms

E. Microbiology of *F. necrophorum*

F. Pathogenic Mechanisms

G. Diagnosis and Identification of *F. necrophorum* infection

H. Treatment

I. Prevention

J. Conclusion

K. References

COURSE OBJECTIVES

After completing this course the participant will be able to:

1. outline the history of *Fusobacterium necrophorum* infection
2. discuss the various types of infections caused by *Fusobacterium necrophorum*
3. explain the pathogenicity factors of *F. necrophorum*
4. outline the clinical features of Lemierre's disease caused by *F. necrophorum*
5. explain how *Fusobacterium necrophorum* is identified
6. state methods to recover *Fusobacterium necrophorum*
7. outline methods of treatment of *Fusobacterium necrophorum* infection

A. INTRODUCTION

Fusobacterium necrophorum is a Gram-negative, non-spore-forming, nonmotile, obligately anaerobic (requires strict anaerobic conditions for growth), pleomorphic (varies in size and shape) bacterium. The current genus name, *Fusobacterium*, comes from the Latin word "fusus," meaning spindle, and was given to describe the most common species, *Fusobacterium nucleatum*, which is spindle shaped. However, the other species of *Fusobacterium*, including *Fusobacterium necrophorum*, are not spindle shaped. The species name "necrophorum" is derived from the organism's frequent association with necrotic lesions in humans and animals (4). *F. necrophorum* is part of the normal flora of the oral cavity, gastrointestinal tract, and genitourinary tract of animals and humans (4,8). The organism is also a normal inhabitant of the rumen of cattle and sheep.

F. necrophorum is a more frequent pathogen in animals than in humans, although as will be described in this paper, infection in humans, although rare, can be severe, life threatening, and often overlooked. Clinicians and laboratory personnel should be aware of *F. necrophorum* infection because it is more prevalent than previously thought and because it can cause extreme illness or death, especially

in teens and young adults. Also, the current decrease in usage of antibiotic therapy to treat pharyngitis has caused a recurrence of *F. necrophorum* infection.

The organism is generally associated with abscesses and various necrotic infections, particularly oral, paraoral and lower respiratory tract infections in animals and in humans. It is recovered in animals as a major pathogen causing infections such as foot rot, calf diphtheria, and liver abscesses in cattle, horses, goats, pigs, and sheep. When *F. necrophorum* is involved in disease it can cause necrotic lesions that are generally referred to as "necrobacillosis" (death of tissue due to the pathogenic factors of the organism). See Section F. Pathogenic Mechanisms.

In humans, *F. necrophorum* infection is most commonly associated with various oral and tonsillar abscesses, persistent or chronic sore throat, and other abscesses throughout the body. A very severe complication of tonsillar abscess or oral infections due to *F. necrophorum* is Lemierre's syndrome, also called Lemierre's disease or post-anginal sepsis, which occurs predominantly in older children or young adults. Note: The definition of the word post-anginal is any extreme pain that feels suffocating (not just heart pain). In Lemierre's disease, the first symptom is an extremely painful sore throat (pharyngitis) that subsides but is followed by thrombophlebitis (a blood clot) of the jugular vein and septicemia (organisms in the blood stream) that travel to and cause distal abscesses (infections in other parts of the body), particularly in the lungs, pleural space, liver and large joints in the body. Other complications from *F. necrophorum* infection include meningitis, thrombosis of the cerebral veins, and infection of the urogenital and the gastrointestinal tract. The classic symptom of Lemierre's syndrome is a sudden acute jugular vein thrombophlebitis, followed by fever and sepsis (inflammatory response including shock and drop in blood pressure). The ability of *F. necrophorum* to stimulate clot formation with subsequent systemic spread is a fundamental feature of the pathogenesis of *F. necrophorum*. See Section F. Pathogenesis Mechanisms. This syndrome is the most important life-threatening manifestation of *F. necrophorum* infection in man. Lemierre's syndrome will be described in greater detail in Section D. Illness/Symptoms.

Recently, *F. necrophorum* has been associated with recurrent or chronic sore throat in humans. Some studies have found that *F. necrophorum* is present in 10% of patients aged 15-24 with chronic pharyngitis, and may be an agent of pharyngitis as often as beta-*Streptococcus* Group A (*Streptococcus pyogenes*) (1,3,9). See Section D. Illness/Symptoms. Consequently, some medical centers and clinics have started to use specific selective media for the isolation of *F. necrophorum* for the workup of chronic sore throat in specific age groups. See Section G. Diagnosis and Identification of *F. necrophorum* infection.

There are two subspecies of *F. necrophorum*. Human infection is usually caused by *F. necrophorum* subsp. *funduliforme*. In animals, the common pathogen is *F. necrophorum* subsp. *necrophorum*. There are some minor differences in the pathogenicity, immunology, and biochemical activity between the two organisms. However, in this course, the two subspecies will be considered the same.

Among all the cases of human infection with Gram-negative anaerobes, *Fusobacterium necrophorum* constitutes only a tiny proportion, perhaps less than 1%, of all anaerobic isolates in various infections (4,5). However, *F. necrophorum* is unique among the Gram-negative anaerobes for its pathogenicity and for its association with

very unusual infections in humans and in animals.

This Distance Learning Course will describe the many diseases caused by *F. necrophorum*. Some of the unusual and often life-threatening types of infection caused by this organism will be outlined. In addition, this course will describe the history, transmission, pathogenesis, diagnosis, identification of the organism, and clinical features of these infections in animals and in humans.

B. HISTORY OF FUSOBACTERIUM NECROPHORUM INFECTION

Since *Fusobacterium necrophorum* is a more common pathogen in animals than in humans, it is not surprising, therefore, that the first reports of infection were from animals. Probably the earliest report of *Fusobacterium necrophorum* infection in animals was in 1876 by G. Dammann (a veterinarian in Germany) when he described diphtheritic (necrotic laryngitis) infections in calves (2). The symptoms and clinical features Dammann observed are typical of *F. necrophorum* infection we see today. Dammann, however, believed he was observing infection due to the organism called, at the time, *Bacillus diphtheria*. In 1884, F. Loeffler (a German microbiologist) demonstrated that calf diphtheria was actually due to a Gram-negative organism called, at the time, *Actinomyces necrophorus*. He identified and described a thin Gram-negative rod with filamentous forms in stained sections from necrotic tissue of diphtheritic material. When Loeffler injected this necrotic material into mice, they developed an infection that generated foul-smelling purulent lesions containing bacilli similar in morphology to the original necrotic material. Loeffler was able to grow the organism in calf serum broth, but was not able to subculture the organism and perform further testing. Other reports in 1886 called the same organism *Bacillus necrophorus*, isolated from infections in hogs.

Probably the first reported human *F. necrophorum* infection was in 1891, when C.G. Schmorl (a German pathologist) reported hand abscesses in himself and his assistant after they handled rabbits they had experimentally infected with *F. necrophorum* to study necrobacillosis (2). Stained smears of the pus from their hand abscesses showed the same characteristic filamentous Gram-negative bacilli as from the rabbits. Schmorl named the organism *Sphaerophorus funduliformis*. Schmorl also noted in his report the ability of *F. necrophorum* to produce a zoonotic infection (an infection passed from animals to humans).

The first isolation and a detailed description of *F. necrophorum* from humans was made by Jean Hallé in 1898 as part of a Ph.D. thesis on the bacteriology of the female genital tract. He called the organism *Bacillus funduliformis*, a descriptive term of the Gram stain morphology, from the Latin fundula (little pockets), because he felt that some of the bacilli resembled irregular shaped sausages (9). His line drawings of the microscopic appearance of the organism under different conditions are still accurate today. See Fig. 1. Line Drawings of *Bacillus funduliformis*.

Two French physicians in 1900 reported the first description of an infection in a patient who had symptoms similar to what we now call Lemierre's syndrome, i.e., a post-anginal septicemic infection (pharyngitis preceding fever, rigors, internal jugular thrombophlebitis, and distal abscesses). The patient initially appeared to be improving after his severe sore throat and acute tonsillitis, but suddenly after 4 or 5 days, the patient had an onset of rigors and progressed to an overwhelming sepsis (inflammatory response leading to shock and decreased blood pressure) and died (9). See Section D. Illness/Symptoms. The two French physicians were able to isolate anaerobic

pleomorphic Gram-negative bacilli from the abscess material and from the patient's blood culture. They may have called the organism *Bacterium necrophorum*. However, their description of the colonies and the Gram stain morphology of the organism are consistent with *F. necrophorum*.

An understanding of the disease and symptoms of post-anginal infection was more clearly characterized by Lemierre in 1936. André Lemierre (1875 to 1956) was a physician and professor of microbiology and infectious diseases at a hospital in Paris. He became aware of serious human infections due to *F. necrophorum*, although the organism at that time was called *Bacillus funduliformis*. Lemierre described a human system-wide septic thrombophlebitis infection with *F. necrophorum*. The sentinel case he reported was a young child with chronic purulent otitis presenting with septic arthritis of the knee, cerebral abscess, and signs of overwhelming systemic infection (9). Smears of the abscess material showed organisms consistent with current descriptions of *F. necrophorum*. Other investigators during this period were calling this organism *Bacillus symbiophiles* or by other synonyms.

Lemierre believed that post-anginal septicemia and systemic septic thrombophlebitis infection were a feature of the anaerobe *F. necrophorum*, and the recovery of the same organism from other body sites of a patient was significant. Lemierre highlighted the confusion of nomenclature for the identical organism, whereby German authors were referring to the organism as *Bacillus symbiophiles* and French workers as *Bacillus funduliformis*. Lemierre suggested that these might be a single organism on the basis of the properties of the organisms and the clinical descriptions of the associated cases.

Lemierre's main contribution to the understanding of this syndrome was the clarity of his clinical description of post-anginal septicemia associated with the organism we now know as *F. necrophorum*. His comment on the ease of clinical diagnosis of the condition defines his understanding of the disease. "To anyone instructed as to the nature of these septicemias it becomes relatively easy to make a diagnosis on the simple clinical findings. The appearance and repetition several days after the onset of a sore throat (and particularly of a tonsillar abscess) of severe pyrexial (fever response) attacks with an initial rigor or still more certainly the occurrence of pulmonary infarcts and arthritic manifestations, constitute a syndrome so characteristic that mistake is almost impossible" (2,9).

Since the early reports of *F. necrophorum* infection in animals and in humans, this organism has been classified under a variety of genera and species. The confused taxonomy for many years has led to a misunderstanding of its significance in infection, and has caused problems in the actual identification of the organism and lack of understanding of its pathogenic mechanisms (2). The various synonyms of *F. necrophorum* are listed in Table 1, Previous Synonyms of *Fusobacterium necrophorum*. Much of the confusion in taxonomy probably has been due to inadequate anaerobic culture methods. Better anaerobic culture methods allowed the isolation and identification of *F. necrophorum* and a clearer understanding of the role of the organism in various infections (4).

By the start of the antibiotic era in the 1940's, the features of Lemierre's syndrome had been established and the incidence of disease began to decline because of appropriate therapy. However, with the decline in the incidence of Lemierre's syndrome, the knowledge, experience, and recognition of the severe disease in humans due to *Fusobacterium necrophorum* was often forgotten and

the disease not immediately diagnosed. The “forgotten” disease of Lemierre’s syndrome still exists and perhaps is undergoing a resurgence in Europe (2,9). It is speculated that the resurgence of Lemierre’s syndrome observed in Europe may be due to their policies restricting antibiotic use in patients with pharyngitis. With the role of *F. necrophorum* in persistent or chronic sore throat, an understanding of the organism is extremely important. See Section D. Illness/Symptoms.

C. TRANSMISSION

F. necrophorum is part of the normal bacterial flora of the gastrointestinal tract, the oral cavity, and the rumen in cattle, goats, and sheep. Therefore, transmission in animals commonly occurs as a result of direct contact with soil contaminated with animal feces, especially in wet, muddy, or unsanitary conditions. *F. necrophorum* is known to survive in the soil of pastures for up to 18 weeks (2), which is unusual from a microbiological standpoint since *F. necrophorum* is a non-spore-forming anaerobe. It is believed that contaminated soil leads to foot disease or foot rot in horses, sheep, and cattle, and it is believed that cattle or sheep diphtheria is likely contracted from contaminated food or food containers such as buckets, stalls, etc., containing *F. necrophorum*. Direct animal to animal contact is also a common transmission method in animals through open sores or from animal bites.

Since *F. necrophorum* is part of the normal bacterial flora of the oral cavity, genitourinary tract, and the gastrointestinal tract in humans, it has been assumed that primary transmission of the organism occurs from direct contact with body fluids or mucous membranes. Another suggestion has been that transmission occurs due to contamination from one’s own normal bacterial flora, and *F. necrophorum* causes infection after either an injury or some type of break or change in immunity (9). Yet another suggestion has been that transmission may occur after surgical procedures, accidental trauma, from animal bites, or infected open sores of animals contaminated with *F. necrophorum*.

However, recent investigations using molecular techniques may show that in some cases severe *F. necrophorum* tonsillitis and its rare consequences, such as Lemierre’s, may be due to acquiring *F. necrophorum* from an exogenous source, perhaps from an animal, or by human to human transmission (9). Other interesting investigations show that severe *F. necrophorum* infection may be the result of another entirely different concurrent infection, such as infection with Epstein-Barr virus (EBV) which causes infectious mononucleosis, or another possible viral infection, which may alter the individual’s normal oral flora or lower one’s resistance so that *F. necrophorum* can begin to initiate disease acquired from another source (9). Approximately 10% of published cases of severe *F. necrophorum* infection are associated with infectious mononucleosis (9). Some investigators believe that alterations or changes in the pharyngeal mucosa during cases of infectious mononucleosis might allow invasion and penetration of *F. necrophorum* into the tonsillar epithelium, leading to potential infection.

Other studies in Finland suggest that dramatic changes of an individual’s normal oral flora, after pneumococcal vaccination for example, may cause a person to be more susceptible to *F. necrophorum* infection either from an exogenous source or from one’s own flora (2,9). The nasopharynx is colonized by multiple microorganisms, and disruption of the microbiota (microbe population at a body site) with its various synergistic and interfering interactions can either facilitate or block respiratory tract infections.

Pneumococcal vaccination has been shown to alter the carriage of other common pathogens such as *Hemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. Finland has seen an increase in severe *F. necrophorum* infection since initiating a very comprehensive vaccination program, including the pneumococcal vaccine, since early 2000 (2,9). Therefore, it is speculated that the pneumococcal vaccine may be one reason for the increase in serious *F. necrophorum* infection.

So it is not clear at this time whether severe *F. necrophorum* infection in humans (such as recurrent sore throat, severe throat infection and tonsillitis leading to Lemierre’s syndrome) is caused by a person’s own normal flora, or is acquired from another source, or is caused by alteration of one’s normal flora.

D. ILLNESS/SYMPTOMS

This section will describe some unique illnesses (“The Many Diseases of *F. necrophorum*”) and symptoms of *F. necrophorum* infection in animals and humans. Although there are many illnesses due to *F. necrophorum*, primarily foot rot and calf diphtheria in animals, and acute tonsillitis leading to Lemierre’s syndrome, Vincent’s angina, and recurrent sore throat in humans will be described.

The animal diseases are described in this course to give an appreciation for how widespread, virulent and economically destructive *F. necrophorum* infection can be. While there are many types of *F. necrophorum* infections in animals, two major infections are foot rot in cattle, horses, and sheep, and calf diphtheria (necrotic laryngitis). For a description of other *F. necrophorum* infections in animals, such as pneumonia, bovine liver abscesses, pericarditis (infection of membrane surrounding the heart), subcutaneous abscesses (abscesses under the skin), chronic fibronecrotic rhinitis (inflammation of nasal membranes which can restrict air intake), and laryngeal chondritis (an upper respiratory tract disease which causes swelling and occlusion of the larynx), see reference 7.

Foot rot

Foot rot is probably one of the major infections in animals due to *F. necrophorum*. It causes foot destruction, leading to lameness in dairy and beef cattle, goats, and sheep. The disease can have a major financial impact on the cattle industry due to weight loss of the animal and reduction in milk production. The clinical signs of foot rot are usually a sudden onset of swelling, erythema (redness and inflammation), tissue destruction, extreme foul odor, and significant pain to the animal. This is often noted in animals when the animal shifts its weight constantly. If the infection becomes chronic, there may be atrophy in the affected limb and further infection of joints, bone, or tendons. Animals with foot rot typically have a terrible smell coming from their feet due to *F. necrophorum* producing butyric acid. Foot rot infection is usually treated with antibiotics and other methods. See Section H. Treatment. It is said that symptoms and clinical features of foot rot are so characteristic, that the presence of *F. necrophorum* may be predicted (7,8).

Calf diphtheria

Calf diphtheria (necrotic laryngitis), is probably the second most important infection in animals due to *F. necrophorum*. Calf diphtheria can be an acute or chronic infection of the laryngeal mucosa and cartilage of young cattle, characterized by fever, cough, and difficulty in breathing. The disease initially starts with swollen cheeks or ulcerations inside the mouth. It can progress to ulcers of the tongue and palate, and then to pneumonia. The ulcerations are very painful so the infected animal generally is unable to eat, causing extreme weight loss. The infection occurs primarily in feedlot cattle

3–18 mo of age. Cases of calf diphtheria are seen worldwide, are more prevalent in the fall and winter, and can have a major financial impact on the cattle industry (7,8).

Acute tonsillitis and Lemierre's syndrome

In man, infection due to *F. necrophorum* is most commonly oral, tonsillar, or the severe post-tonsillar infection called Lemierre's syndrome. If *F. necrophorum* gets into the bloodstream (bacteremia), generally as a consequence of Lemierre's syndrome, the organism may then initiate secondary infections in the bone, lung, liver, or other body sites. Although Lemierre's syndrome has been well documented for over a century, it is quite a rare condition and modern-day clinicians are frequently unaware of *Fusobacterium necrophorum* and the severity of symptoms it can cause. Acute tonsillitis can be caused by a variety of bacteria and viruses. In many instances, anaerobes--in particular, *Fusobacterium necrophorum*--have a role in an acute inflammatory process in tonsillitis (9). The inflammatory process can progress to more serious infection of nearby tissues and structures, leading to further infection.

Lemierre's syndrome occurs when a *Fusobacterium necrophorum* throat infection progresses to the formation of a peritonsillar abscess, and this infection spreads to nearby tissues and into the jugular vein (a major blood vessel that drains blood from the brain, face, throat and neck and transports it to the heart). (2,9). Infection of the jugular vein may lead to systemic clot formation, or spread of bacteria through the bloodstream (septic thrombophlebitis). *F. necrophorum* has a strong tendency to form clots because of its pathogenic factors. See Table 2. Summary of Pathogenic Mechanisms of *Fusobacterium necrophorum*. Pieces of the infected clot break off and travel to the lungs as emboli, blocking branches of the pulmonary artery, or to other sites.

Lemierre's syndrome generally develops in previously healthy young people (typical age group is 15-24). The pharyngitis of the patient is typically severe and exudative (fluid filled and generally containing pus), followed by a high fever (101-103°F) and rigors (sudden feeling of cold, shivering and sweating) beginning on the fourth or fifth day after the initial sore throat symptoms (2,9). This is usually accompanied by a one-sided thrombophlebitis of the internal jugular vein due to the spread of the infection from the throat. There is swelling at the angle of the jaw and tenderness and pain along the overlying muscles. Metastatic abscesses are usually present and are most often in the lungs. Other sites of distant abscess frequently include the long bones and skeletal joints, which are often very painful. The patient's condition often declines to extreme prostration or coma, and an untreated infection commonly ends in death within 7-15 days. Although severe tonsillar abscess and Lemierre's syndrome are rare the mortality is extremely high. Therefore, the diagnosis of Lemierre's syndrome should be considered in a young septicemic patient with *Fusobacterium necrophorum*, and in cases of isolated *F. necrophorum* infection occurring at unusual sites throughout the body.

Lemierre's syndrome was relatively common in the pre-antibiotic era with a high mortality rate of up to 90% (2,7). The syndrome seemed to disappear with widespread use of antibiotics for upper respiratory tract infection, including pharyngitis. However, in the last 15 years there has been a rise in incidence, possibly related to restriction of antibiotic use for sore throat. Lemierre's is often referred to as a "forgotten" disease", although it is probably best described as a "repeatedly discovered" disease, as it may not always be included in medical curricula, and often is not mentioned in some major medical textbooks, so many physicians are not familiar with

the disease. There is some evidence that Lemierre's syndrome may be on the increase, particularly in the United Kingdom, Finland, and France (2).

For a syndrome that is so characteristic, a diagnosis of Lemierre's is often missed until an anaerobic Gram-negative rod is isolated from a blood culture or from another body site. Many clinicians and even laboratory personnel have never seen a case and are unaware of the condition (I have seen only one case in 30 years as a microbiologist). In many cases the original sore throat has resolved by the time a patient presents with septicemia. The various clinical manifestations of Lemierre's syndrome can be difficult for physicians to single out; therefore, there can often be a diagnostic delay until more laboratory results are available.

Vincent's angina

Another unusual human infection due to *Fusobacterium necrophorum* is called Vincent's angina (also known as trench mouth). Angina means intense local pain. Vincent's angina is often seen in children, in patients who have poor dental hygiene, or in patients who have a compromised immunity. Vincent's angina infections were initially reported from soldiers during WW I, and are very painful, with ulceration and tissue necrosis, and bleeding and swelling and sloughing off of dead tissue from the mouth and throat due to *F. necrophorum* and an oral spirochete (*Borrelia vincentii*). A foul discharge with consequent foul odor to the breath is a common symptom in Vincent's angina. Some studies show that a severe case of Vincent's angina can lead to Lemierre's syndrome (2,8,9). There may be other secondary infections due to *F. necrophorum* in patients with Vincent's angina, such as otitis (ear infections) or sinus infections.

Recurrent or persistent sore throat

Lastly, *F. necrophorum* in humans has been recently identified as a significant cause of persistent sore throat syndrome. This disease is characterized by a chronic, persistent sore throat, negative for beta-hemolytic Group A *Streptococci*, that has persisted for longer than 5 days with no improvement. Sometimes the patient's sore throat symptoms can become increasingly worse during the episode. Studies in the United Kingdom and in other parts of Europe have investigated the cause of persistent sore throat (1,2,3). A recent surge in complicated cases of pharyngitis, particularly in adolescents, prompted more elaborate microbiological testing (1,3). DNA studies revealed that *Fusobacterium necrophorum* is as common as Group A *Streptococci* in the 15-24 year old age group. In one study, the investigators cultured for beta-hemolytic *Streptococcus* groups A, C and G, and *F. necrophorum* (1,3). Among a total of 248 samples, 27 were positive for beta-hemolytic *Streptococcus* Group A, two were positive for beta-hemolytic *Streptococcus* group C, five for beta hemolytic *Streptococcus* G, and 24 were positive for *F. necrophorum*. In this same study, *F. necrophorum* was recovered in 10% of patients with acute sore throat, 21% of recurrent sore throats and 23% of peritonsillar abscesses. Group A *Streptococci* were recovered in 10% of patients 15-24 years of age. The study points out that the consequences of acute rheumatic fever from streptococci are very rare, while the consequences of severe tonsillitis due to *Fusobacterium necrophorum* causing Lemierre's are 1 in 400 cases (1,2,3). In other words, *F. necrophorum* can cause Lemierre's syndrome at an incidence higher than that at which Group A *Streptococcus* can cause acute rheumatic fever. The consequences of severe *F. necrophorum* infection have a greater morbidity and mortality than Group A *Streptococcus* infection. Therefore, the authors suggest that the diagnostic workup for adolescent pharyngitis should be

expanded to consider *F. necrophorum* (1,2,3).

Typically, physicians have considered Group A *Streptococcus* as the only important cause of sore throat because it can lead to acute rheumatic fever. Physicians typically ignore other organisms because those organisms cause either self-limited symptoms without serious sequelae (consequences of disease), or in the case of viruses there is no treatment available. The guidelines for treating Group A *Streptococcus* have evolved into algorithms for sore throat management that encourage many offices, urgent care centers, and emergency departments to use a rapid strep test for patients with sore throat symptoms. Often the rapid strep test is ordered routinely for patients with sore throat, in lieu of basing the decision to test on a targeted history and physical examination that takes into account the patient's age. This strategy results in patients being diagnosed only as having or not having strep throat, resulting in a decision that antibiotics are not needed if the patient does not have strep throat (1,3).

But when the patient's symptoms persist or worsen, and if the patient is in a certain age group, these criteria no longer apply and more careful workup is needed. When an adolescent or young adult (ages 15-24 years) patient has a sore throat, the physician needs to pay special attention to the following red flags: rigors, shaking chills, high fever (greater than 102°F), night sweats, and unilateral neck swelling (1,3). These red flags should indicate that the patient may have a more serious illness. *Fusobacterium necrophorum* pharyngitis and chronic sore throat occurs predominately in the same age group as patients who have Lemierre's syndrome. Though much remains unknown about *F. necrophorum*, it appears to cause sore throats just as commonly as strep does in adolescents and young adults, and Lemierre's syndrome in this age group appears to be more common than acute rheumatic fever. Dealing with adolescent and young adult pharyngitis is more complicated than many practitioners realize.

E. MICROBIOLOGY

The colony size of *Fusobacterium necrophorum* on brucella blood agar is 2-3 mm in diameter in 24 to 48 hours (*E. coli*, by comparison, is 3-4 mm in diameter) if the medium used is enriched with vitamin K₁ and hemin, and if the medium is pre-reduced (a process in which the medium is never exposed to oxygen). The morphology of the colony is circular, convex to umbonate (a conical or surface elevation like half an egg and raised), and the surface often can be bumpy and uneven, with a margin of scalloped to erose (irregular notched margin) (5,8). Colonies are cream-yellow in color, smooth and round, and they often produce an odor of cabbage due to the production of butyric acid. There can be some alpha-hemolysis (greening around colonies on blood agar plates) following exposure to oxygen. *F. necrophorum* may not grow on media that is not enriched or has not been manufactured pre-reduced, since the organism is an obligate anaerobe (6).

The cellular morphology of *F. necrophorum* is a Gram-negative, nonsporeforming, pleomorphic bacillus that ranges in size from 0.5 to 0.7 µm in diameter, with swelling round bodies to 1.75 µm in diameter, and with some of the cell filaments longer than 10 µm in length (5,8). See Fig 2. Gram-stain of *Fusobacterium necrophorum*. The bacterial cells are usually extremely pleomorphic (vary in size from small, almost coccoid bodies to long filaments with parallel sides and blunt ends). The morphology will be affected by the type of media used and the age of the culture. Filamentous forms are usually seen more frequently in young cultures and from a broth medium, while bacilli forms are more common in older cultures and when

grown on agar. With standard stains, e.g., safranin or carbol fuchsin, irregular staining or beading may be seen. Older cultures appear to stain more irregularly; some attribute the loss of staining ability to aging and degeneration. Some speculate that the irregular staining characteristics and extreme pleomorphic nature of *F. necrophorum* may be due to exposure to oxygen, and that when the organism is stained under an anaerobic atmosphere, the cells may be more regular. The highly pleomorphic Gram stain morphology of *F. necrophorum* allows differentiation from *F. nucleatum*, which is seen as long, thin, spindle shaped rods. See Table 3. Features and Biochemical Characteristics of Most Common *Fusobacterium* species.

Biochemically, *F. necrophorum* is indole positive, nitrate reduction test negative, esculin hydrolysis negative, and produces lipase on egg yolk agar (5,8). See Fig 3. Photograph of Positive Lipase Reaction of *Fusobacterium necrophorum*. All species of *Fusobacterium* produce butyric acid in high amounts from a broth medium containing glucose, and ferment lactate to propionate, detected by gas liquid chromatography. Use of special potency antibiotic disks as a means to initially group anaerobes show all species of *Fusobacterium* resistant to vancomycin 5 µg, but susceptible to kanamycin 1000 µg and colistin 10 µg (5). See Table 4 for Special Potency Disk Reactions. Under a long-wave UV Woods Lamp, *Fusobacterium* species colonies fluoresce with a vivid greenish-yellow color (chartreuse) (5). The most direct means of identification of *F. necrophorum* is with Gram stain, indole production, and lipase reaction. See Table 3. Features and Biochemical Characteristics of Most Common *Fusobacterium* species. Other biochemical tests show that some strains grow in 20% bile and are catalase negative. *F. necrophorum* does not generally ferment carbohydrates or can be variable and often weak in its fermentation reactions. The organism's major energy substrate is lactic acid, which is converted mainly to acetate, butyrate, and small amounts of propionate (8,9).

Some rapid commercial identification kits for anaerobic bacteria incorrectly identify *Fusobacterium necrophorum*. Often this is because too small a number of *F. necrophorum* isolates is included in the data base of the commercial anaerobic identification system to permit adequate differentiation and separation from other anaerobes, and because *F. necrophorum* often does not utilize many of the substrates employed in the commercial system. So take this into account if you only use a commercial anaerobic identification system to identify this organism.

F. PATHOGENIC MECHANISMS

F. necrophorum has a variety of unusual and complex pathogenic mechanisms to cause disease in man and in animals. See Table 2. Summary of Pathogenic Mechanisms of *Fusobacterium necrophorum*.

One of the major virulence factors of *F. necrophorum* is leukotoxin, a secreted protein active specifically against leukocytes. The leukotoxin of *F. necrophorum* is a unique protein that does not share DNA sequence similarity with any other leukotoxin (8,9). Leukotoxin produces degeneration and lysis of the leukocyte cellular membrane, releasing the cellular contents of the leukocyte and causing death of the cell. Leukotoxin contributes to the formation of necrotic abscesses characteristic of *F. necrophorum* infection. The production of necrosis helps *F. necrophorum* evade the host immune response and to proliferate.

Another major pathogenic factor of *F. necrophorum* is a cell wall lipopolysaccharide endotoxin (LPS) which is chemically similar to the LPS seen in aerobic gram-negative bacteria. An endotoxin is part of the outer membrane of the cell wall of Gram-negative bacteria.

The biological potency of the lipopolysaccharide resembles that of *Salmonella* endotoxin in capacity to produce local and generalized reactions (2,9). The lipopolysaccharide of *F. necrophorum* produces abscesses, hemolysis, and tissue destruction. Lipopolysaccharide also produces fever, shock, and a decrease in blood pressure, which are clinical features commonly seen in patients with Lemierre's syndrome.

Other pathogenic mechanisms in *F. necrophorum* include various exotoxins (secreted toxins that damage normal host cell metabolism) that destroy leukocytes and prevent migration of leukocytes into the infected area. Some of these exotoxins are hemolysin, leucocidin, hemagglutinin, plasminogen, platelet aggregation factor, and several other extracellular enzymes, such as phosphatases, proteases, and deoxyribonucleases (2,9). All these pathogenic mechanisms contribute to entry, colonization, proliferation, and establishment of the organism, and to the development of lesions and abscesses. Leucocidin is likely one of the most significant exotoxins of *F. necrophorum* and is linked particularly to the destruction of polymorphonuclear leukocytes (PMNs). This exotoxin appears to function by causing the formation of pores (holes) in PMNs so that the cellular contents leak out.

Activation of the other important exotoxins, such as plasminogen, platelet aggregation factor, and the production of hemagglutinin by *F. necrophorum*, cause abnormal coagulation and the formation of the clots and thrombotic complications that are common features seen in Lemierre's syndrome. The exotoxins of *F. necrophorum* first initiate an inflammatory response that causes platelets to aggregate and blood vessels to dilate, and then finally produce clotting abnormalities. In addition, *F. necrophorum* produces various proteolytic enzymes that aid in the pathogenesis of the organism by breaking down proteins, leading to tissue destruction and permitting invasion and entry into other tissues.

Lastly, *F. necrophorum* has two clever ways of attaching itself to host cells so that the above pathogenic factors can cause disease. One is adhesions (cell-surface molecules that allow adherence to specific host cell sites) and another is fimbriae (hair like structures on the perimeter of the organism) for attachment to the host cell (2,9). Once attachment occurs, the pathogenic mechanisms of *F. necrophorum* can start to function on host cells.

However, one of the most striking and unanswered aspects of the pathogenesis of human *F. necrophorum* infection is the tightly clustered age distribution in late teens and early 20's. This applies not only to Lemierre's syndrome, but also to tonsillitis, peritonsillar abscess, and persistent sore throat. There are interesting questions that remain unanswered such as, is there a change in exposure to *F. necrophorum* at that age group, is there a change in the individual's immunity at this age, is this young person likely exposed to a more virulent strain with different pathogenic factors, are there structural changes to the tonsil at this age that make infection more likely, and is there an increase in possible concomitant viral infections such as EBV that might lead to *F. necrophorum* infection. All are interesting questions that scientists cannot answer yet.

G. DIAGNOSIS AND IDENTIFICATION OF *F. NECROPHORUM* INFECTION

Some *F. necrophorum* infections in animals or in man can be initially suspected by observing specific symptoms and history. For example, clinical signs of foot rot in an animal plus physical examination of the foot will lead to a presumptive diagnosis of *F. necrophorum* infection. Similarly, in humans, a history of recent

severe sore throat followed by sepsis and distal infections in a teenager or young adult will lead to a presumptive diagnosis of Lemierre's syndrome. The symptoms of specific syndromes are so characteristic and unique that *Fusobacterium necrophorum* may be speculated before any diagnostic testing is performed.

However, in most cases the diagnosis of infection and identification of *F. necrophorum* must be obtained by laboratory diagnostic tests. The clinical laboratory plays an important role in obtaining a diagnosis in Lemierre's syndrome by using blood cultures, joint aspirates or specimens from other sites to test for the causative agent of the infection. The recovery of an anaerobic pleomorphic Gram-negative rod with filaments and swellings from blood cultures and/or from other sites is helpful in the preliminary identification of the organism.

Since *F. necrophorum* is an obligate anaerobe and sensitive to oxygen, the clinical specimen should be transported in an anaerobic transport device (such as those manufactured by Anaerobe Systems, BD Diagnostics, Hardy Diagnostics, Remel, and others) to maintain specimen quality. Further, a good quality specimen (obtained by needle and syringe) free of contamination is important in the identification of the organism involved in the infection.

Once in the laboratory, the specimen should be plated onto enriched, pre-reduced anaerobic media (Anaerobe Systems, Morgan Hill, CA) to ensure good growth. Media such as pre-reduced Brucella enriched with vitamin K₁ and hemin is a good choice. One study showed poor growth or no growth of *F. necrophorum* on media that was not pre-reduced (6). Some laboratories are not able to recover *F. necrophorum* from clinical specimens because good anaerobic media along with the use of good anaerobic technique (jars or chambers) are not used. Anaerobic bacteria vary considerably in their sensitivity to oxygen. *F. necrophorum* is strictly anaerobic and is not able to tolerate the oxygen concentration found in most media (6).

In some instances, the laboratory may choose to use special media specifically formulated to provide presumptive identification of *F. necrophorum*. One such medium is egg yolk with kanamycin and vancomycin (EYKV), a selective and differential medium that contains a suspension of egg yolk as well as vancomycin and kanamycin for the isolation and presumptive identification of *Fusobacterium necrophorum* (one source of this medium is Anaerobe Systems). The egg yolk suspension allows for the detection of lipase activity, which if positive hydrolyzes the fats within the egg yolk medium, resulting in an iridescent sheen on the surface or surrounding the colony. See Fig 3. Photograph of Positive Lipase Reaction of *Fusobacterium necrophorum*. EYKV also contains vancomycin to inhibit the growth of gram-positive microorganisms and kanamycin to inhibit the growth of gram-negative, facultatively-anaerobic bacilli. The medium will not provide complete identification of *F. necrophorum*; additional biochemical testing must be performed to confirm the identity of the organism.

Another medium that can be used is Fusobacterium Selective Agar (FSA), a selective medium for the isolation and presumptive identification of *Fusobacterium* species. FSA medium contains josamycin, neomycin and vancomycin at concentrations that inhibit most gram-positive and most gram-negative anaerobes (one source of this medium is Anaerobe Systems). FSA should be inoculated directly with clinical material and streaked to obtain isolated colonies. A non-selective medium such as Brucella blood agar should also be inoculated with the clinical specimen to permit growth of all organisms present.

Isolated colonies should be subcultured to aerobic media to ensure that the organism recovered is an anaerobe, since most aerobic organisms will grow under anaerobic conditions. Therefore the step called "aerotolerance testing" is necessary to determine if the isolate is an anaerobe. See reference number 5 for technique on how to perform aerotolerance testing. Once it is determined that the isolated colony is an anaerobe, further testing using special potency disks, indole, lipase, and other biochemical testing should be utilized to identify the isolate. See Section E. Microbiology of *F. necrophorum*, and Tables 3 and 4. For other biochemical testing and other methods which might be used to identify *Fusobacterium necrophorum*, see reference numbers 5 and 8.

H. TREATMENT

To some extent, the choice of treatment of *F. necrophorum* infection depends upon the type and location of the infection, and upon the patient, but in general, treatment usually involves an antibiotic. *F. necrophorum* is susceptible to β -lactam antibiotics (penicillins and cephalosporins) and tetracyclines, clindamycin, and metronidazole. There have been some reports in the literature from Great Britain, Finland, and France that suggest that some isolates of *F. necrophorum* may produce β -lactamase (an enzyme that provides resistance to penicillin-like antibiotics) (2,9). In one study, about 2% of *F. necrophorum* isolates from blood cultures were resistant to penicillin (2). For the most part, resistance to penicillin-like antibiotics has not been observed in the United States. Studies in the United States consistently find that 100% of *F. necrophorum* strains are sensitive to metronidazole, ticarcillin-clavulanate, piperacillin/tazobactam, 3rd generation cephalosporins (ceftriaxone, ceftazidime, cefotaxime and others), clindamycin, cefoxitin, and imipenem, all of which can be used therapeutically to treat infections associated with *Fusobacterium necrophorum* (2,9). Often in patients with Lemierre's syndrome or other systemic infections, penicillin or antibiotics that are combined with a beta-lactamase inhibitor such as clavulanic acid or tazobactam are used along with metronidazole or clindamycin. Lemierre's syndrome is primarily treated with antibiotics given intravenously. In some instances, clindamycin can be given alone and can be used as the primary treatment in penicillin-allergic patients.

F. necrophorum is intrinsically resistant to gentamicin and quinolones (e.g., ciprofloxacin, levofloxacin), and tetracyclines show relatively poor activity against the organism. Unfortunately, *F. necrophorum* is not sensitive to erythromycin nor other macrolides that are often used for suspected Group A *Streptococcal* pharyngitis in penicillin-allergic patients, so these drugs would be ineffective in patients with a potential *F. necrophorum* infection. Penicillin or a cephalosporin remains the first treatment choice for adolescents and young adults with pharyngitis, and the addition of clindamycin is indicated for those with evidence of sepsis or neck swelling. Appropriate antibiotics should not be delayed, as mortality and morbidity are improved with the prompt administration of antibiotics (2,9). Generally, treatment for serious *F. necrophorum* infection, such as Lemierre's syndrome, is treated for a total of 6 weeks, with about 2 to 3 weeks of intravenous antibiotic therapy because of the possibility of relapse (2,9).

Since septic thrombophlebitis is such a key part of Lemierre's syndrome, the ability of antibiotics to penetrate and be active in fibrin clots is essential. Because clots are dense and not vascular they are hard for antibiotics to penetrate. It is also difficult for antibiotics to penetrate abscess and necrotic lesions. If antibiotic therapy does not improve the clinical picture, it may necessary to

perform surgical drainage of any abscesses or focal infection, and/or perform ligation (surgical closure of a blood vessel) of the internal jugular vein where the antibiotic cannot penetrate.

Treatment in animals for foot rot, calf diphtheria, and other infections due to *F. necrophorum* is usually with antibiotics, such as penicillin, sulfadiazine/trimetoprim, and sulfadimethoxine (6). All should be given by injection for three days as topical therapy is not effective. Most cases of foot rot are usually treated with antibiotics plus foot baths using solutions of 1% sodium hypochlorite, 0.2% chlorhexidine, 70% ethanol, 2% glutaraldehyde, 3% hydrogen peroxide, iodophores, and others (7).

I. PREVENTION

There are some good prevention and control measures for *F. necrophorum* infection in animals. Vaccinations against *F. necrophorum*, for example, are available to help prevent foot rot and liver abscesses in animals and are very effective (7). Although controversial, the addition of antibiotics to animal feed to prevent foot rot and calf diphtheria helps reduce *F. necrophorum* infection (7). There are various preventive measures that can be taken around animals to prevent contact with *Fusobacterium necrophorum* contaminated soil and manure by improving the drainage around drinking and feeding areas. Also, the isolation of cows when infected, and the use of a protective boot during the early infectious stages of foot rot helps prevent other animals from obtaining the infection. The use of preventive foot baths using 5% copper sulphate or formalin, zinc methionine, and paraformaldehyde is also helpful (7). The antimicrobials most commonly used for animal prophylaxis are bacitracin, methylene disalicylate, chlortetracycline, oxytetracycline, and virginiamycin (7).

In humans, the most important preventive measure to reduce the impact of Lemierre's syndrome is early recognition of symptoms of severe tonsillitis and tonsillar abscess in patients age 15-24. Once symptoms are recognized, it is important to start appropriate antibiotic therapy early since mortality and morbidity are reduced with rapid treatment. Although this infection is rare, researchers agree that this diagnosis should be considered in a septicemic patient with thrombosis from an unusual site, and in patients age 15-24. Prevention in humans is difficult, so physicians need to be on the alert for symptoms that predispose a patient to Lemierre's disease. For example, in a 15-24 year old patient with persistent sore throat, a clinician should consider a possible infection *F. necrophorum* and culture for this organism (1,3).

CASE STUDY

A 16-year-old girl who had been in excellent health consulted her physician because she developed a five-day minor fever and very sore throat. He found that she had large, swollen tonsils with pus on the exterior surface. The physician considered that, based on her symptoms and history, she might have Group A *Streptococcus* pharyngitis. A rapid strep test performed in the office test was negative. In light of this finding the doctor thought she might have a viral pharyngitis, so he gave her no antibiotic, only medicine for her throat pain, fever, and nasal congestion.

Her symptoms and her sore throat abated temporarily; however, on the 8th day after her office visit, she suddenly developed a fever with chills, and pain on one side of her neck that progressed down toward her clavicle. She returned to her physician, who examined her again and felt she might have Group A *Streptococcal* pharyngitis even though her rapid strep test was negative. The

physician was aware that the reported sensitivity of the rapid strep test is 85-90%. He started her on erythromycin since she was allergic to penicillin, and the patient went home.

The following day she was unable to get out of bed. She had a headache, a fever of 102°F, chills, painful joints, and a painful red area on the right side of her face with some neck stiffness. Her parents took her to the emergency department at the local hospital. The ER physician was concerned about her sepsis and fever, so he ordered two sets of blood cultures. He was able to aspirate fluid from the abscess in her neck. A stat Gram stain ordered on the neck abscess fluid showed many WBCs and some pleomorphic Gram-negative rods. Cultures were requested from the clinical material that had been obtained by needle and syringe and submitted to the laboratory in an anaerobic transport tube.

In the emergency room the examination of the patient showed there was marked neck stiffness and tenderness extending to the soft tissue of her shoulder. The patient had markedly purulent and inflamed tonsils in addition to the abscess in her neck area. The patient also complained about pain in her legs and knees. Further examination revealed that she had low blood pressure and symptoms of shock. Stat laboratory tests were ordered, including a CBC, coagulation studies, erythrocyte sedimentation rate, liver function tests, and a C-reactive protein test. Radiological studies of the patient's neck were requested. A computer tomography scan (CT) showed a thrombosis of the right internal jugular vein.

The laboratory test results showed a WBC count of 20,000/ μ l, thrombocytopenia, impaired renal function, increased erythrocyte sedimentation rate, elevated C-reactive protein and a normal coagulation profile. This teenage girl not only had an infection but was in shock. Based on clinical features of the patient and preliminary laboratory work, a diagnosis of Lemierre's syndrome was made and antimicrobial therapy was initiated. She was treated with intravenous ceftriaxone and metronidazole and anticoagulant therapy and was transferred to a hospital room for further management.

The following day, laboratory data showed the blood cultures were positive with pleomorphic Gram-negative rods, which were subcultured to aerobic and anaerobic media. The neck abscess specimen showed growth of anaerobic pleomorphic Gram-negative rods. The laboratory was able to perform some spot tests on the isolate from the neck abscess, as well as subculture the isolate to a brucella sheep blood agar plate with special potency disks and to an egg yolk agar plate. Spot tests on the neck abscess material showed the isolate was indole positive and nitrate reduction test negative. The next day, growth from the anaerobic subculture plate that contained the special potency antibiotic disks showed the isolate was resistant to the vancomycin 5 μ g disk, but susceptible to both kanamycin 1000 μ g and colistin 10 μ g disks. Growth on the egg yolk plate produced an oily iridescent sheen on top and adjacent to isolated colonies, indicative of a positive lipase test. Based upon these tests, the laboratory determined that the isolate was *Fusobacterium necrophorum*. The laboratory also performed a rapid 4 hr anaerobe identification kit on the isolate; however, the commercial test kit was not able to determine the name of the isolate.

Meanwhile, the patient began to slowly improve. Her fever came down to 100°F, and some of her symptoms of sepsis (shock, fever, and chills) were reduced, although her neck abscess still looked red and swollen. Her doctors were still concerned about the abscess in her neck and jugular vein. A surgeon performed a ligation of the

vein and removed any clots. The young girl did well with her surgery and slowly became less febrile and more responsive.

Subsequent blood cultures were obtained, but were negative for any growth. Her doctor felt she was improving but needed to be on IV antibiotics for several weeks to prevent any chance of relapse. The patient was discharged with IV antibiotics for two weeks but needed to see her doctor after one week to make sure that the IV given on an outpatient basis was satisfactory.

Case Study Review

This case illustrates the problem of diagnosing *Fusobacterium* infection. Her physician was not aware of the possibility of pharyngitis in her age group being due to anything other than Group A *Streptococcus*, or a viral infection. Therefore, the patient was not worked up further.

When the symptoms of this patient became worse, she was taken to a hospital emergency department, where the ER physician was able to perform some tests to determine her diagnosis. The ER physician performed the correct steps to make a diagnosis and began appropriate antibiotics. Her symptoms of shock, her age, her one-sided neck pain and her history of having had a severe case of pharyngitis that abated temporarily provided clues to the ER physician. In addition, laboratory results from the stat Gram stain led the doctor in the right direction, suggesting what additional tests to perform and which antibiotics to prescribe. The aspirate of the neck abscess showed pleomorphic Gram-negative rods, information the ER physician used to select antibiotics that covered most aerobic and anaerobic Gram-negative organisms.

The laboratory used the correct anaerobic transport system, enriched media, and environmental conditions to recover *Fusobacterium necrophorum* an obligate anaerobe. It is likely that less optimal transport conditions, media, and environmental systems would not have allowed isolation of the organism.

Lemierre's syndrome historically had a high mortality rate, up to 90% before the advent of antibiotics. Because the syndrome is seldom seen now it is called a "forgotten disease." Further diagnostic delay could have been devastating to this patient. However, the patient did recover with surgical treatment of the jugular vein and the use of appropriate antibiotics for a prolonged time.

J. CONCLUSION

Human *F. necrophorum* infection, once less prevalent, is on the rise. It is important that clinicians and laboratory personnel become aware of the symptoms, course of disease, diagnosis and treatment of *F. necrophorum* infection because when it is missed, this organism can cause severe illness or death. *F. necrophorum* is now recognized as a causative agent in many cases of recurrent sore throat syndrome and tonsillitis. One of the aims of this course is to provide information about *F. necrophorum* and Lemierre's disease.

As devastating as the disease can be in humans, the prevalence of *F. necrophorum* is far greater in animal populations and causes symptoms and diseases in cattle, sheep, and horses. Many of these infections have a significant economic impact.

F. necrophorum is unique among non-spore-forming anaerobes for its virulence, and for its ability to form necrotic tissue due to its many pathogenic factors. *Fusobacterium necrophorum* are part of the normal flora of the oropharyngeal, gastrointestinal and genital tracts of animals and humans.

Transmission of *F. necrophorum* in animals is due primarily to contaminated soil or food with manure. *F. necrophorum* can survive many weeks in the soil, even though it does not produce spores.

Transmission of *F. necrophorum* in humans was believed to be due to acquiring the organism from one's own normal flora. Recent studies have shown that this is not always the case. Other factors such as concurrent infection with a virus like EBV, or from an exogenous source may be factors.

F. necrophorum in humans causes a variety of infections that are often overlooked. The most recent finding is that *F. necrophorum* can be a cause in persistent sore throat. In one study, it was found that *F. necrophorum* is responsible for acute sore throats with the same incidence as caused by Group A *Streptococci* in specific age groups.

For the most part, the diagnosis of *F. necrophorum* infection depends upon the laboratory to isolate and identify the organism. It is important to use a good transport system to maintain viability of the organism, and once in the laboratory, to plate the specimen on good enriched media using good anaerobic techniques. Since *F. necrophorum* is a strict anaerobe many laboratories are not able to recover the organism unless good anaerobic techniques are used.

In humans early diagnosis and recognition of symptoms in patients in specific age groups is important to prevent Lemierre's and further disease. The use of antibiotics early helps to prevent symptoms. Unfortunately, in many cases, the diagnosis of Lemierre's syndrome is made after a positive blood culture or after the recovery of *F. necrophorum* from other body sites. Awareness of the potential complications of Lemierre syndrome and prompt management are crucial in preventing disease. Lemierre's syndrome carries a significant rate of morbidity and mortality. The high rate of morbidity and mortality is due not only the virulence of the organism, but also because of lack of awareness of the symptoms by a physician, its uncommon presentation, and because of the use of inadequate anaerobic culture methods.

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Table 1 Previous Synonyms of *Fusobacterium necrophorum**

Actinomyces necrophorus
Actinomyces cuniculi
Bacillus filiformis
Bacillus funduliformis
Bacillus diphtheria
Bacillus necrophorus
Bacillus pyogenes anaerobius
Bacillus symbiophiles
Bacterium necrophorum
Bacterium necrophorus
Bacteroides funduliformis
Bacteroides necrophorus
Fusiformis necrophorus
Necrobacterium funduliforme
Streptothrix cuniculi
Sphaerophorus necrophorus
Sphaerophorus funduliformis
Streptothrix necrophora

*adapted from reference number 4.

Table 2. Summary of Pathogenic Mechanisms of *Fusobacterium necrophorum**

Factor	Mechanism of Pathogenicity
Adhesion	Cell surface molecules which permit attachment to host cells. This is key first step in pathogenesis.
Fimbriae	Hair-like structures on surface of <i>F. necrophorum</i> that permit attachment to host cell.
Leucocidin	Probably most significant exotoxin. It destroys leukocytes by causing pores in the leukocyte cell membrane so that cellular material leaks out.
Leukotoxin	This exotoxin contributes to the characteristic necrotic abscesses in <i>F. necrophorum</i> infection. Produces degeneration and lysis of the leukocyte cellular membrane causing death to the cell.
Lipopolysaccharide (LPS)	Important endotoxin within the cell wall of <i>F. necrophorum</i> . It produces abscesses, hemolysis, and tissue destruction. It also elicits a strong immune response which may include fever, shock, and decreased blood pressure characteristic of Lemierre's syndrome.
Proteolytic enzymes	Various exotoxins that break down proteins in body tissue and lead to destruction, which permits invasion of organism into other tissues.
Plasminogen, platelet aggregation factor, hemolysin, and hemagglutinin	Important exotoxins that cause abnormal coagulation and the formation of clots characteristic of Lemierre's syndrome.

*Adapted from references number 7 and 8.

Table 3. Features and Biochemical Characteristics of Most Common Fusobacterium species*

Species name	Cellular morphology	Indole	Growth in 20% bile	Lipase	Esculin hydrolysis
<i>F. mortiferum</i>	bizarre, round bodies	-	+	-	+
<i>F. necrophorum</i>	pleomorphic with swellings and long rods	+	- ⁺	+ ⁻	-
<i>F. nucleatum</i>	slender pointed end rods	+	-	-	-
<i>F. varium</i>	bizarre, round bodies	V	+	V ⁺	-

Key: - is negative, + is positive, V is variable, V⁺ is variable, V⁻ is variable, V⁺ most strains are positive, V⁻ most strains are negative, V⁺ some strains may be positive after 5 days of incubation.

Indole: Some organisms are able to oxidize tryptophan into indole. A positive test of indole is indicated in this chart by a +.

Growth in 20% bile: Some anaerobic gram-negative rods can grow in the presence of high concentrations of bile. Growth is indicated by turbidity and is indicated in this chart by +. Lipase: Some organisms produce the enzyme lipase that can break down triglycerides in media, producing an oily iridescent sheen on and surrounding colonies on a medium such as egg yolk agar.

Esculin hydrolysis: Some organisms are able to hydrolyze esculin to esculetin, which reacts with iron in the medium to produce a dark brown or black complex. A positive test is indicated in this chart by a +.

* adapted from references number 4 and 7.

Table 4. Special Potency Antibiotic Disks Reactions*

Organism	Response ^a to:		
	Kanamycin 1,000 µg	Vancomycin 5 µg	Colistin 10 µg
Gram positive organisms		S ^b	R
<i>Bacteroides fragilis</i> group	R	R	R
<i>Bacteroides ureolyticus</i> group	S	R	S
<i>Fusobacterium</i> sp.	S	R	S
<i>Porphyromonas</i> sp.	R	S ^c	R
<i>Prevotella</i> sp.	R	R	V
Gram-negative cocci	S	R	S

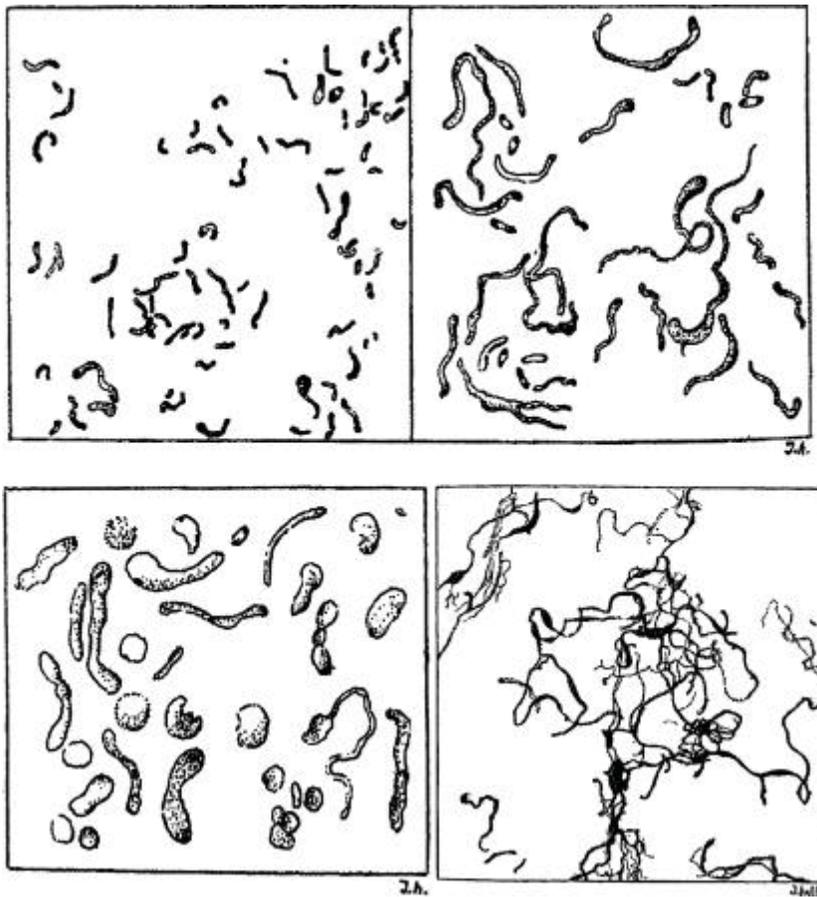
^a R, resistant; S, susceptible; V, variable

^b Exceptions: Rare strains of *Lactobacillus* sp. and *Clostridium* sp. may be vancomycin resistant

^c *Porphyromonas* sp. is vancomycin sensitive, but fluoresces or is pigmented

*adapted from reference number 4.

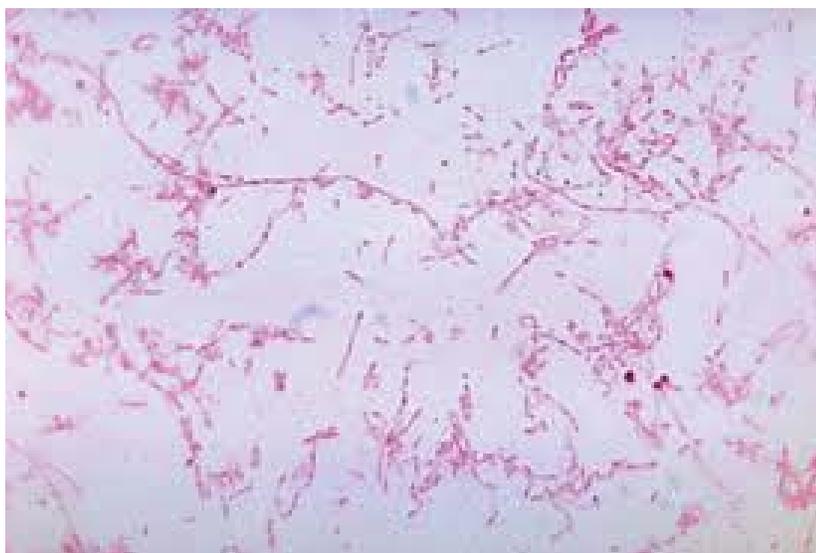
Fig. 1. Line drawings of *Bacillus funduliformis**



*Line drawings from J. Halle, 1898. Adapted from reference number 8.

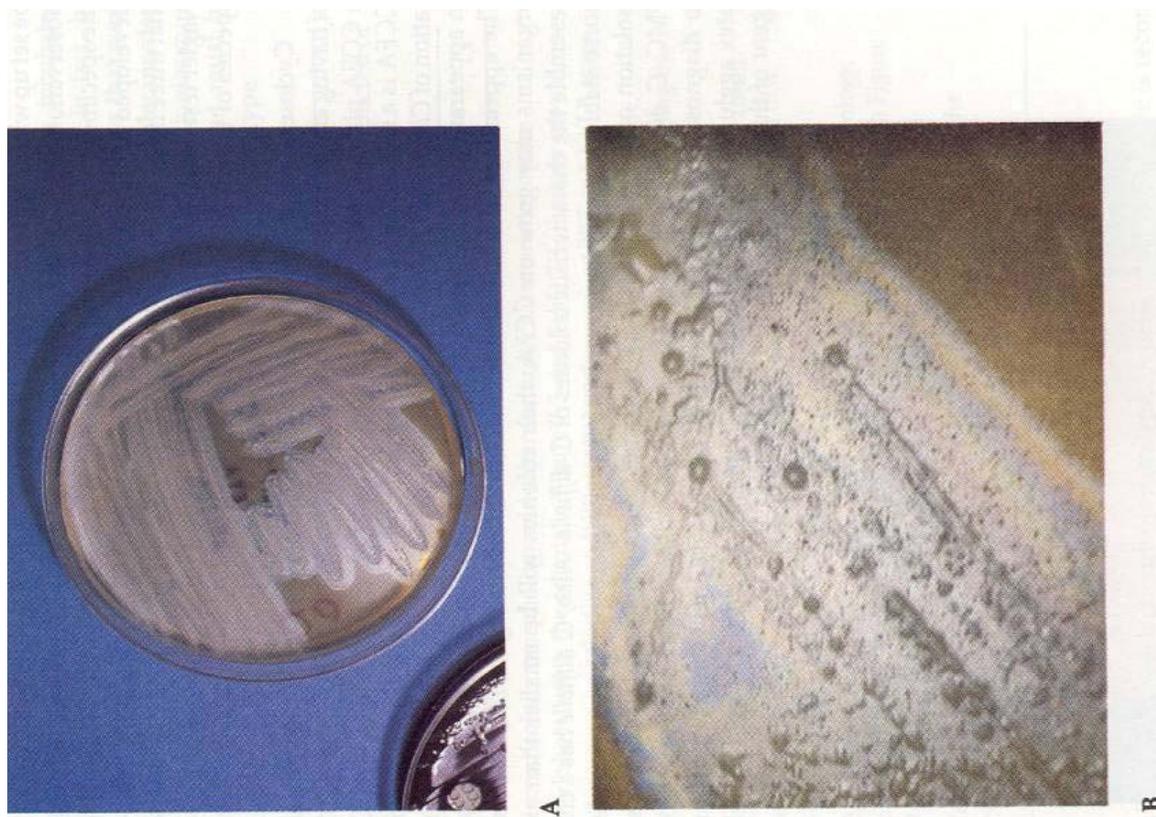
Comments from a thesis by J. Halle: "Top left, common short, slightly curved rods seen in pus and tissues; top right, rare giant forms; bottom left, poorly staining vesicular forms sometimes seen in culture; bottom right, bizarre pleomorphic forms commonly seen in culture."

Fig 2. Gram stain of *Fusobacterium necrophorum**



*Adapted from archival photos from Centers for Disease Control and Prevention, 2014.

Fig. 3. Photograph of Positive Lipase Reaction of *Fusobacterium necrophorum**



*adapted from Anaerobe Systems and Hardy Diagnostics. Photo on left is looking at whole plate; photo on right is close up of positive lipase reaction observing oily sheen and iridescence.

REVIEW QUESTIONS

Course #DL-012

Choose the **one** best answer

1. Which one of the following statements about *Fusobacterium necrophorum* infections is correct?
 - a. causes abortion in cattle
 - b. causes necrotic lesions
 - c. more common infection in humans than animals
 - d. causes infections that are difficult to treat
2. Which set of symptoms is most typical of foot rot in cattle due to *F. necrophorum*:
 - a. fever, chills, swelling
 - b. swollen cheeks, fever, pneumonia
 - c. erythema, tissue destruction, foul odor
 - d. pain, lameness, chronic arthritis
3. Which is not a pathogenic factor of *Fusobacterium necrophorum*:
 - a. lipopolysaccharide
 - b. leukotoxin
 - c. hemagglutinin
 - d. fibronectin
4. Which set of symptoms is most typical of Lemierre's syndrome?
 - a. peritonsillar abscess, fever, septic thrombophlebitis
 - b. pharyngitis, flu-like symptoms, chronic arthritis
 - c. sudden fever, chills, pneumonia
 - d. peritonsillar abscess, fever, meningitis
5. Which set of biochemical test results best fits the profile of *Fusobacterium necrophorum*:
 - a. indole positive, nitrate reduction test positive, lipase negative
 - b. indole positive, nitrate reduction test negative, lipase positive
 - c. indole negative, nitrate reduction test negative, lipase negative
 - d. indole positive, nitrate reduction test positive, lipase positive
6. In what age group is recurrent sore throat due to *F. necrophorum* most prevalent:
 - a. individuals >65 years old
 - b. children 8-12 years of age
 - c. 15-24 years of age
 - d. 24-30 years of age
7. One pathogenic function of lipopolysaccharide from *F. necrophorum* is;
 - a. attachment
 - b. production of exotoxins
 - c. chemotaxis of macrophages
 - d. tissue destruction
8. One characteristic of *F. necrophorum* is the ability to fluoresce:
 - a. black
 - b. red
 - c. chartreuse
 - d. depending upon the medium used
9. Which set of features best describes the appearance of *F. necrophorum* on brucella blood agar:
 - a. colonies are umbonate, scalloped margin, cream color
 - b. colonies are circular, smooth edge, white color, smell of lemon
 - c. colonies are rough, gray to translucent, with a narrow zone of beta hemolysis
 - d. colonies are oval, smooth margin, yellow color
10. Which are the correct Gram stain morphological features of *Fusobacterium necrophorum*:
 - a. Gram negative rod, sometimes in pairs, sometimes very small
 - b. Gram negative coccobacillus, sometimes with large swellings
 - c. Gram negative pleomorphic rod, sometimes with long cells, sometimes with swellings
 - d. Gram negative rod, long, thin, spindle-shaped large cells
11. Attachment of *F. necrophorum* to host cells is initiated by:
 - a. D-galactose residues
 - b. Fimbriae
 - c. the protein internalin
 - d. leucocidin
12. What is a recommended antibiotic therapy for *Fusobacterium necrophorum*?
 - a. penicillin plus metronidazole
 - b. penicillin plus gentamicin
 - c. cephalosporin plus ciprofloxacin
 - d. penicillin plus macrolide
13. The function of leucocidin in the pathogenesis of *F. necrophorum* is that it:
 - a. produces phagosome enzymes
 - b. causes fever and shock
 - c. promotes attachment to host cells
 - d. causes destruction of polymorphonuclear leukocytes
14. The most common manifestations of Vincent's angina include:
 - a. high fever, bleeding and swelling of tissues from mouth, blood clot in lungs
 - b. high fever, shock, one-sided thrombophlebitis of jugular vein
 - c. painful ulcerations, swelling and sloughing off of dead tissue
 - d. painful ulcerations, high fever, spread of infection throughout the body
15. The typical clinical history for a patient with Lemierre's syndrome is:
 - a. prior pharyngitis, high fever, one-sided thrombophlebitis
 - b. prior pharyngitis, flu-like symptoms, meningitis
 - c. flu-like symptoms, fatigue, bacteremia, pneumonia
 - d. flu-like symptoms, high fever, one-sided thrombophlebitis

16. The most optimal preventive measures to reduce the impact of Lemierre's syndrome are:
- early recognition of symptoms and start appropriate antibiotic
 - treat patient after obtaining rapid strep testing result
 - obtain X-ray and sonogram of jugular vein
 - obtain blood cultures
17. One measure to prevent *F. necrophorum* infection in animals is not:
- to reduce the animal's contact with manure
 - to treat animals with macrolides
 - to improve drainage
 - to add antibiotics to food
18. The isolation and identification of *F. necrophorum* in the laboratory does not depend upon:
- a good specimen transported in an anaerobic transport device
 - use of good enriched anaerobic media
 - use of correct anaerobic environment
 - use of commercial anaerobic identification system
19. The formation of clots and abnormal coagulation characteristics of *F. necrophorum* are due to:
- the activation of leukotoxin
 - the activation of adhesion molecules and fimbriae
 - the activation of plasminogen, platelet aggregation factor; and hemagglutinin
 - the activation of leucocidin
20. The special potency antibiotic disk reactions of *Fusobacterium necrophorum* are:
- resistant to vancomycin 5 µg, and susceptible to kanamycin 1000 µg and colistin 10 µg
 - susceptible to vancomycin 5 µg, and resistant to kanamycin 1000 µg and colistin 10 µg
 - susceptible to vancomycin 5 µg, and kanamycin 1000 µg, but resistant to colistin 10 µg
 - resistant to vancomycin 5 µg, kanamycin 1000 µg, and colistin 10 µg
21. Typically *F. necrophorum* produces:
- isobutyric acid from glucose broth
 - formate from glucose broth
 - acetone from glucose broth
 - butyric acid from glucose broth
22. Lemierre's syndrome usually occurs when:
- a peritonsillar abscess spreads to nearby tissues and to the jugular vein
 - a peritonsillar abscess spreads to the lungs
 - Vincent's angina spreads to the oral cavity
 - recurrent sore throat persists past 5 days and causes meningitis
23. One reason the diagnosis of Lemierre's syndrome is often overlooked is because:
- symptoms are vague and mimic bacterial pneumonia
 - the syndrome is rare and many clinicians have never seen a case
 - the patient has flu-like symptoms
 - the patient is thought to have Group B *Streptococcus* infection
24. Which statement is not correct to best describe recurrent or persistent sore throat:
- a sore throat that has persisted for longer than 5 days and is negative for *Streptococcus* Group A
 - a pharyngitis particularly in patients 15-24 years of age
 - 10% of cases in certain age groups may be due to *F. necrophorum*
 - always caused by viruses
25. Which statement is not correct to describe the transmission of *Fusobacterium necrophorum* in animals:
- obtained from contaminated soil
 - organism can survive in soil of pastures for up to 18 weeks
 - direct animal to animal contact through open sores or bites
 - obtained from contaminated water source
26. Professor Lemierre's most important contribution to our understanding of *F. necrophorum* infection was:
- the clarity of his clinical description of post-anginal septicemia
 - his clarity in determining the cause of foot rot infection in animals
 - his clarity in his clinical description of bacterial pneumonia
 - his understanding of the clinical impact of bleeding abnormalities in infection
27. Which statement is not correct to describe the transmission of *Fusobacterium necrophorum* in humans:
- obtained from our own normal flora
 - obtained after a break or change in immunity
 - obtained from contaminated food
 - obtained during a concurrent infection with a virus
28. The term zoonotic infection means:
- an infection that occurs in animals from a zoo
 - an infection that occurs in animals that can be transmitted to humans
 - an infection that is transmitted to animals from man
 - an infection caused by an organism in animals that can cause disease in other species of animals
29. Which statement is most likely the reason a clinical laboratory was not able to isolate *F. necrophorum* from a specimen after observing from a direct Gram stain:
- not using media enriched with sheep blood
 - not using media enriched with vitamin K, hemin and was pre-reduced
 - not using media that contains egg products
 - not incubating media under 5% carbon dioxide
30. The production of a positive lipase reaction depends upon:
- the ability of organism to break down triglycerides in media
 - the ability of organism to split tryptophan in egg yolk agar
 - the ability of organism to break down lecithin in egg yolk agar³
 - the ability of organism to hemolyze fats in egg yolk agar

Record your answers on the Answer Sheet on page 28

EBOLA VIRUS DISEASE – Worldwide Implications

DL-013

1.0 CE

Level of Difficulty: Basic

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The information in this course is from <http://www.cdc.gov/vhf/ebola/healthcare-us/index.html>. Please refer to this reference for further information.

OBJECTIVES:

At the end of this course the participant will be able to

1. Give the host and susceptible mammals of Ebola virus disease
2. Identify the countries involved in the present epidemic
3. List the symptoms of Ebola
4. Describe how Ebola is transmitted
5. Identify the laboratory tests for diagnosis of Ebola
6. Discuss the procedures for prevention of transmission of Ebola
7. Outline the equipment and procedures for personal prevention equipment
8. Summarize the roles of the various healthcare facilities involved in identification, diagnosis, and treatment of Ebola
9. Outline the history of Ebola virus disease
10. Discuss the viral make-up and species of Ebola

INTRODUCTION:

Ebola (Ebola hemorrhagic fever) is a severe disease that is caused by a virus. Ebola is named for the river in Africa where the disease was first recognized in 1976. The exact origin and natural host of Ebola virus are unknown. There are five kinds of Ebola virus named for the places they are associated with. Four cause disease in humans. Ebola-Reston, the only kind that does not cause disease in humans, was brought to the United States in 1989. Monkeys that were brought to a Reston, Virginia research facility had the disease. They were very sick and died. None of the people who worked with the monkeys got sick.

People who get Ebola can have a high fever, body aches, rash, vomiting, and chest pain. They can also go blind, go into shock, and hemorrhage. They can die within one week of catching the virus. There is no vaccine or treatment for Ebola.

In March 2014 an epidemic of Ebola began in West Africa. This distance learning course will cover the history of the epidemic as well as the disease and procedures for caring for patients and preventing the spread of the disease.

EBOLA VIRUS

Ebola, previously known as Ebola hemorrhagic fever, is a rare and deadly disease caused by infection with one of the Ebola virus strains. Ebola can cause disease in humans and nonhuman primates (monkeys, gorillas, and chimpanzees).

Ebola is caused by infection with a virus of the family *Filoviridae*, genus *Ebolavirus*. It is single-stranded RNA in filaments or branched filaments. There are five identified Ebola virus species, four of which are known to cause disease in humans: Zaire virus (*Zaire ebolavirus*), Sudan virus (*Sudan ebolavirus*), Tai Forest virus (*Tai Forest ebolavirus*, formerly *Cote d'Ivoire ebolavirus*), and Bundibugyo virus (*Bundibugyo*

ebolavirus). The fifth, Reston virus (*Reston ebolavirus*), has caused disease in nonhuman primates, but not in humans.

Ebola viruses are found in several African countries. Ebola was first discovered in 1976 near the Ebola River in what is now the Democratic Republic of the Congo. Since then, outbreaks have appeared sporadically in Africa. See Table 1.

The natural reservoir host of Ebola virus remains unknown. However, on the basis of evidence and the nature of similar viruses, researchers believe that the virus is animal-borne and that bats are the most likely reservoir. Four of the five virus strains occur in an animal host native to Africa.

EBOLA OUTBREAK IN WEST AFRICA

The 2014 Ebola epidemic is the largest in history, primarily affecting the West African countries of Guinea, Liberia, and Sierra Leone. There have been a small number of cases reported in Nigeria and Mali and a single case in Senegal. Two imported cases, including one death, and two locally acquired cases in healthcare workers were reported in the United States. Several other countries reported imported cases.

In March 2014 the Ministry of Health of Guinea reported Ebola fever in four southeastern districts. There were reports of suspected cases in the neighboring countries of Liberia and Sierra Leone. Guinea reported a total of 86 suspected cases including 59 deaths. The Pasteur Institute made a preliminary report of *Zaire ebolavirus* as the causative agent. By the end of March there were 112 cases and 70 deaths. Over the ensuing months the number of cases in Guinea, Liberia, and Sierra Leone continued to increase, with the fatality rate over 55%.

Various outside organizations including Doctors Without Borders, WHO-led international response organizations, and CDC helped set up Ebola treatment centers, awareness campaigns, and provided equipment. Other organizations provided laboratory diagnostics.

On September 30 a man who had returned from Liberia was first diagnosed with Ebola at Texas Presbyterian Hospital. He died 9 days later. On October 10 a healthcare worker at the hospital developed Ebola and recovered. A second healthcare worker who had cared for the index case developed Ebola and subsequently recovered. On October 23 a case was reported in a medical aid worker who had returned from Guinea. The patient was treated at Bellevue Hospital in New York City and recovered. In addition to these patients, six other U.S. citizens who were diagnosed in Africa were brought to the U.S. for treatment. One died and the other five recovered.

Since November there were 8 cases reported in Mali and 20 in Nigeria. The number of cases in Guinea, Liberia, and Sierra Leone continued to increase. However, the report at the end of January 2015 showed that for the first time since the end of June 2014, there have been fewer than 100 new cases reported in a week in Guinea, Liberia, and Sierra Leone.

As of 3/20/15 the total cases (suspected, probable, and confirmed) in the three countries was 24,754 with 10,236 deaths, a 41.4% fatality rate. In addition to the U.S., there were imported cases in four other countries, England, Scotland, Senegal, and Spain. A country is considered to be free of Ebola virus transmission when 42 days (double the 21-day incubation period) has lapsed since the last patient in isolation became laboratory negative for EVD.

EBOLA DISEASE

Symptoms:

Symptoms include: fever, severe headache, joint and muscle pain, weakness, fatigue, diarrhea, vomiting, abdominal (stomach) pain, and unexplained hemorrhage. Symptoms may appear anywhere from 2 to 21 days after exposure to Ebola, but the average is 8-10 days.

Treatment:

No FDA-approved vaccine or medicine (e.g., antiviral drug) is available for Ebola as of this writing. Experimental vaccines and treatments for Ebola are under development, but they have not yet been fully tested for safety or effectiveness. Recovery from Ebola depends on good supportive clinical care and the patient's immune response. Symptoms of Ebola and complications are treated as they appear. The following basic interventions, when used early, can significantly improve the chances of survival:

- Providing intravenous fluids and balancing electrolytes
- Maintaining oxygen status and blood pressure
- Treating other infections if they occur

People who recover from Ebola infection develop antibodies that last for at least 10 years, possibly longer. It is not known if people who recover are immune for life or if they can become infected with a different species of Ebola. Some people who have recovered from Ebola have developed long-term complications, such as joint and vision problems.

Diagnosis:

Diagnosing Ebola in a person who has been infected for only a few days is difficult because the early symptoms, such as fever, are nonspecific to Ebola infection and often are seen in patients with more common diseases, such as malaria and typhoid fever.

However, if a person has the early symptoms of Ebola and has had contact with the blood or body fluids of a person sick with Ebola; contact with objects that have been contaminated with the blood or body fluids of a person sick with Ebola; or contact with infected animals, they should be isolated and public health professionals notified. Samples from the patient can then be collected and tested to confirm infection.

Ebola virus is detected in blood only after onset of symptoms, most notably fever, which accompany the rise in circulating virus within the patient's body. It may take up to three days after symptoms start for the virus to reach detectable levels.

Laboratory Tests:

<u>Timeline of Infection</u>	<u>Diagnostic tests available</u>
Within a few days after symptoms begin	Antigen-capture enzyme linked immunoassay IgM ELISA Polymerase chain reaction (PCR) Virus isolation
Later in disease course or after recovery	IgM and IgG antibodies
Retrospectively in deceased patients	Immunohistochemistry testing PCR Virus isolation

Transmission:

Because the natural reservoir host of Ebola viruses has not yet been identified, the way in which the virus first appears in a human at the start of an outbreak is unknown. However, scientists believe that the first patient becomes infected through contact with an infected animal, such as a fruit bat or primate (apes and monkeys),

which is called a spillover event. Person-to-person transmission follows and can lead to large numbers of affected people. In some past Ebola outbreaks, primates were also affected and multiple spillover events occurred when people touched or ate infected primates.

When an infection occurs in humans, the virus can be spread to others through direct contact (through broken skin or mucous membranes in, for example, the eyes, nose, or mouth) with

- Body or body fluids (including but not limited to urine, saliva, sweat, feces, vomit, breast milk, and semen) of a person who is sick with Ebola
- Objects (like needles and syringes) that have been contaminated with the virus
- Infected fruit bats or primates (apes and monkeys)

Ebola is not spread through the air, by water, or in general, by food. However, in Africa, Ebola may be spread as a result of handling bushmeat (wild animals hunted for food) and contact with infected bats. There is no evidence that mosquitoes or other insects can transmit Ebola virus. Only a few species of mammals (e.g., humans, bats, monkeys, and apes) have shown the ability to become infected with and spread Ebola virus.

Healthcare providers caring for Ebola patients and family and friends in close contact with Ebola patients are at the highest risk of getting sick because they may come in contact with infected blood or body fluids, which may be through contact with objects like bedding, clothes, needles, syringes, sharps, or medical equipment. During outbreaks of Ebola, the disease can spread quickly within healthcare settings (such as a clinic or hospital). Exposure to Ebola can occur in healthcare settings where hospital staff is not wearing appropriate personal protective equipment.

Dedicated medical equipment (preferably disposable, when possible) should be used by healthcare personnel who provide patient care. Proper cleaning and disposal of instruments, such as needles and syringes, also are important. If instruments are not disposable, they must be sterilized before being used again. Without adequate sterilization of instruments, virus transmission can continue and amplify an outbreak.

Scientists know that the Ebola virus can stay in semen and in vaginal fluids even after recovery. Scientists continue to study whether and for how long Ebola can be spread through sex. Until more is known, Ebola survivors should not have sex (oral, vaginal, or anal) for at least three months after recovery. If abstinence is not possible, a condom should be used every time.

PREPARATION AND PREVENTION:

Acute care facilities

During the epidemic the CDC developed protocols for hospitals in the United States that might have to treat a patient with Ebola. All U.S. acute care facilities have an important role in preparing to identify, isolate, and evaluate patients under investigation (PUI) for Ebola and promptly inform public health authorities. However, the roles and the preparations required will differ by facility. Acute healthcare facilities can serve one of three roles:

1. Frontline healthcare facility
2. Ebola assessment hospital
3. Ebola treatment center

The capabilities of each are as follows:

Frontline healthcare facility:

- Identify patients with relevant exposure history and Ebola-compatible symptoms

- Isolate patients
- Inform health department
- Initiate testing if low-risk; high risk should be transferred for evaluation and testing
- Staff trained on specimen transport, waste management, Standard Precautions; proficient in donning and doffing personal protection equipment (PPE)

Ebola assessment hospitals

- Evaluate and care for patient for up to 96 hours or until discharged or transferred
- Initiate Ebola testing and transport patient to Ebola treatment center if lab-confirmed Ebola Virus Disease
- Staff trained and proficient in donning/doffing PPE, proper waste management, infection control practices, and specimen transport

Ebola treatment centers

- Care for and manage patient throughout disease process

Personal Protective Equipment (PPE) needs for each facility level.

Since in healthcare settings Ebola virus is spread through direct contact (through broken skin or through mucous membranes of the eyes, nose, or mouth) with blood or body fluids of a person who is sick with Ebola or with objects (needles, syringes, bedding, clothes) that have been contaminated with the virus, all healthcare workers caring for patients with Ebola, PPE with full body coverage including respiratory protection and double gloving is recommended to reduce the risk of contamination. The PPE procedure most likely to result in contamination of the healthcare worker is doffing (taking off) of PPE. There are different personal protection equipment needs for each of these care facilities, as follows:

Frontline healthcare facility

- The use of PPE should be based on the patient's clinical status
- PPE for clinically stable patients should be sufficient for most patients
- Maintain access to Ebola PPE sufficient for 12-24 hours of patient care, to be used if needed

Ebola assessment hospitals

- The use of PPE should be based on the patient's clinical status
- Maintain Ebola PPE sufficient for 4-5 days of patient care

Ebola treatment centers

- Maintain Ebola PPE sufficient for at least 7 days of patient care

Outpatient facilities

Most patients with fever and other symptoms coming to an ambulatory care facility don't have Ebola, but it is important that staff members know how to identify and manage patients who might have Ebola. Staff members should be ready to take three steps: Identify, Isolate, and Inform by doing the following:

- Ask every patient if, in the last 21 days, they traveled to a country with widespread transmission or uncertain control measures (Guinea, Liberia, or Sierra Leone) or had contact with someone with confirmed Ebola.
- If a patient appears to be at risk for Ebola, isolate the patient immediately, avoid unnecessary direct contact, determine personal protective equipment needed, and notify the health department to arrange a transfer to a facility that can further assess the patient.
- Do not transfer the patient without first notifying the health department; these patients should only be transferred to

a facility approved by public health authorities.

Clinical Laboratory Testing of Clinical Specimens when Ebola is a concern

Note: for more information see <http://www.cdc.gov/vhf/ebola/healthcare-us/laboratories/safe-specimen-management.html>

If a patient meets the criteria for persons under investigation for Ebola (has the early symptoms including fever, severe headache, vomiting, diarrhea, abdominal pain or unexplained hemorrhage and has an epidemiological risk factor, had contact with the blood or body fluids of a person sick with Ebola, contact with objects that have been contaminated with the blood or body fluids of a person sick with Ebola, or contact with infected animals), they should be isolated and local and/or state public health authorities notified. A blood specimen is drawn and sent to the indicated Laboratory Response Network laboratory. If it is determined that testing for Ebola virus is indicated, at least 4 mL whole blood collected in a plastic tube and preserved with EDTA is the preferred sample. Specimens should be shipped with refrigerant to maintain 2°-8°C.

CDC considers the risk of acquiring Ebola virus disease or other hemorrhagic diseases through laboratory testing to be low, but not zero risk. Recommended measures to minimize the risk of laboratory transmission include: limiting the number of staff engaged in testing, evaluating, and segregating equipment used for testing and performing testing in a dedicated space. The decision to perform testing in a hospital care laboratory using existing instrumentation, or alternatively, acquiring dedicated point of care instrumentation should be carefully evaluated because of the consequence of testing contaminated blood might lead to core laboratory instruments being removed from service. The planning should include how to mitigate such potential outcomes.

Although laboratory testing for patients for which there is a clinical suspicion of Ebola, or a patient with confirmed Ebola will likely vary, assessment and treatment facilities should consider how they might safely perform the following laboratory tests, if indicated, or, if unable to safely perform specific tests, identify alternative approaches to patient management (e.g., empiric treatments, alternative diagnostic strategies):

- A complete blood count including differential, and platelet count
- Sodium, potassium, chloride, bicarbonate, calcium, blood urea nitrogen, creatinine, and glucose
- Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin
- Coagulation testing, specifically prothrombin (PT) expressed as INR
- Blood culture for bacterial pathogens
- Malarial testing (smear or rapid tests)
- Influenza virus testing
- Respiratory syncytial virus and other respiratory virus testing
- Rapid group A strep testing on throat swabs
- Urinalysis

Ebola treatment hospitals should be able to provide the above tests, as well as additional testing required to manage a patient with Ebola.

SUMMARY:

Ebola virus disease was first described in 1976 at the Ebola River in Africa. It is a very severe disease that is spread by contact

with body fluids from an infected person. The Ebola epidemic, which started in the West African countries of Guinea, Sierra Leone, and Liberia in March 2014, presented challenges of control, prevention, and treatment. Several cases were imported to the United States, which led to two secondary cases. The CDC developed procedures for healthcare facilities to prepare for possible cases. These include how to diagnose, treat, and prevent the spread of the disease and who to notify. Laboratories need to be prepared to test patients and to be aware of the consequences of infected blood in their core instruments.

REFERENCES:

<http://www.cdc.gov/vhf/ebola/healthcare-us/index.html>

Outbreaks Chronology: Ebola Virus Disease					
Known Cases and Outbreaks of Ebola Virus Disease, in Reverse Chronological Order:					
Year(s)	Country	Ebola subtype	Reported number of human cases	Reported number (%) of deaths among cases	Situation
August – November 2014	Democratic Republic of the Congo	Ebola virus	66	49 (74%)	Outbreak occurred in multiple villages in the Democratic Republic of the Congo. The outbreak was unrelated to the outbreak of Ebola in West Africa.
March 2014 – Present	Multiple countries	Ebola virus	24281	9976	Ongoing outbreak across multiple countries in West Africa. Number of patients is constantly evolving due to the ongoing investigation.
November 2012 – January 2013	Uganda	Sudan virus	6*	3* (50%)	Outbreak occurred in the Luwero District. CDC assisted the Ministry of Health in the epidemiologic and diagnostic aspects of the outbreak. Testing of samples by CDC's Viral Special Pathogens Branch occurred at UVRI in Entebbe.
June – November 2012	Democratic Republic of the Congo	Bundibugyo virus	36*	13* (36.1%)	Outbreak occurred in DRC's Province Orientale. Laboratory support was provided through CDC/UVRI lab in Uganda. The outbreak in DRC had no epidemiologic link to the near contemporaneous Ebola outbreak in the Kibaale district of Uganda.
June – October 2012	Uganda	Sudan virus	11*	4* (36.4%)	Outbreak occurred in the Kibaale District of Uganda. Laboratory tests of blood samples were conducted by the UVRI and the CDC.
May 2011	Uganda	Sudan virus	1	1 (100%)	The Uganda Ministry of Health informed the public a patient with suspected Ebola Hemorrhagic fever died on May 6, 2011 in the Luwero district, Uganda. The quick diagnosis from a blood sample of Ebola virus was provided by new CDC Viral Hemorrhagic Fever laboratory installed at the Uganda Viral Research Institute.
December 2008 – February 2009	Democratic Republic of the Congo	Zaire virus	32	15 (47%)	Outbreak occurred in the Mwaka and Luebo health zones of the Province of Kasai Occidental.
November 2008	Philippines	Reston virus	6 (asymptomatic)	0	First known occurrence of Ebola-Reston in pigs. Strain closely similar to earlier strains. Six workers from the pig farm and slaughterhouse developed antibodies but did not become sick.

December 2007 – January 2008	Uganda	Bundibugyo virus	149	37 (25%)	Outbreak occurred in Bundibugyo District in western Uganda. First reported occurrence of a new strain.
2007	Democratic Republic of the Congo	Zaire virus	264	187 (71%)	Outbreak occurred in Kasai Occidental Province. The outbreak was declared over November 20. Last confirmed case on October 4 and last death on October 10.
2004	Russia	Zaire virus	1	1 (100%)	Laboratory contamination.
2004	Sudan (South Sudan)	Sudan virus	17	7 (41%)	Outbreak occurred in Yambio county of southern Sudan. This outbreak was concurrent with an outbreak of measles in the same area, and several suspected EHF cases were later reclassified as measles cases.
November – December 2003	Republic of the Congo	Zaire virus	35	29 (83%)	Outbreak occurred in Mbomo and Mbandza villages located in Mbomo district, Cuvette Quest Département.
December 2002 – April 2003	Republic of the Congo	Zaire virus	143	128 (89%)	Outbreak occurred in the districts of Mbomo and Kélé in Cuvette Quest Département.
October 2001 – March 2002	Republic of the Congo	Zaire virus	57	43 (75%)	Outbreak occurred over the border of Gabon and the Republic of the Congo. This was the first time that Ebola hemorrhagic fever was reported in the <u>Republic of the Congo</u> .
October 2001 – March 2002	Gabon	Zaire virus	65	53 (82%)	Outbreak occurred over the border of Gabon and the Republic of the Congo.
2000 – 2001	Uganda	Sudan virus	425	224 (53%)	Occurred in Gulu, Masindi, and Mbarara districts of Uganda. The three most important risks associated with Ebola virus infection were attending funerals of Ebola hemorrhagic fever case-patients, having contact with case-patients in one's family, and providing medical care to Ebola case-patients without using adequate personal protective measures.
1996	Russia	Zaire virus	1	1 (100%)	Laboratory contamination.
1996	Philippines	Reston virus	0	0	Ebola-Reston virus was identified in a monkey export facility in the Philippines. No human infections were identified.
1996	USA	Reston virus	0	0	Ebola-Reston virus was introduced into a quarantine facility in Texas by monkeys imported from the Philippines. No human infections were identified.
1996	South Africa	Zaire virus	2	1 (50%)	A medical professional traveled from Gabon to Johannesburg, South Africa, after having treated Ebola-infected patients and having been exposed to the virus. He was hospitalized, and a nurse who took care of him became infected and died.

1996-1997 (July – January)	Gabon	Zaire virus	60	45 (74%)	Occurred in Booué area with transport of patients to Libreville. Index case-patient was a hunter who lived in a forest camp. Disease was spread by close contact with infected persons. A dead chimpanzee found in the forest at the time was determined to be infected.
1996 (January – April)	Gabon	Zaire virus	37	21 (57%)	Occurred in Mayibout area. A chimpanzee found dead in the forest was eaten by people hunting for food. Nineteen people who were involved in the butchery of the animal became ill; other cases occurred in family members.
1995	Democratic Republic of the Congo (formerly Zaire)	Zaire virus	315	250 (81%)	Occurred in Kikwit and surrounding area. Traced to index case-patient who worked in the forest adjoining the city. The epidemic spread through families and hospitals
1995	Côte d'Ivoire (Ivory Coast)	Tai Forest virus	1	0	Scientist became ill after conducting an autopsy on a wild chimpanzee in the Tai Forest. The patient was treated in Switzerland.
1994	Gabon	Zaire virus	52	31 (60%)	Occurred in Mékouka and other gold-mining camps deep in the rain forest. Initially thought to be yellow fever; identified as Ebola hemorrhagic fever in 1995.
1992	Italy	Reston virus	0	0	Ebola-Reston virus was introduced into quarantine facilities in Sienna by monkeys imported from the same export facility in the Philippines that was involved in the episodes in the United States. No humans were infected.
1989-1990	Philippines	Reston virus	3 (asymptomatic)	0	High mortality among cynomolgus macaques in a primate facility responsible for exporting animals in the United States. Three workers in the animal facility developed antibodies but did not get sick.
1990	USA	Reston virus	4 (asymptomatic)	0	Ebola-Reston virus was introduced once again into quarantine facilities in Virginia and Texas by monkeys imported from the Philippines. Four people developed antibodies but did not get sick.
1989	USA	Reston virus	0	0	Ebola-Reston virus was introduced into quarantine facilities in Virginia and Pennsylvania by monkeys imported from the Philippines.
1979	Sudan (South Sudan)	Sudan virus	34	22 (65%)	Occurred in Nzara, Maridi. Recurrent outbreak at the same site as the 1976 Sudan epidemic.
1977	Zaire	Zaire virus	1	1 (100%)	Noted retrospectively in the village of Tandala.
1976	England	Sudan virus	1	0	Laboratory infection by accidental stick of contaminated needle.

1976	Sudan (South Sudan)	Sudan virus	284	151 (53%)	Occurred in Nzara, Maridi, and the surrounding area. Disease was spread mainly through close personal contact within hospitals. Many medical care personnel were infected.
1976	Zaire (Democratic Republic of the Congo – DRC)	Zaire virus	318	280 (88%)	Occurred in Yambuku and surrounding area. Disease was spread by close personal contact and by use of contaminated needles and syringes in hospitals/clinics. This outbreak was the first recognition of the disease.

*Numbers reflect laboratory confirmed cases only.

References available from: <http://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html>

REVIEW QUESTIONS:

COURSE # DL-013

Choose the one best answer

- The species of Ebola virus that causes the present epidemic in West Africa is
 - Reston ebolavirus
 - Sudan ebolavirus
 - Tai Forest ebolavirus
 - Zaire ebolavirus
- The present epidemic is primarily in which three countries?
 - Guinea, Liberia, and Sierra Leone
 - Guinea, Mali, and Liberia
 - Liberia, Sierra Leone, and Nigeria
 - Sierra Leone, Senegal, and Nigeria
- Which of the following is not a symptom of Ebola?
 - fever
 - stomach pain
 - jaundice
 - hemorrhage
- Which of the following is thought to be the most likely reservoir of Ebola virus?
 - monkeys
 - chimpanzees
 - humans
 - bats
- Transmission of Ebola virus disease is by all the following except
 - blood
 - mosquitoes
 - contaminated bedding
 - vomitus
- Which of the following is correct in the use of personal protective equipment (PPE)?
 - Doffing equipment presents more hazard than donning
 - PPE is used primarily in treatment hospitals
 - PPE consists of full body coverage including a face-mask
 - Frontline healthcare facilities need to have PPE sufficient for 4-5 days of patient care
- In the history of Ebola virus disease in Africa, what year had the worst epidemic before the present epidemic?
 - The Congo in 1995
 - Sudan in 1976
 - Uganda in 2000-2001
 - The Congo in 2007
- Laboratory tests for the identification of Ebola virus shortly after symptoms begin are all of the following except
 - IgM ELISA
 - PCR
 - IgG antibodies
 - Antigen-capture enzyme linked immunoassay
- Which of the following is not in the province of Ebola Assessment Hospitals?
 - Initiate Ebola testing of possible infected patient
 - Evaluate and care for patient during entire disease process
 - Train staff in use of PPE
 - Transport of specimen
- The preferred sample for Ebola virus testing is
 - sputum collected in a sterile tube
 - 4 ml EDTA whole blood
 - first voided urine in the morning
 - serum from clotted blood specimen

Record your answers on the Answer Sheet on page 28

DISTANCE LEARNING

(Home Study)

Course Registration Form

&

Answer Sheet

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2. Evaluation Form, and
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Course Title: _____

Course Number: _____-_____

SIGNATURE (REQUIRED FOR PROCESSING)

circle the one best answer

1. a	7. a	13. a	19. a	25. a
b	b	b	b	b
c	c	c	c	c
d	d	d	d	d

2. a	8. a	14. a	20. a	26. a
b	b	b	b	b
c	c	c	c	c
d	d	d	d	d

3. a	9. a	15. a	21. a	27. a
b	b	b	b	b
c	c	c	c	c
d	d	d	d	d

4. a	10. a	16. a	22. a	28. a
b	b	b	b	b
c	c	c	c	c
d	d	d	d	d

5. a	11. a	17. a	23. a	29. a
b	b	b	b	b
c	c	c	c	c
d	d	d	d	d

6. a	12. a	18. a	24. a	30. a
b	b	b	b	b
c	c	c	c	c
d	d	d	d	d

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1. Overall, I was satisfied with the quality of this course.

5 4 3 2 1

2. The objectives of this course were met.

5 4 3 2 1

3. Difficulty was consistent with the no. of CE hours.

5 4 3 2 1

4. I will use what I learned from this course.

5 4 3 2 1

5. It took me _____ hours to complete this course.

6. What did you like or dislike about this program?

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Level of Difficulty: [B] = Basic / [I] = Intermediate / [A] = Advanced

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DL-013 [] Ebola Virus Disease [B]	DL-010 [] MMWR Report – Chlamydia & Neisseria [A]	DL-012 [] Diseases Caused by Fusobacterium necrophorum [I]
DL-011 [] Measles – United States 2014 [B]	DL-004 [] Viral Hepatitis: Causes, Diagnosis, and Treatment [I]	DL-009 [] Listeriosis – A Foodborne Disease [I]
DL-008 [] Cryptosporidiosis [B]	DL-002 [] Diagnosis of Malaria in the U.S. [I]	DL-005 [] Q Fever, Diagnosis and Management [I]
DL-007 [] Giardiasis [B]	DL-001 [] Hantavirus – A Special Pathogen [I]	DL-003 [] Update on Salmonella Foodborne Gastroenteritis [I]
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DL-995 [] Hemolytic Disease of the Newborn [B]	DL-993 [] Coccidioidomycosis (Valley Fever) [I]	DL-990 [] Clostridium difficile 027 [I]
DL-992 [] Organ Specific Autoimmune Diseases [I]	DL-988 [] The Great Imposter – Lyme Disease [I]	DL-984 [] Cost Effective Clinical Microbiology [I]
DL-989 - Neutrophilia – available on CAMLT website only [B]	DL-986 [] Candida & its Role in Opportunistic Mycoses [I]	DL-980 [] E. coli O157:H7 Infection [I]
DL-987 [] An Update on Autoimmune Diseases [B]	DL-982 [] Chlamydiae & Their Role in Human Disease [I]	DL-974 [] Anaerobic Bacteriology for the Clinical Laboratory [I]
DL-985 - Hematology Case Studies/Platelets – on website only [B]	DL-979 [] Papillomaviruses & Cervical Cancer [I]	DL-968 [] Intro to HIV, HIV Infection & AIDS - 2014 [B]
DL-983 [] Prion Diseases [I]	DL-967 [] Thyroid Hormones and Thyroid Diseases [I]	
DL-976 [] Update on West Nile Virus [I]	DL-966 [] Cystic Fibrosis and Microbial Infections [I]	
DL-975 [] Megaloblastic Anemia [I]	DL-957 [] A Bacterial Carcinogen – Helicobacter pylori [I]	
DL-973 [] Hb A1c Testing of Patients with Hemoglobinopathies [I]	DL-956 [] Patty Pancreas [I]	
DL-965 [] Infectious Disease: A Gender Bias [B]	DL-950 [] Smallpox [B]	
DL-963 [] Patient Identification [B]	DL-906 [] Ergonomics – Intermediate Self-Study Package for Safety [I]	
DL-958 [] A Plague on U.S. [B]	DL-905 [] The Bug Everyone Has/ Epstein-Barr Virus Case Study [B]	
DL-954 [] Updated Review of Blood Collection Equipment [B]		
DL-922 [] Hematology Case Study/Hypochromic, Microcytic Anemia [B]		
DL-909 [] A Safety Plan for Laboratories [B]		

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Sign here to enroll for the automatic renewal option: _____ Date: _____

Checks to: CAMLT, LAB-PAC and/or E & R as appropriate – OR -

Credit Card Payment: Visa Master Card

Card# _____ Exp. _____

Three-digit security code (on back of credit card): _____

Date _____ Signature _____

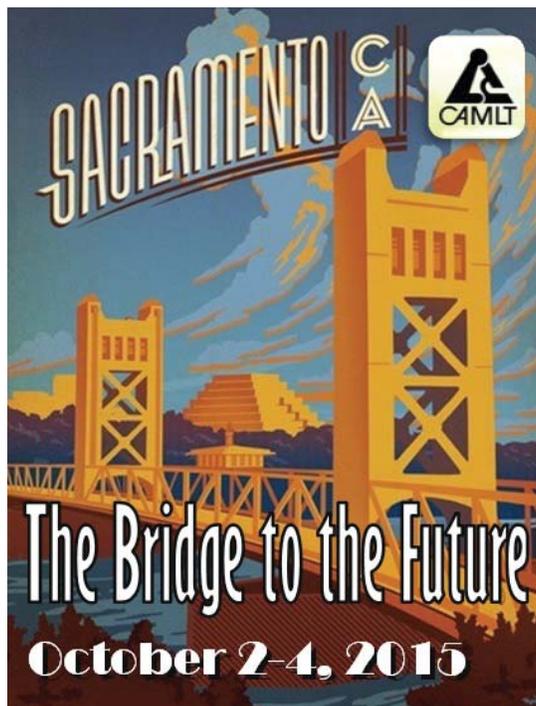
2015 CONTINUING EDUCATION CALENDAR

Program planning in progress

Watch www.camlt.org/calendar for details

October 2-4

76th Annual Meeting, Exhibits & Workshops
Embassy Suites Sacramento – Riverfront Promenade



WANT MORE INFORMATION? CONTACT:

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