1. PURPOSE and SCOPE

1.1 Wayne State University (WSU) research personnel and animal care personnel who handle or care for pregnant sheep are at risk of inadvertently contracting Coxiella burnetii (C. burnetii). Pregnant sheep, cattle, and goats are the primary reservoirs of C. burnetii.

1.2 This SOP is designed to provide research personnel and animal care personnel practical guidelines that can prevent transmission of C. burnetii from potentially infectious laboratory sheep.

2. PRINCIPLE

2.1 C. burnetii is a gram-negative rickettsial bacterium which causes Q fever disease. During birthing pregnant sheep can shed C. burnetii organisms in amniotic fluid and placental tissue. The organism may also be shed in urine, feces, blood, or milk products of infected animals. C. burnetii can survive for long periods in the environment: the bacterium is resistant to heat, drying, and disinfectants. Ticks also serve as vectors for C. burnetii by transmitting the bacterium among animals and humans.

2.2 Q fever symptoms: Acute fever, chills, headache, weakness, fatigue, muscle soreness, or severe sweats. Pneumonitis, pericarditis, hepatitis, or generalized infections can occur. A chronic Q fever infection can develop and may involve an endocarditis. Q fever infections can also be asymptomatic in animals and humans.

2.3 Transmission: Human exposures to C. burnetii usually occur by way of inhalation of aerosols generated by infected animals' placental tissue or placental fluid. Contact with contaminated urine, feces, or animal bedding are also sources of exposure. Direct contact with contaminated objects and surfaces can result in an exposure, tick bites also transmit C. burnetii.

2.4 Laboratory hazard: Inhalation of C. burnetii aerosols or an accidental cut by a contaminated sharp object are the most common sources of laboratory associated exposures.

2.5 Incubation period: Following exposure to C. burnetii signs and symptoms of Q fever usually present within 2 to 3 weeks.

2.6 When to seek treatment: Report to Occupational Health Services-4K clinic (OHS-4K) if you handle pregnant sheep and develop a high fever or flu like symptoms or if you have handled pregnant sheep within 6 weeks of illness. Inform the clinician that you handle pregnant sheep and request a medical evaluation for Q fever disease. If you become ill after regular business hours (7am-5pm) report to Detroit Receiving Hospital Emergency Room (DRH-ER). Complete WSU ‘Report of Injury’ form: submit this form to your supervisor. If you are a research personnel also notify your Principal Investigator (PI). On the next business day follow-up at OHS-4K clinic. Notify WSU Occupational Health Specialist at 313-577-5917 and DLAR Senior Director at 313-993-6769. Note antibiotic drug therapy is most effective when started as soon as possible: within three days of onset of illness. If you are not in Detroit, see a local physician and notify WSU Occupational Health Specialist as soon as possible.
3. **Containment Precautions**

3.1 WSU Division of Laboratory Animal Resources (DLAR) procures pregnant sheep from flocks that are serologically negative for *C. burnetii*. However, it has been established that serologically negative sheep and asymptomatic sheep can still shed *C. burnetii*. **Therefore when handling pregnant sheep it is necessary to handle/treat the sheep as if they are infectious.**

3.2 Personnel in high risk categories include those who are immunocompromised, women who are pregnant or actively attempting to become pregnant, personnel with a history of valvular heart disease, or valvular grafts, and those with a history of hepatitis. High risk personnel will not be assigned work duties involving pregnant sheep. High risk personnel will not work in sheep housing rooms, OR room, nor the necropsy room during procedures involving pregnant sheep.

3.3 DLAR provides animal care personnel and research personnel education on potential hazards associated with handling pregnant sheep and training on safety precautions that will prevent exposure to *C. burnetii*. DLAR documents personnel training sessions.

3.4 Personnel are required to review this SOP and sign documentation indicating they are aware of potential hazards and will comply with health and safety precautions.

3.5 Personnel caring for pregnant sheep and research staff using pregnant sheep must be enrolled in WSU Animal Contact Occupational Health and Safety Program (AniCon).

3.6 Access to sheep housing rooms is restricted to personnel meeting specific entry requirements. Hazardous warning signs with the universal biohazard symbol indicating that pregnant sheep have the potential to shed *C. burnetii* will be posted on doors.

3.7 Telephone numbers of the PI, DLAR Senior Director, a Veterinarian, and Office of Environmental Health & Safety (OEHS) will be listed on emergency contact signage.

3.8 Biosafety Level 2 practices and facilities are adhered to for laboratory procedures involving tissue/fluids from pregnant sheep, e.g., serological examinations or staining impression smears.

3.9 Modified Biosafety Level 2+ practices are adhered to for surgical procedures, necropsy, and manipulation of pregnant sheep tissues or fluids.

3.10 A ventilation control system will be in place to prevent exhaust air from re-circulating to other areas of the facility. The sheep housing rooms, operating room, and necropsy room will be maintained at negative air pressure with respect to adjoining corridors and rooms. Used exhaust filters must be disposed of as biohazard waste after they are changed.

3.11 A visual monitoring device (vanometer) confirming inward air flow will be used. Personnel will be assigned to check sheep housing rooms’ airflow at least twice a day and if necessary notify appropriate staff to readjust the airflow to negative air pressure with respect to adjoining corridors and rooms.

3.12 Don N95 mask, gloves, protective clothing, and shoe covers when receiving or transporting sheep. Restrict transport areas and post signage along transport paths.
4. **PERSONAL PROTECTIVE EQUIPMENT (PPE)**

4.1 PPE must be worn when; handling pregnant sheep, working in the sheep housing rooms, working in surgical prep room, and during surgical procedures. A hand washing sink and bottles of eyewash solution are located in sheep rooms. A hand washing sink and an eyewash station is located near the OR. Personnel are required to be fit-tested for an N-95 Mask.

4.2 Required PPE when entering sheep rooms;

- 4.2.1 Gloves
- 4.2.2 Protective Clothing
- 4.2.3 Shoe Covers
- 4.2.4 N-95 Mask

4.3 Required PPE when performing surgery, necropsy, or cleaning sheep rooms;

- 4.3.1 Gloves (Double glove during surgical procedures)
- 4.3.2 Protective Clothing
- 4.3.3 Shoe Covers
- 4.3.4 N-95 Mask (Don a new N-95 Mask during sheep surgery, then discard)
- 4.3.5 Goggles or Face Shield (Also mandatory if you wear contacts or have an eye irritation)
- 4.3.6 Surgical Scrubs (during surgery)
- 4.3.7 Waterproof Suit and Boots (When cleaning the sheep room)
- 4.3.8 Disposable Head Cover (During surgery and cleaning sheep rooms)

5. **PERSONAL HYGIENE**

5.1 Hand washing is a fundamental aseptic technique that will decrease the spread of infectious disease. Using appropriate PPE combined with good hygiene will decrease the spread of *C. burnetii*. Sheep housing rooms are equipped with a hand washing sink and bottles of eyewash solution. A hand washing sink and eye irrigation equipment are near the OR room. A shower room/facility is located in sheep housing facilities.

5.2 Mechanical pipetting devices must be used: mouth pipetting is prohibited.

5.3 Eating, drinking, smoking, handling contact lenses, or applying cosmetics is not permitted in sheep housing areas.

5.4 Personnel must wash hands each time gloves are removed. This includes before and after providing sheep care, after handling items in sheep rooms, after work in surgical prep rooms, and immediately after providing assistance in the OR. Change gloves and wash hand between servicing sheep rooms. Do not reuse gloves.

5.5 All procedures involving sheep must be performed carefully to minimize the creation of aerosols.

5.6 Place contaminated sharps in puncture-resistant sharp containers. Contact (OEHS) for disposal of sharp containers.
6. **DECONTAMINATION PROCEDURES**

6.1 Place disposable surgical equipment in an autoclave bag. Wet the exterior of autoclave bags with 1:100 bleach dilution (sodium hypochlorite). Autoclave this bag and its content. After autoclaving, the bag can be incinerated or placed in a lined biohazard container/bin for disposal by OEHS. Bleach dilutions must be made fresh daily*.

6.2 Liquid waste, amniotic fluid, the content of suction bottles must be diluted with 1:100 bleach dilution; after 30 minutes this liquid waste can be disposed down a sink drain. Soak suction bottles in a fresh 1:100 bleach dilution for 30 minutes then autoclave.

6.3 Biohazard stickers must be used to label containers containing biohazard materials. Do not place biohazard bags in regular trash container: biohazard bags must be disposed of by OEHS.

6.4 After transporting sheep to the OR, remove shoe covers before entering the OR. Place used shoe covers in either an autoclave bag or biohazard container lined with a biohazard bag.

6.5 Spills and accidents that involve sheep birthing products, sheep waste, or sheep bedding must be reported to DLAR Senior Director or a Veterinarian, PI, and OEHS. A written report of the incident must be maintained. Fresh bleach dilutions must be available for decontamination.

6.6 Spills or splatters involving **sheep blood or body fluids** must be decontaminated with 1:10 bleach dilution.

6.7 After leading/transporting sheep to surgical prep room or the OR immediately disinfect the transport path using 1:100 bleach dilution. If the sheep’s path becomes soiled with **sheep blood or body fluids** immediately disinfect the soiled areas with a 1:10 bleach dilution.

6.8 Decontaminate sheep surgical prep room and OR with 1:100 bleach dilution: floors, walls, tables, and equipment in rooms must be decontaminated.

6.9 Place items with visible blood in biohazard container lined with autoclave bag, autoclave these items, then contact OEHS for disposal.

6.10 Non-disposable sharps and surgical equipment including clipper blades must soaked in fresh 1:100 bleach dilution for 30 minutes before autoclaving. Wipe the body of the clippers with 1:100 bleach dilution then gas sterilize if applicable.

6.11 Decontaminate non-disposable surgical instruments in a 1:100 bleach dilution. Ensure that instrument transport carts are clean: wipe carts with 1:100 bleach dilution. Don PPE when transporting used surgical instruments and during decontamination. Non-disposable goggles can be decontaminated by wiping with 1:100 bleach dilution.

6.12 Decontaminate surgical equipment; IV poles, OR tables, instrument stands, anesthetic machines, and OR lights with 1:100 bleach dilution immediately after sheep surgery. Also decontaminate exterior surfaces and prep tables with 1:100 bleach dilution.

6.13 The exterior surface of bags and transport containers containing sheep products for further study must be decontaminated with 1:100 bleach dilution before leaving the sheep housing area. Wipe the outer plastic covers of records and papers with 1:100 bleach dilution.

6.14 Mop floors and clean walls with fresh 1:100 bleach dilution. Floors, walls, or items contaminated with **sheep blood or body fluids** must be decontaminated with 1:10 bleach dilution.
DECONTAMINATION PROCEDURES – continued from page 4

6.15 Non-disposable surgical scrubs and lab coats worn during sheep surgery must be soaked in 1:100 dilution of bleach for 30 minutes prior to washing in a machine with bleach and detergent.

6.16 When cleaning sheep rooms don a waterproof suit, N95 mask, head cover goggles, gloves, and boots. Use a low-pressure garden hose with a ‘HydroFoamer’ calibrated to dispense 1:100 bleach dilution. Bleach dilution dispensing apparatus will be color coded. Allow bleach dilution to contact surfaces for 30 minutes, and then rinse down surfaces: take precautions to minimize aerosols. Remove PPE at exit door and place in appropriate containers.

6.17 Safety Guidelines For Mixing Bleach Dilution;

6.17.1 Don: gloves, mask, goggles, and protective clothing when mixing bleach with water.
6.17.2 Avoid mixing bleach with other cleaners and disinfectants.
6.17.3 Dilute bleach with water in well ventilated areas.
6.17.4 Avoid spray bottle dispensers: spray bottles produce aerosols.
6.17.5 Use “Pour” or “pump” bottle dispensers: pump bottle do not produce aerosols.
6.17.6 Mix fresh bleach dilutions daily for effective decontamination.
6.17.7 Label bleach dilution containers: indicate bleach dilution ratio and date of preparation.
6.17.8 1:100 bleach dilution ratio** = 1 part bleach to 99 parts water (H2O).
6.17.9 1 gal. H2O + 0.25 cup bleach = 1:100 bleach dilution.
6.17.10 1:10 bleach dilution ratio*** = 1 part bleach to 9 parts water (H2O).
6.17.11 1 gal. H2O + 1.5 cups bleach = 1:10 bleach dilution.
6.17.12 Bleach dilution can be dispensed in 8 oz. or 16 oz. ‘pour’ or ‘pump’ bottles.

DLAR Approval: ___________________________ Date: ___________________________

* Fresh bleach dilution indicates the bleach dilution was prepared within 24hours.
** 1:100 bleach dilution ratio corresponds to .25 cup of household bleach added to 1gallon water.
*** 1:10 bleach dilution ratio corresponds to 1.5 cups of household bleach added to1gallon water.

References:
http://www.cdc.gov/qfever/symptoms/index.html
http://www.cdc.gov/qfever/stats/index.html
http://www.cdc.gov/qfever/prevention/index.html
I have read the Pregnant Sheep Standard Operating Procedure (SOP). I understand there is a risk that serologically negative sheep can shed *Coxiella burnetii* (*C. burnetii*). While handling pregnant sheep I will adhere to safety precautions outlined in DLAR Pregnant Sheep SOP. I will handle/treat pregnant sheep as if they are infectious.

I understand pregnant women and women actively attempting to become pregnant should not work with pregnant sheep because contracting *C. burnetii* can result in Q fever disease. Q fever disease is associated with premature deliveries, stillbirths, and fetal abortions. I understand Q fever can cause cardiac inflammation which can be fatal for individuals with a history of valvular heart disease. I understand personnel with a history of valvular heart disease, and valvular grafts should not work with pregnant sheep. I understand Q fever disease can develop into a chronic long term illness: immunocompromised individuals should not work with pregnant sheep. I understand Q fever can result in inflammation of the liver: personnel with a history of hepatitis should not work with pregnant sheep.

I acknowledge my responsibility to notify the Occupational Health Specialist for Research of changes in my health status or reproductive plans that would preclude me from working with pregnant sheep. I acknowledge I can decline working with pregnant sheep without disclosing my specific medical condition if I provide the Occupational Health Specialist for Research medical certification from my physician indicating my health could be compromised by working with potentially infectious pregnant sheep.

Currently in the United States there is no commercially available vaccine for *C. burnetii*. In the United States the current treatment for Q fever disease consist of antibiotic drug therapy. I am responsible to report suspected incident of exposure to *C. burnetii* to the appropriate authorities. I am responsible to seek treatment as soon as possible if I think I was exposed to *C. burnetii* or if I think I am ill due to exposure to *C. burnetii*. I am responsible to follow medical treatment plans if a doctor determines I have a probable case of Q fever disease based on my presenting signs and symptoms or results of serologic test.

**WSU Animal Research Personnel**

Signature: ____________________________

Printed name: ____________________________ Job title: ____________________________

Date: ____________________________

**WSU DLAR Animal Care Personnel**

Signature: ____________________________

Printed name: ____________________________ Job title: ____________________________

Date: ____________________________

Copy signed “Pregnant Sheep SOP Acknowledgement to: AniCon Files and DLAR Files.”