*ECVM Examination Information – Spring 2022*

## *SETUP OF THE EXAMINATION*[1]

All parts of the examination will be held in person and will be conducted by 3 members of the Examination Committee. The candidates will be supervised at all times by one or more invigilators.

## CONTENT OF THE EXAMINATION

### *CONTENT OF THE EXAMINATION*

The examination consists of the following two parts, each carrying equal weight:

### *I. General Microbiology (worth 100%)*

This part of the Examination shall deal with (i) nomenclature, classification, morphology, geographical distribution, biological characteristics (including cell and molecular biology), detection and identification of bacteria, fungi and viruses of animals and animal products, and (ii) pathogenesis, immunology, pathology, epizootiology, clinical presentation, diagnosis and control of bacterial, fungal and viral diseases of animals.

### *II. Applied Microbiology (worth 100%)*

This part of the Examination consists of two sections, Sections A & B.

**Section A:** shall be used to evaluate the candidate's clinical expertise in veterinary microbiology, and their communication skills. It shall be based on the preparation of four case reports dealing with different species of domesticated animals. The case reports must be organised as follows: clinical history, clinical presentation, differential diagnosis, diagnosis, control and outcome. Preparation of this material will rely on instructions available in this document and on the ECVM website. Other contributions such as a research project report, field trials (drugs/vaccines), evaluation of an infection control scheme, etc., may be acceptable in exceptional cases but prior agreement must be obtained from the Examination Committee.

**Section B**: the Examination shall be used to evaluate the candidate's ability to understand and use in an appropriate way different diagnostic techniques. Section B consists of Diagnostic scenarios and Microbial identification.

The pass mark for each exam is 50%. Distinction is awarded for a mark in excess of 80%.



[1] The instructions provided here refer to the Qualifying Examination. The respective details of the mock exam are provided in this text as footnotes.

**European College of Veterinary Microbiology (ECVM)**

**Qualifying Examination, 2022**

**Part I – General Microbiology**

Examination Committee: Prof. John Ikonomopoulos (Chair), Prof. Bryan Markey, Dr Ana Cláudia Coelho, Dr Els Broens.

**Time Allowed: 3 Hours**

**INSTRUCTIONS FOR CANDIDATES**

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- There are **three Sections**: A, B and C
- Candidates must answer **all 40** single best answer (SBA) / extended matching set questions (EMSQ) in **Section A.**
- Candidates must answer **two** essay questions out of four from **Section B** and **four** short answer questions out of six from **Section C.**
- All sections carry equal marks (candidates should aim to spend approx. 1 hour per section – each section is worth 60 marks – 180 marks in total for the exam)

## Section A

(each correct answer is worth 1.5 marks)

For SBA questions highlight or ring the most correct answer.
For EMSQs, insert the letter(s) of your chosen answer beside the question in the space indicated.

1. Which features differ between containment level 2 and level 3 laboratories?

   a. **Maintenance of a negative pressure cascade between the laboratory and the external environment**
   b. The requirement for handwashing facilities
   c. The use of class 2 microbiological containment cabinets
   d. Users wearing positive pressure respirators
   e. Floors and benches to be sealed and easily cleanable

2. The primers used in the PCR assay are composed of:

   a. **Single stranded DNA oligonucleotides**
   b. Double stranded DNA oligonucleotides
   c. Single stranded RNA oligonucleotides
   d. Double stranded RNA oligonucleotides
   e. Double stranded DNA dideoxynucleotides

3. The European Medicines Agency has classified antimicrobial agents into 4 categories A to D. What agents are included in Category B ("Restrict") ?

   a. Fluoroquinolones, macrolides and tetracyclines
   b. **Fluoroquinolones, 3rd and 4th generation cephalosporins and polymixins**
   c. 3rd and 4th generation cephalosporins, aminopenicillins and polymixins
   d. 3rd and 4th generation cephalosporins, aminopenicillins and macrolides
   e. 3rd and 4th generation cephalosporins, sulphonamides, tetracyclines and polymixins

4. Bacteria most commonly acquire antimicrobial resistance genes by conjugation, which means;

   a. Free DNA in the near environment of the bacteria is encapsulated by the bacterium
   b. DNA is transferred from a bacteriophage infecting the bacterium

c. **DNA is transferred through a pilus from another bacterium in close contac**t

d. Substitution in the nucleotide sequence

e. None of the above

5. Strangles is a bacterial disease caused by the following microorganism:

a. *Streptococcus equi* subspecies *zooepidemicus*

**b. *Streptococcus equi* subspecies *equi***

c. *Streptococcus dysgalactiae* subspecies *equisimilis*

*d. Streptococcus equinus*

*e. Staphylococcus pseudintermedius*

6. *Mycoplasma* spp. are intrinsically resistant to which antimicrobials?

a. Fluoroquinolones
b. Macrolides
c. Tetracyclines
d. Aminoglycosides
e. **β-Lactams**

7. *Pasteurella multocida* is the primary aetiological agent of:

a. Necrotic rhinitis in pigs
b. Suppurative bronchopneumonia in horses
c. Fowl coryza in chickens
**d. Haemorrhagic septicaemia in cattle**
e. Glasser's disease in pigs

8. The transmission of *Salmonella enterica* ssp*. enterica* Sv. Gallinarum is a typical example of:

a. Airborne transmission
b. Transspermal transmission
c. Oral transmission
**d. Transovarial transmission**
e. Lactogenic transmission

9. What pathogen invades and colonises actively growing hair-shafts in cats?

*a. Dermatophilus congolensis*
***b. Microsporum canis***
*c. Pasteurella multocida*
d. *Staphylococcus aureus*
e. *Staphylococcus pseudointermedius*

10. Which of the following viruses are spread by *Culicoides* species midges?

**a. Bluetongue virus and Schmallenberg Virus**
b. Hantaviruses and Bluetongue virus
c. Rift valley fever and Bluetongue virus
d. Hantaviruses and Arenaviruses
e. Arenaviruses and Schmallenberg virus

11. Highly pathogenic avian influenza A viruses replicate:
a. Exclusively in the respiratory and digestive tracts
b. Exclusively in the respiratory tract
**c. Throughout the body (systemically)**
d. Exclusively in the digestive tracts
e. Exclusively in epithelia

**Extended matching set question. Subject: Bacteriology.**

*For each of the questions 12 to 17, select the **most appropriate answer** from the table below.*

*Each option may be used once, more than once, or not at all.*

| | |
|---|---|
| A | Rappaport Vassiliadis medium |
| B | Arabinogalactan |
| C | Coagulase test |
| D | Friis medium |
| E | Satellite haemolysis |
| F | Frey medium |
| G | IS*900* |
| H | ESI |
| I | IS*711* |
| J | Ring phenomenon |
| K | Positive CAMP reaction |
| L | Catalase test |
| M | MALDI-TOF |
| N | Ellinghausen-McCullough-Johnson-Harris medium |
| O | X factor dependent haemolysis |
| P | Outer lipids |
| Q | API test |
| R | IS6*110* |

| | |
|---|---|
| S | Peptidoglycan |
| T | Mycolic acids |
| U | IS*901* |
| V | Fluoroscopy |
| W | CAMP test |
| X | Oxidase test |
| Y | FT-IR |

12. A simple test for the differentiation of *Staphylococcus* from *Streptococcus* is **[ L ]**
13. A widely used target sequence for the specific detection and identification of *Mycobacterium avium* ssp. *paratuberculosis* is **[ G ]**
14. A commonly used medium for the cultivation of porcine mycoplasmas is **[ D ]**
15. An enhanced beta-hemolysis produced by *Brachyspira hyodysenteriae* around slits or holes in the agar is referred to as **[ J ]**
16. The acid-fastness of mycobacteria identified by Ziehl-Neelsen stain is attributed to what in their cell wall ? **[ T ]**
17. A spectroscopic method for the differentiation and identification of bacterial isolates is **[ Y ]**

18. Why are antibodies not detected in the sera of sheep infected with jaagsiekte sheep retrovirus (JSRV)?

a. Antibodies are sequestered in the lungs
b. Antibodies are tightly bound to JSRV antigen
c. Retrovirus infection suppresses the humoral response
d. Retrovirus infection suppresses cell mediated immunity
**e. Similar retrovirus sequences present in ovine genome**

19. Which of the following statements is correct for *MERS-CoV*?

**a. Camels are an important reservoir**
b. Cattle are an important reservoir
c. Goats are an important reservoir
d. Bats are an important reservoir
e. Sheep are an important reservoir

20. A viral condition also known as avian infectious laryngotracheitis is caused by:

**a. Gallid Herpesvirus 1**

**b.** Avian encephalomyelitis virus

c. Avian hepatitis E virus

d. Avian reticuloendotheliosis virus

e. Avian nephritis virus

21. The *Ixodes* species tick is a vector for which of the following microorganisms?

a. *Leishmania infantum*

**b. *Anaplasma phagocytophilum***

c. African swine fever virus

d. West Nile virus

e. *Coxiella burnetii*

22. Which of these viruses may be associated with cerebellar hypoplasia in kittens ?

**a. Feline parvovirus**

b. Feline herpesvirus

c. Feline calicivirus

d. Feline leukaemia virus

e. Feline immunodeficiency virus

23. The Coggins test is the prescribed tests for international trade of horses for ?

a. Glanders

b. Salmonellosis

c. African horse sickness

d. Equine herpesvirus type 1

e. **Equine infectious anemia**

24. In 2016, Smith and colleagues published a study involving 863 dog owners. Each dog owner completed a survey that included questions on vaccination, deworming and frequency of consulting a veterinarian. This study design is best described as:

a. Cohort

b. Case-control

**c. Cross-sectional**

d. Clinical trial

e. Prospective study

25. What additional clinical signs/lesions would be most likely to occur in a dairy herd where an outbreak of salmonellosis has caused detachment of hooves and ear tips in a number of calves?

**a. Abortion**

b. Endocarditis

c. Mastitis

d. Metritis

e. Pneumonia

26. Which of the following bacteria is the cause of contagious equine metritis in mares?

*a. Escherichia coli*

*b. Rhodococcus equi*

*c. Trueperella pyogenes*

***d. Taylorella equigenitalis***

*e. Enterobacter cloacae*

27. What bacterium is a major cause of non-suppurative arthritis in pigs?

*a. Haemophilus somni*

*b. Anaplasma phagocytophilum*

*c. Staphylococcus hyicus*

***d. Erysipelothrix rhusiopathiae***

*e. Pasteurella multocida*

**Extended matching set question. Subject: Antimicrobial susceptibility testing (AST), antimicrobial therapy and resistance (AMR).**

*For each of the questions 28 to 32, select the **most appropriate answer** from the table below. Each option may be used once, more than once, or not at all.*

| | |
|---|---|
| A | Cephalotin |
| B | Cefazolin |

| | |
|---|---|
| C | Ceftiofur |
| D | Cefoxitin 30µg |
| E | Tetracycline |
| F | Imipenem |
| G | Mueller–Hinton agar |
| H | Extended-spectrum beta-lactamases |
| I | Minimum inhibitory concentration |
| J | Broth dilution method |
| K | E-test |
| L | Aminoglycosides |
| M | Transposons |
| N | Beta-lactam antibiotics |
| O | Efflux pumps |
| P | Chloramphenicol |
| Q | Fluoroquinolones |
| R | Lincomycin |

28. A screening agent for detection of Methicillin Resistant Staphylococci (MRS) [**D**, **Cefoxitin 30µ**g]

29. Enzymes with hydrolytic activity against 3rd generation cephalosporins [**H**, **Extended-spectrum beta-lactamases**]

30. A mechanism of antimicrobial resistance to aminoglycosides and fluoroquinolones [**O**, **efflux pump**]

31. Prolonged therapy with this agent could be ototoxic, nephrotoxic [**L**, **Aminoglycoside**s]

32. Persistent infection of what type of horse facilitates the dissemination of equine arteritis virus?

a. Foals
b. Geldings
c. Non-pregnant mares
d. Pregnant mares

**e. Stallions**

33. Which bacterium is the most common cause of canine pyoderma?

*a. Staphylococcus aureus*

*b. Malassezia pacydermatitis*

*c. Streptococcus canis*

***d. Staphylococcus pseudintermedius***

*e. Corynebacterium amycolatum*

34. Which veterinary application of antimicrobial agents is currently forbidden in the European Union?

a. Prophylaxis

b. Metaphylaxis

c. Therapy

**d. Growth promotion**

e. None of the above

35. Which of the following occupational groups has a high risk of acquiring resistant bacteria of animal origin?

a. Pig farmers

b. Abattoir workers

c. Animal caretakers

d. Veterinarians

**e All of the above**

36. Which of the diagnostic methods listed below is most appropriate to use for herd diagnosis of Johne's disease?

a. PCR detection of the specific IS900 element in composite faecal samples from a selection of calves

**b. ELISA to detect antibodies in blood drawn from a selection of adult cows**

c. Culture of composite feacal samples from a selection of calves

d. Smears of faecal material stained with Ziehl-Neelsen staining from 20 % of cows

e. Clinical examination of all adult cows

37. Which microbial species produces the most potent biological toxin?

a. *Aspergillus flavus*
b. ***Clostridium botulinum***
c. *Clostridium chauvoei*
d. *Shiga-toxin producing Escherichia coli*
e. *Vibrio cholerae*

38. In the event of an outbreak of foot-and-mouth disease which presents a reasonable risk of spreading widely, the use of emergency vaccination may be sanctioned in order to block viral replication, avoid new infectious sources and reduce clinical disease. Once the outbreak is under control, all vaccinated animals are killed. Why?

a. All EU countries are free of FMD
b. Importation from countries where there are FMD outbreaks into the EU is banned
c. Importation from countries vaccinating against FMD into the EU is banned
d. Vaccination protects against clinical FMD, but does not prevent infection too.
**e. In the EU only cloven-hoofed animals serologically negative to FMDV are allowed**

39. Which of the following bacteria is a commonly implicated organism in bovine cystitis?

a. *Pseudomonas aeruginosa*
b. *Salmonella* Dublin
c. *Proteus mirabilis*
***d. Corynebacterium renale***
*e. Klebsiella pneumoniae*

40. Fluoroquinolones are a group of broad-spectrum antibacterial agents, effective against multi-drug resistant Gram-negative bacteria in the absence of acquired resistance. Identify their mode of antibacterial action:

a. Interference with folic acid mechanism
b. Inhibition of protein synthesis (30s subunit)
**c. Inhibition of DNA gyrase enzyme**
d. Disruption of penicillin binding proteins
e. Inhibit cell wall synthesis

## Section B

Answer two of the four essay questions (EQs) that follow. (Each essay answer is worth 30 marks.)

**1**. Discuss the criteria used historically to confirm the aetiology of infectious diseases and the reasons why they have come to be considered obsolete? Explain how these criteria for establishing causation have been re-formulated and improved.

**2**. *Coxiella burnetti* is the causative agent Q fever, which is a zoonotic disease. Review disease transmission, clinical signs, pathology, diagnosis, public health importance, and control measures.

**3**. A manufacturer is offering a new molecular diagnostic platform for the detection of *Streptococcus equi* subspecies *equi* using loop-mediated isothermal amplification (LAMP) technology. The manufacturer claims that the test is fast, simple, reliable, and easy-to-use and shows you data that the performance is comparable to SYBR Green real time PCR assays for this pathogen.

a) Describe the main differences between LAMP and real time PCR. Your answer should refer but not necessarily be limited to detection methodology, turn-around time, resources, minimum detection limit, cost of equipment, and number of primers.

Currently, your laboratory uses real time PCR for the detection of *S. equi* subs. *equi*.
Before you introduce this new LAMP assay in your laboratory, you want to validate it by comparing the LAMP results of 20 clinical samples (nasal washes from horses suspected of strangles) with the results of your current real time PCR. The validation is done prospectively, and the samples are split in two equal aliquots before further processing.

The table below shows the results:

| sample ID | LAMP | realtime PCR |
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| 3 | negative | negative |
| 4 | negative | positive |
| 5 | negative | positive |
| 6 | positive | positive |
| 7 | negative | negative |
| 8 | negative | negative |
| 9 | negative | positive |
| 10 | positive | positive |
| 11 | positive | positive |
| 12 | negative | negative |

| | | |
|---|---|---|
| 13 | negative | negative |
| 14 | negative | positive |
| 15 | positive | positive |
| 16 | negative | negative |
| 17 | negative | positive |
| 18 | positive | positive |
| 19 | negative | negative |
| 20 | negative | negative |

b) Analyse the results recorded with LAMP, using real time PCR as the reference method (provide a table to assess agreement between the two methods and calculate the concordance rate). Elaborate on the potential consequences of replacing real time PCR with this LAMP assay in your laboratory.

**4**. Explain what a dermatophyte is. Indicate the dermatophyte species of importance in animals and explain with relevant examples how knowledge of the species involved in an infection can assist in preventing further infection. Review the laboratory diagnosis of dermatophytes.

……

**Model answers**

**1.** Discuss the criteria used historically to confirm the aetiology of infectious diseases and the reasons why they have come to be considered obsolete? Explain how these criteria for establishing causation have been re-formulated and improved.

*Indicative key-points points to be covered:*

Part 1

In 1890, Robert Koch recorded the criteria that must be satisfied for a pathogenic agent to be recognised as the cause of a disease.

The Koch's postulates:

1. The microorganism or other pathogen must be present in all cases of the disease and not in healthy individuals.
2. The pathogen can be isolated from the diseased host and grown in pure culture.
3. The pathogen from the pure culture must cause the disease when inoculated into a healthy, susceptible laboratory animal.
4. The pathogen must be reisolated from the new host and shown to be the same as the originally inoculated pathogen.

With regards to the 1st postulate:

The validity of this postulate is contradicted by the condition referred to as “carrier state”, which denotes either the period of incubation of an infectious disease or the biological association between a host and an opportunistic pathogen.

Until recently it was believed that a carrier state is not possible for pathogens found in the blood of immune competent hosts. Like so many others, this conviction was also overturned, since it was discovered that there are pathogens, such the human herpes virus, that remain dormant in blood cells without causing any symptoms. The presence of a pathogen in the blood of clinically healthy individuals has also been confirmed in connection with bacteria. This is the case for *Bartonella* spp., that lives in blood of a wide range of animal hosts that become infected through scratches and bites from infected cats and rats, or the bite of arthropod vectors, such as ticks, fleas, and lice.

With regards to the 2nd postulate:

Uncultivable infectious agents, *Mycobacterium leprae*. Many other pathogens, including *Erysipelothrix*, *Mycoplasma*, and *Ureoplasma* do not grow in conventional media, and of course the same applies to viruses, the cultivation of which requires the presence of live cells.

With regards to the 3rd postulate:

The 3rd of Koch’s postulates implies conviction that all pathogens are obligate. There are however diseases, the causation of which is linked to certain predisposing factors, such as for example vascular damage predisposing to bacterial endocarditis or immune suppression leading to systemic infection induced by members of the normal microbial flora.

In addition to the pathogen’s ability to cause a disease, the 3rd of Koch’s postulates introduces another parameter in disease pathogenesis, namely “susceptibility”. The reference to susceptibility is a great scientific breakthrough recorded in the 19th century since it implies understanding of a pathogen’s ability to induce an infection only to certain animal species. What happens though when it becomes evident that within any given animal species there are individuals with varying degrees of susceptibility to certain pathogens? How could anyone precisely determine the sensitivity of animal species to a certain disease, if it occurs only or more commonly in individuals with certain genetic characteristics?

With regards to the 4th postulate:

In many cases, the pathogen associated with the causation of a condition is not present when the latter is observed, which means that it will not be possible to isolate the pathogen from the lesions after these appear. This is confirmed in several cases for viral or bacterial infections, such as human papillomavirus and streptococci causing cervical cancer and rheumatic fever, respectively.

Part 2

Today, the parameter of genetic predisposition is under investigation in connection with most diseases, infectious or not. In some cases, this investigation has provided evidence that certain genomic regions appear consistently only in affected or non-affected individuals. Depending on whether these regions that deviate from normal i.e., from what is considered the “wild type”, are functional, they can be recognised either as genetic markers/indicators or genetic factors conferring resistance/sensitivity to the disease.

A context more suitable compared to the Koch's postulates, to assess the significance of these indicators in connection with the causation of a disease, is that which was proposed by Bradford Hill in 1965 i.e., the Hill's criteria for causation.

Reading the Hill's criteria, it becomes evident that in the years that passed after the publication of Koch's postulates, the understanding of the complexity and degree of uncertainty in establishing causal association between a presumed cause and an observed affect, increased significantly. As one would expect, just like the Koch's postulates, the Hill's criteria have been greatly debated and questioned. Exactly because of the complexity of multifactorial disease aetiology, there are those who propose that an association analysis should not rely on any predefined criteria but on scientific common-sense deduction. In this regard, the Hill's criteria are deemed more suitable for prediction models rather than defining whether a pathogenic agent is the cause of a disease:

**The Bradford Hill criteria**

1. Strength (effect size): A small association does not mean that there is not a causal effect, though the larger the association, the more likely that it is causal.
2. Consistency (reproducibility): Consistent findings observed by different persons in different places with different samples strengthens the likelihood of an effect.
3. Specificity: Causation is likely if there is a very specific population at a specific site and disease with no other likely explanation. The more specific an association between a factor and an effect is, the bigger the probability of a causal relationship.
4. Temporality: The effect has to occur after the cause (and if there is an expected delay between the cause and expected effect, then the effect must occur after that delay).
5. Biological gradient (dose-response relationship): Greater exposure should generally lead to greater incidence of the effect. However, in some cases, the mere presence of the factor can trigger the effect. In other cases, an inverse proportion is observed: greater exposure leads to lower incidence.
6. Plausibility: A plausible mechanism between cause and effect is helpful.
7. Coherence: Coherence between epidemiological and laboratory findings increases the likelihood of an effect. However, Hill noted that "... lack of such [laboratory] evidence cannot nullify the epidemiological effect on associations".
8. Experiment: "Occasionally it is possible to appeal to experimental evidence".
9. Analogy: The use of analogies or similarities between the observed association and any other associations.
10. Some authors consider, also, Reversibility: If the cause is deleted then the effect should disappear as well.

**2.** *Coxiella burnetti* is the causative agent Q fever, which is a zoonotic disease. Review the disease transmission between humans and animals, clinical signs, pathology, diagnosis, public health importance, and control measures.

*Indicative key points to be covered:*

Disease transmission

Primary reservoirs are cattle, sheep and goats but reservoirs of infection also include a wide variety of animal species, including arthropods (mainly ticks), wildlife (including rodents, birds, marine mammals and wild ruminants), as well as companion animals (dogs and cats). Q fever has been described as a disease with worldwide distribution, except for a few countries such as New Zealand. The main route of transmission to humans is airborne. Aborted foetuses, birth fluids, and the placenta of affected animals carry large amounts of the pathogen and act as potent sources of infection. The pathogen is present in all the bodily secretions and excretions of affected individuals and can spread via aerosols.

Major clinical signs and pathology

Placentitis leading to abortion or stillbirth occurs in cattle, sheep and goats. In humans, clinical disease is manifested with flu-like symptoms caused by atypical pneumonia. Common pathological findings include hepatitis and life-threatening endocarditis. Aborted foetuses may display hepatitis, myocarditis, and interstitial pneumonia. The placenta is often presented with opacity and thickening due to exudative inflammation, necrosis, and intercotyledonary fibrosis.

Diagnosis

Isolation of *C. burnetii* is possible only in embryonated eggs, experimental animals, or mammalian cell cultures. Polymerase chain reaction (PCR) testing can be performed for the detection of the pathogen in DNA isolated from vaginal mucus, embryonic tissues milk, colostrum, blood, urine, and faeces. Inflammatory exudates can be stained using modified Ziehl– Neelsen, Gimenez, Stamp, Giemsa or modified Koster stain. Methods of choice for clinical diagnosis: PCR and ELISA.

Control measures

Sanitary and isolation measures should be applied to limit disease transmission within and between humans and animals: ssegregation of parturient ruminants and careful disposal of placentas and aborted foetuses are essential, following confirmation of disease.

Abortion in animals can be prevented with annual vaccination of non-pregnant ruminants, using inactivated egg-yolk vaccines.

Public Health importance

The primary route of infection for humans is airborne via inhalation of aerosols contaminated with the pathogen released by the affected individuals in their bodily secretions and excretions. Transmission is also possible through the oral route via ingestion of unpasteurised milk. Q fever is recognised as occupational zoonosis affecting veterinarians, veterinary technicians, livestock farmers, dairy workers, slaughterhouse workers and researchers. *C. burnetti* is deemed a category B biological terrorism agent.

**3.** A manufacturer is offering a new molecular diagnostic platform for the detection of *Streptococcus equi* subspecies *equi* using the loop-mediated isothermal amplification (LAMP) technology. The manufacturer claims that the test is fast, simple, reliable, and easy-to-use and shows you data that the performance is comparable to SYBR Green real time PCR assays for this pathogen.

a) Describe the main differences between LAMP and real time PCR. Your answer should refer but not necessarily be limited to detection methodology, turn-around time, resources, minimum detection limit, cost of equipment, and number of primers.

*Indicative key points to be covered:*

| | LAMP | Realtime PCR |
|---|---|---|
| Detection methodology | DNA amplification at constant temperature | DNA amplification conducted at repeating cycles of 3 different temperatures |
| Turn-around time | Less than an hour | 45-90min |
| Resources | Can be done on-site | Laboratory needed |
| Minimum detection limit (analytical sensitivity) | Depending on the targeted analyte, the MDL of real time PCR is usually lower | |
| Cost of equipment | Equipment much cheaper | Expensive equipment needed |
| Number of primers | Up to 6 primers | 2 primers (mostly) |

Currently, your laboratory uses real time PCR for the detection of *S. equi* subs. *equi*.
Before you introduce this new LAMP assay in your laboratory, you want to validate it by comparing the LAMP results of 20 clinical samples (nasal washes from horses suspected of strangles) with results of your current real time PCR. The validation is done prospectively, and the samples are split in two equal aliquots before further processing.

The table below shows the results:

| **sample ID** | **LAMP** | **realtime PCR** |
|---|---|---|
| 1 | positive | positive |
| 2 | negative | negative |
| 3 | negative | negative |
| 4 | negative | positive |
| 5 | negative | positive |
| 6 | positive | positive |
| 7 | negative | negative |
| 8 | negative | negative |
| 9 | negative | positive |
| 10 | positive | positive |
| 11 | positive | positive |
| 12 | negative | negative |
| 13 | negative | negative |
| 14 | negative | positive |
| 15 | positive | positive |
| 16 | negative | negative |
| 17 | negative | positive |
| 18 | positive | positive |
| 19 | negative | negative |

| | | |
|---|---|---|
| 20 | negative | negative |

b) Analyse the results recorded with LAMP, using real time PCR as the reference method (provide tables to assess agreement between the two methods). Elaborate on the potential consequences of replacing real time PCR with this LAMP assay in your laboratory.

3b. *Indicative key points to be covered:*

| | | ***S. equi equi*** | | |
|---|---|---|---|---|
| | | ***PCR*** | | |
| | | pos | Neg | Total |
| ***LAMP*** | Pos | 6 | 0 | 6 |
| | neg | 5 | 9 | 14 |
| | Total | 11 | 9 | 20 |
| | Concordance between +ve results | | 55% | |
| | Concordance between -ve results | | 100% | |
| | Overall concordance | | 75% | |

The reliability of the results will be affected negatively with regards only to the real time PCR-positive results, since 45% of the real time PCR-positive samples are likely to be characterized as negative if tested with LAMP.

**4**. Explain what a dermatophyte is. Indicate the dermatophyte species of importance in animals and explain with relevant examples how knowledge of the species involved in an infection can assist in preventing further infection. Review the laboratory diagnosis of dermatophytes.

Key points to be covered:

**Dermatophytes (Ringworm)**

- *Ascomycota*
  - ringworm species were formerly Fungi Imperfecti
- About 40 species known
  - may be grouped
    - Geophilic (opportunistic pathogens)
    - Zoophilic (obligate pathogens)
    - Anthrophilic (obligate pathogens)

  Two genera (9 species) of veterinary interest
  - *Microsporum* spp. & *Trichophyton* spp.

**Table** Dermatophytes of animals, their main hosts and reported geographical distribution.

| Dermatophyte | Hosts | Geographical distribution |
|---|---|---|
| *Microsporum canis* var. *canis* | Cats, dogs | Worldwide |
| *M. canis* var. *distortum* | Dogs | New Zealand, Australia, North America |
| *M. equinum* (considered to be identical to *M. canis*) | Horses | Africa, Australasia, Europe, North and South America |
| *M. gallinae* | Chickens, turkeys | Worldwide |
| *M. gypseum* | Horses, dogs, rodents | Worldwide |
| *M. nanum* | Pigs | North and South America, Europe, Australasia |
| *Trichophyton equinum* | Horses | Worldwide |
| *T. equinum* var. *autotrophicum* | Horses | Australia and New Zealand |
| *T. mentagrophytes* var. *mentagrophytes* | Rodents, dogs, horses and many other animal species | Worldwide |
| *T. mentagrophytes* var. *erinacei* | European hedgehogs, dogs | Europe, New Zealand |
| *T. verrucosum* | Cattle | Worldwide |

- Identification of the dermatophyte involved is based on:
    - Host species
    - Colonial appearance
        - examine obverse and reverse
    - Microscopy for macroconidia
        - *Microsporum* species: spindle-shaped
        - *Trichophyton* species: cigar-shaped, low numbers produced
    - PCR

**Relevant example** – dermatophyte species affecting horses

- *Trichophyton mentagrophytes var. mentagrophytes* - rodents
- *Trichophyton verrucosum* - cattle
- *Microsporum canis* - stable cats
- *Microsporum gypseum* - rolling in soil

**Diagnosis**

- History - more common in young animals
- Clinical signs
- Wood's lamp (UV light)
    - 50%+ *M. canis* cases fluoresce along hair shafts
- Specimens
    - plucked hairs (not cut)
    - skin scrapings (edge of lesions)
- Direct microscopy
    - 10-20% KOH wet preparation - arthrospores
- Isolation

    - Sabouraud dextrose agar + additives
    - 27°C for 3 weeks (*T. verrucosum* 37°C for 5 weeks)
    - Aerobic
- PCR

## Section C

Answer four short answer questions (SAQs) out of the following list of six[2]. (Each short answer question is worth 15 marks)

**SAQ1**. A 3-year-old, mid-term pregnant ewe was submitted for post-mortem examination in late November. It was one of a flock of approximately 50 Suffolk-cross ewes which were outside on pasture and were receiving supplementary silage feeding. The ewe had shown signs of depression, profuse salivation, ataxia, circling behaviour and at times had stood with her head pressed against objects and appeared as though she were blind. The farmer kept no other farm animals but had three Border Collie dogs.

a) List the differential diagnosis for this case given the history and clinical signs described.

*Indicative key points to be covered:*

Listeriosis, gid (coenurosis), nervous ketosis, polioencephalomalacia, brain abscesses (less likely: middle ear disease, scrapie, Visna Maedi, aflatoxicosis).

b) The farmer is concerned that the silage may be contributing to the disease. Explain why the farmer might think this and what your advice would be to the farmer?

*Indicative key points to be covered:*

The disease is referred to in some countries as "silage disease" because it is typically associated with the consumption of silage of poor quality. Listeria can multiply in acidic environment, and the conditions for this become ideal in silage that is not adequately acidic. The farmer must be advised to monitor routinely the pH of the silage, which should always be lower than 5.5. When the pH of the silage is relatively high, even if this occurs temporarily, listeria multiplies and will remain infectious even if the pH drops to the acceptable levels (< 5.5).

c) Examine the images of the organism cultured from the brain of the sheep and the Gram stain. Identify the organism, giving reasons for your answer.

Photo 1. Blood agar

[2] This part of the mock exam consists of 4 short answer questions, all to be answered.

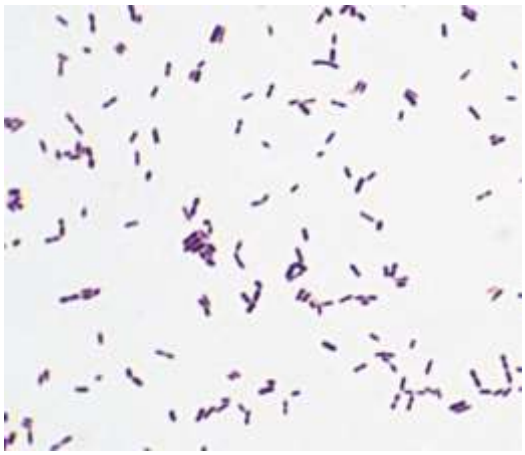


Photo 2. Gram stained smear

Photo 3. Aesculin broth. Left: uninoculated control. Right: inoculated with isolate

*Indicative key points to be covered:*
Small non-haemolytic colonies on BA, Gram positive, aesculin positive. Based on the disease characteristics and the results of the microbiology tests, the bacterium fits the profile of *Listeria monocytogenes*.

d) The organism was isolated only after subculture from a nutrient broth which had been held at 4°C for two weeks. Explain why this procedure is necessary.

*Indicative key points to be covered:*
This is an enrichment procedure – the organism is usually present in small numbers in brain tissue and may not grow directly on BA; Listeria can multiply at 4°C but this temperature inhibits the growth of other contaminating organisms and positive growth is obtained after 1-12 weeks incubation, with weekly subculture.

e) A section of the midbrain was prepared for histopathological examination. What characteristic change can you see in each histological image ?
**Photo 4 - Microabscess**
**Photo 5 - Vascular cuffing**

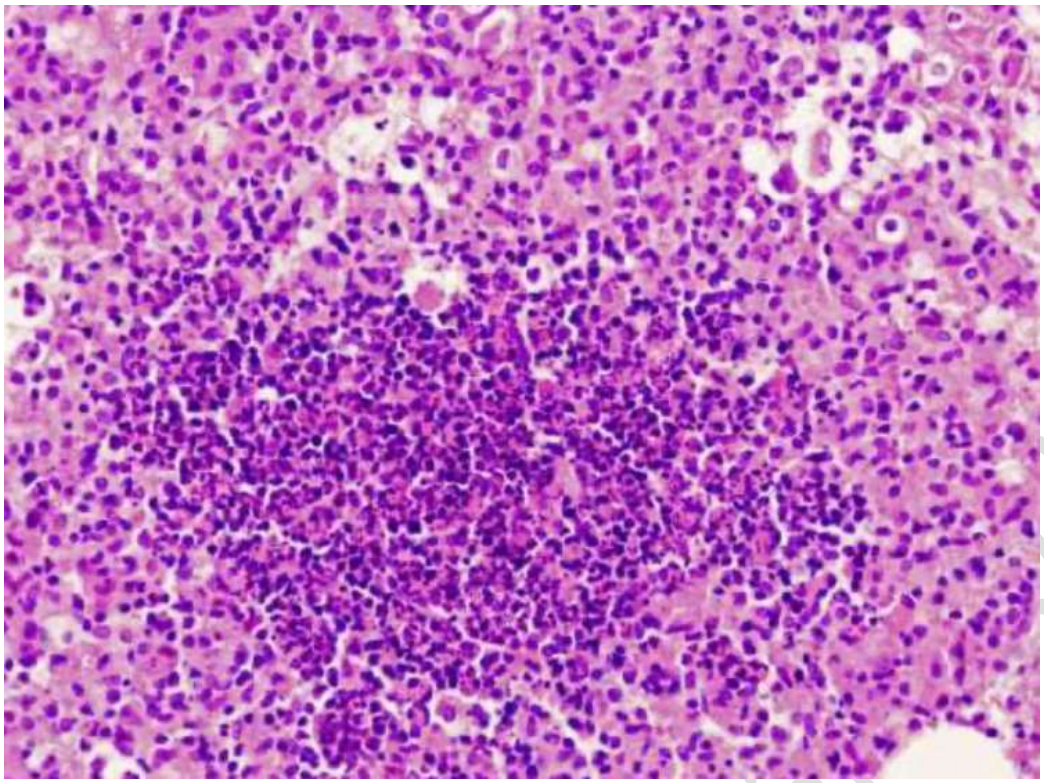


Photo 4. H&E section of midbrain

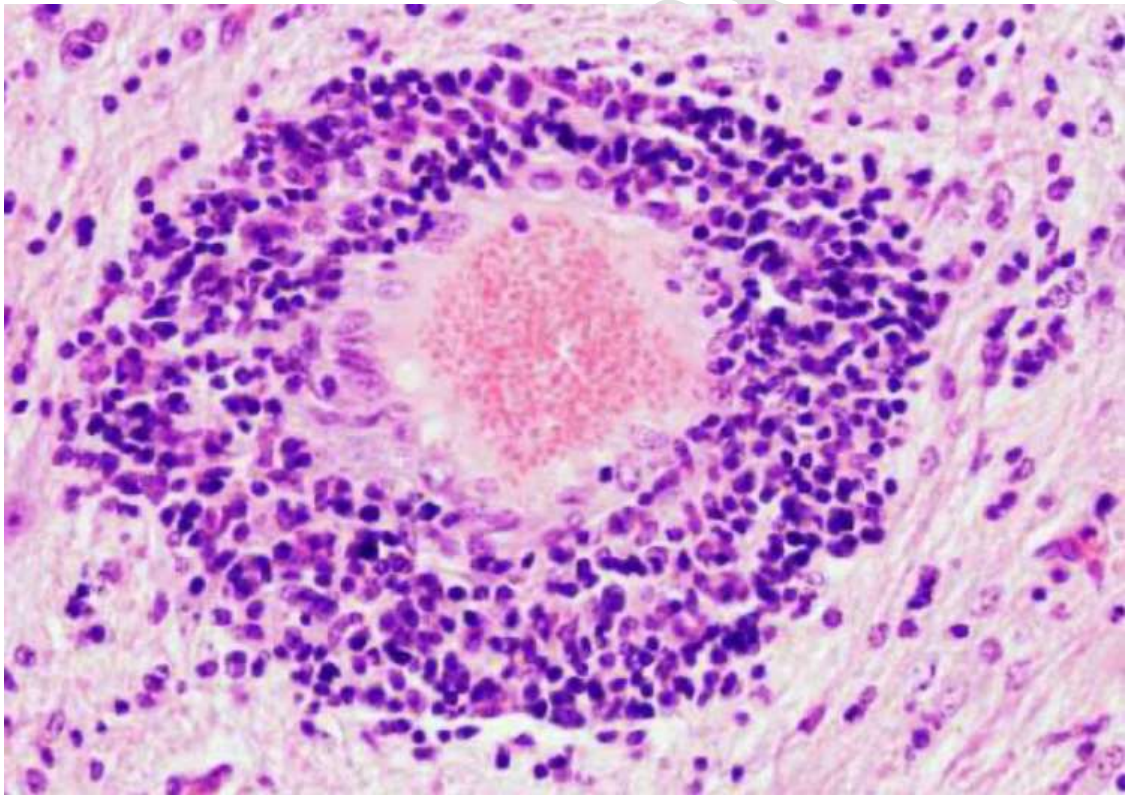


Photo 5. H&E section of midbrain

f) What is your final diagnosis and what advice would you give the farmer for prevention of this disease in the future

**Listeriosis – probably contracted from silage. Ensure silage of good quality, adequately preserved to prevent multiplication of Listeria. Ensure ewes well managed so not immunosuppressed etc**

**SAQ2**. An outbreak of upper respiratory disease (Photo A) has occurred in a large cattery that takes in strays.

a) Name the two most important respiratory viruses that affect cats.

Photo B: **feline herpesvirus 1**

Photo C: **feline calicivirus**

**Photo A**

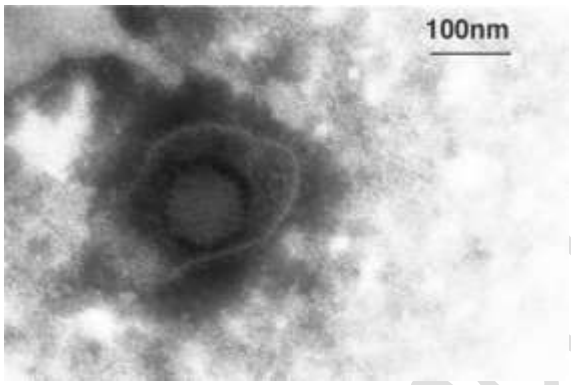



**Photo B (Electron micrograph)**

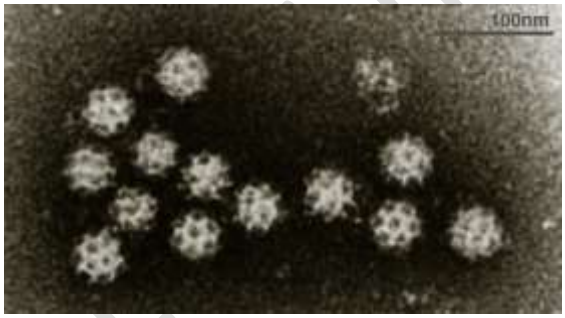



**Photo C (Electron micrograph)**



b) Compare and contrast the epidemiology of these two viruses.

- **Close contact, direct and indirect transmission (calicivirus more resistant than herpesvirus).**
- **Oronasal and conjunctival routes**
- **Outbreaks in crowded catteries, animal shelters**

- **FVR - 80% of recovered cats are latent carriers; 50% of carriers shed intermittently, especially after periods of stress (postpartum reactivation of infection in queens). Kittens infected as maternal immunity wanes (5-8 weeks).**
- **FCV - persistent carriers are important (mutation and recombination allow virus to escape the host immune response), majority of cats in a colony undergo sequential re-infection with viral variants**

c) What specimens should you take and what laboratory tests should you request if you wish to confirm the diagnosis ?

Specimens: **Nasopharyngeal and conjunctival swabs (place in virus transport medium for virus isolation):**

Diagnostic Tests:

- **PCR (for herpesvirus)**
- **RT-PCR (for calicivirus**
- **Virus isolation (both viruses)**

d) A large number of kittens are at risk of picking up this infection in the cattery. What can be done?

- **Intranasal vaccination (rapid onset of protection)**
- **Disinfect and improve hygiene.**
- **Segregate vulnerable kittens.**
- **Isolate affected cats.**
- **Avoid stress and overcrowding.**

**SAQ3**. A yearling Friesian heifer has developed a profuse, intermittent diarrhoea (Photo A) that has been going on for several days in a large dairy herd. The animal has a nasal discharge (Photo B), is depressed and has lost weight but is continuing to eat and drink. The farmer has administered anthelminthic treatment but with no improvement.
Used in the mock


Photo A

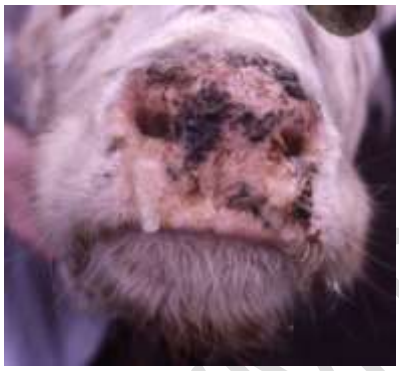


Photo B

a) Compile a list of the top three (3) most likely infectious causes ?
1. Mucosal disease, 2. Salmonellosis, 3. Malignant catarrhal fever

b) Unresponsive to supportive and antibiotic treatment, the animal has been euthanized and sent for post mortem examination. Look at the photographs C, D and E of some of the lesions found. Provide a provisional diagnosis, giving reasons ?

Mucosal disease. The photographs show linear erosions of the gastrointestinal tract and buccal cavity, these are typical of this disease.

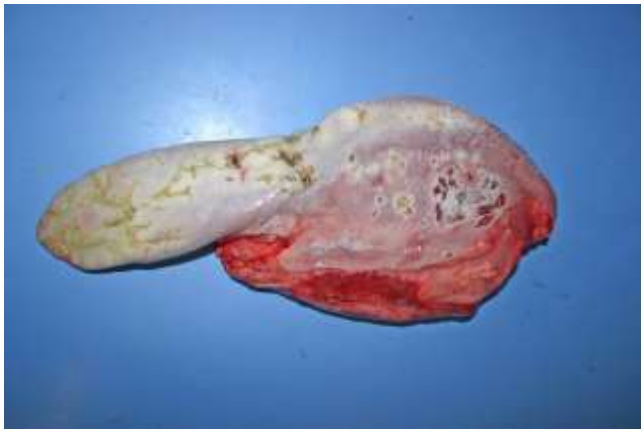


Photo C Tongue

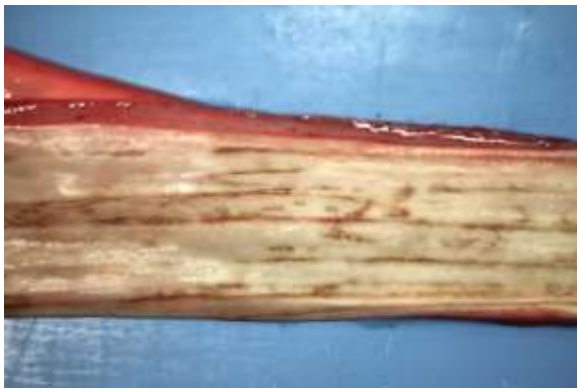


Photo D Oesophagus

Photo E Abomasum



c) Indicate three tissue samples you should take and three tests available to confirm your diagnosis in this animal ? State what each test is designed to detect ?

Samples of gut (with lesions), spleen and lymph node are best.
Available tests: ELISA to detect viral antigen; immunohistochemistry to detect viral antigen in sections of the tissues; Reverse transcription -polymerase chain reaction assay to detect viral RNA; virus isolation to detect viable virus.

d) Outline how you would set about reducing infection in an endemically infected herd, including the animals you would sample and the tests you would employ ?

Identification and culling of PI animals [blood sample or ear notch test to detect viral antigen (ELISA) or viral RNA (RT-PCR]. Can either test whole herd (expensive) or concentrate on newborn calves.
In the case of pregnant animals, testing may not detect an infected foetus and calves born to these dams should be tested when possible after birth.

**SAQ4.** A 4-year-old English setter presented with a 2-month history of bilateral, viscid, green-yellow nasal discharge, which contained flecks of blood. A nasal swab was collected and submitted for microbiological culture.

a) Given this clinical history, provide a list of the likely differential diagnoses?

**Chronic bacterial or fungal infection/ Nasal aspergillosis**
**Foreign body rhinitis(grass awns/seeds/twigs etc)**
**Neoplasia**

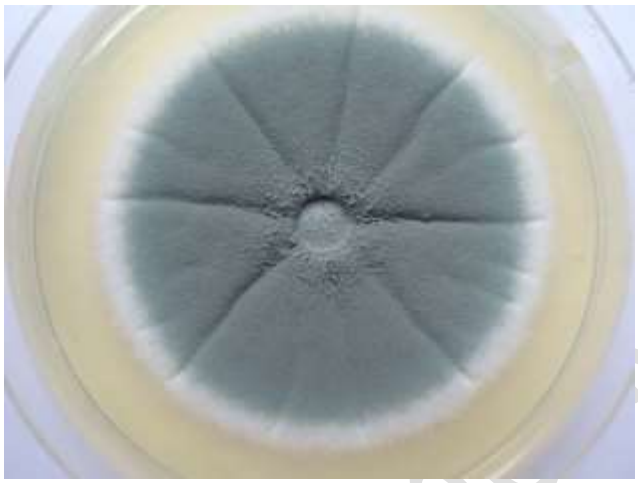


Photo A: Sabouraud's dextrose agar

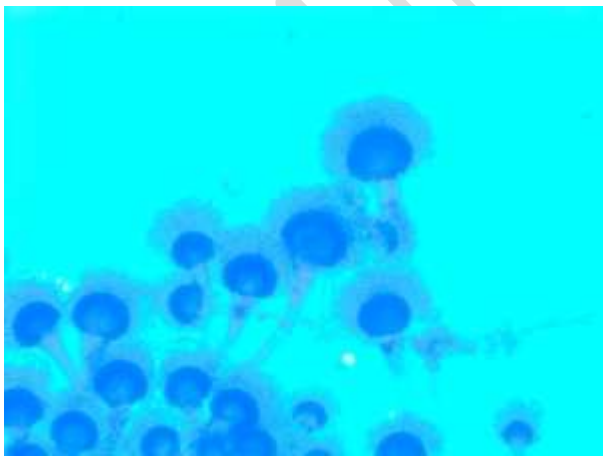


Photo B: Lactophenol cotton blue (LPCB) stained wet preparation (x40 objective)

b) Identify the organism presented (Photos A and B) which was isolated from the swab, giving reasons for your answer.

***Aspergillus fumigatus*** **– cultural appearance and appearance of fruiting heads stained with LPCB.**

c) Explain why detection of the organism from a nasal swab does not provide a definitive diagnosis in this case

***Aspergillus* ubiquitous and commonly present in the nares of normal dogs – need to culture from lesion itself**

d) Describe *two* other diagnostic procedures that could be used to support / confirm your diagnosis.

**Rhinoscopy and visualisation of fungal plaques**
**Biopsy of lesions and identification of fungal hyphae invading the tissues**
**Radiographic examination**

e) How is the condition you suspect in this particular case definitively diagnosed?

**Nasal biopsy and demonstration of fungal hyphae invading the tissues by histopathology**

**European College of Veterinary Microbiology (ECVM**

**Qualifying Exam, 2022**

**Examination 2 – Applied Microbiology**

**Examination Committee: Prof. John Ikonomopoulos (Chair), Prof. Bryan Markey, Dr Ana Cláudia Coelho, Dr Els Broens**



## Section A – Oral Examination[3] (worth 50%)

A dossier of 4 case reports is to be provided by the candidate who is expected to prepare a presentation of all four case reports (using PowerPoint or similar software) before the examination. The particular case-report selected by the Examination Committee for oral presentation will be announced to the candidate during the examination.

The duration of the Oral Examination is approximately 30 minutes (15 minutes for the presentation and 15 minutes for questions).

## Section B – Practical Examination (worth 50%)

**Time Allowed: 3 Hours[4]**

Section B consists of the following two parts (all questions to be answered, each part should take approximately 1.5 hours to complete, therefore each part is worth 90 marks or 180 marks in total for the exam):

- Diagnostic scenarios: Ten diagnostic scenarios (consisting of up to 200 words of text plus appropriate laboratory data)[5] each with four associated MCQs based on the critical analysis of clinical, serological, isolation, molecular or biochemical data (a total of forty MCQs).

[3] This part of the examination is not included in this mock exam.
[4] The time allowed for the part of the Qualifying examination is 3h; the respective time for the mock exam should be 1.5h.
[5] Five questions of this type have been included in this mock exam.

- Microbial identification: Ten cultures[6], microscopic preparations or pictures to be examined. Specimens to be identified will be accompanied by a brief history and relevant background details. It is expected that the pathogen will be identified as far as possible, to genus level and possibly species level. In some cases, it may only be possible to narrow the identification down to a small number of possible microorganisms.



[6] Five questions of this type have been included in this mock exam. The candidate is expected to use the supporting material provided (photographs) to answer the questions.

## Section B - Diagnostic scenarios[7]

**(2 marks per correct answer plus 1 bonus mark for each set of 4 i.e. each case, that is fully correct).**

**1. Case Summary[8]:** A somnolent European brown hare with central nervous signs is presented for post-mortem examination. Gross pathological signs include a massively swollen spleen without further. Examine the photos (A, B, C and D) which illustrate some key findings from this case.

Photo A. Lateral view of the deceased European brown hare



[7] This part of the Qualifying Examination consists of 10 diagnostic scenarios, all to be answered; 5 questions have been included in this mock exam.

[8] The ECVM specialisation is primarily focused to domestic animals. However, this case has been included in the mock examination because it refers to a disease that is currently considered emerging and a potential threat to public health.

Photo B. Massive splenomegaly found during post-mortem examination

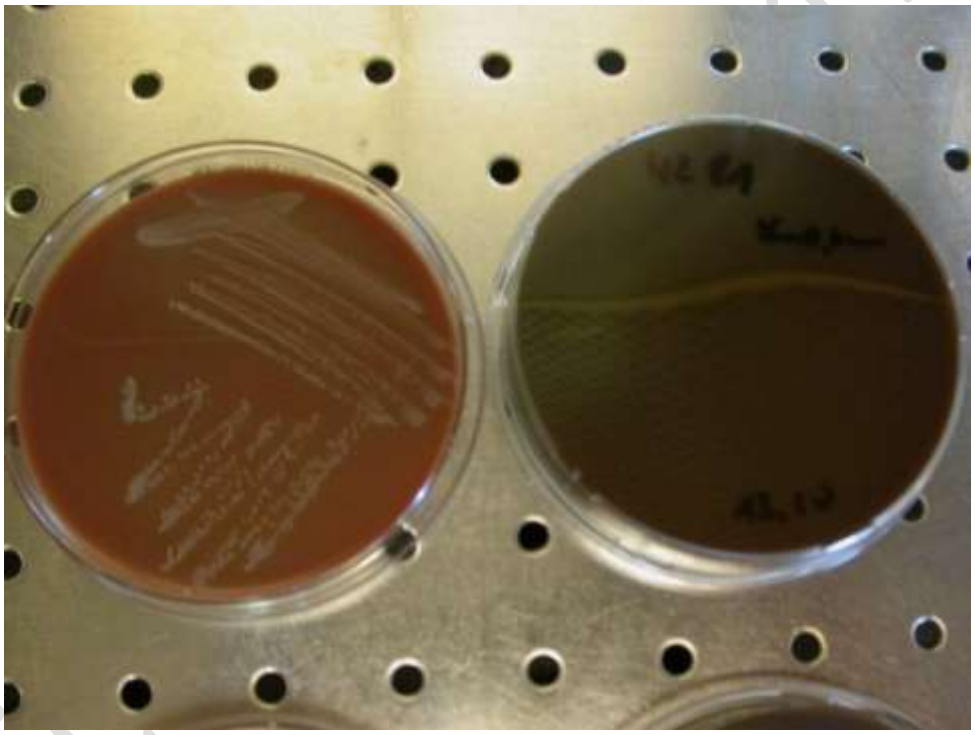


Photo C. High-grade pure growth of greyish, slightly olive coloured convex colonies in aerobic cultures of all major tissues

Photo D. Close-up of respective greyish, slightly olive-coloured convex colonies from photo C grown on Martin-Lewis agar

I. Given the information you have been supplied with, what is your diagnosis:

a. *Brucella suis* infection
b. Myxomatosis
c. European brown hare syndrome
**d. Tularaemia**
e. *Yersinia pseudotuberculosis* infection

II. What is the typical course of infection in the European brown hare:

**a. Acute infection, often accompanied by bacteraemia and sepsis**
b. Chronic infection
c. Co-infection, following rabies
d. Local infection of eyes and skin
e. Infections are observed only in winter season

III. Which of the following statements is **false**:

a. It is a rare zoonosis
b. The infection is transmitted by direct contact as well as by arthropod bites
c. The causative organism is highly contagious and potentially fatal
d. This bacterium has been included in the list of warfare pathogens (dirty dozen)
**e. Humans and livestock are resistant**

IV. What is considered to be the most frequent route of transmission to humans in Scandinavia?

a. Ingestion
b. Aerosol
**c. Mosquito bite**
d. Tick bite
e. Direct contact

2. **Case summary:** A canary owner experienced non-specific signs of illness in some birds of his valuable breeding flock of 20 birds. The signs included lethargy, ruffled feathers and chronic or recurring diarrhoea. Faecal samples were investigated repeatedly in a diagnostic lab, showing heavy growth of coliform bacteria and yeasts. The treatment of individual birds with antibiotics and/or antifungals was not effective. A few birds found dead were submitted for post-mortem examination. They had a good body condition but spleen and liver were enlarged. Photos A+B illustrate this case.

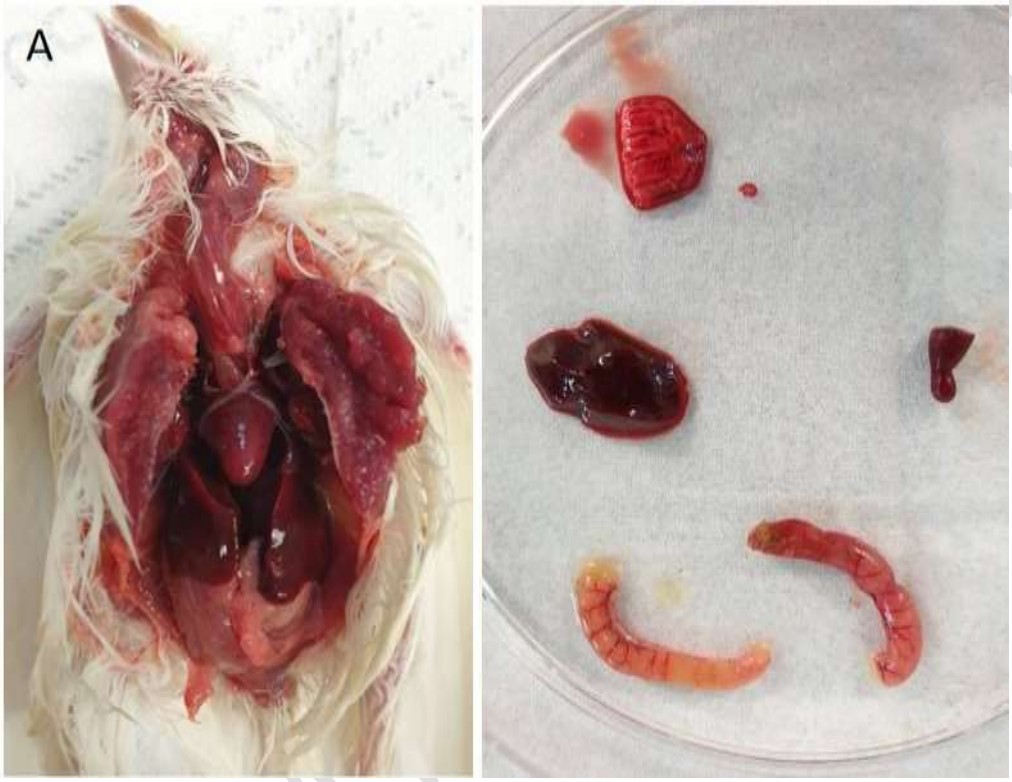



Photo A: Post-mortem examination of a deceased canary

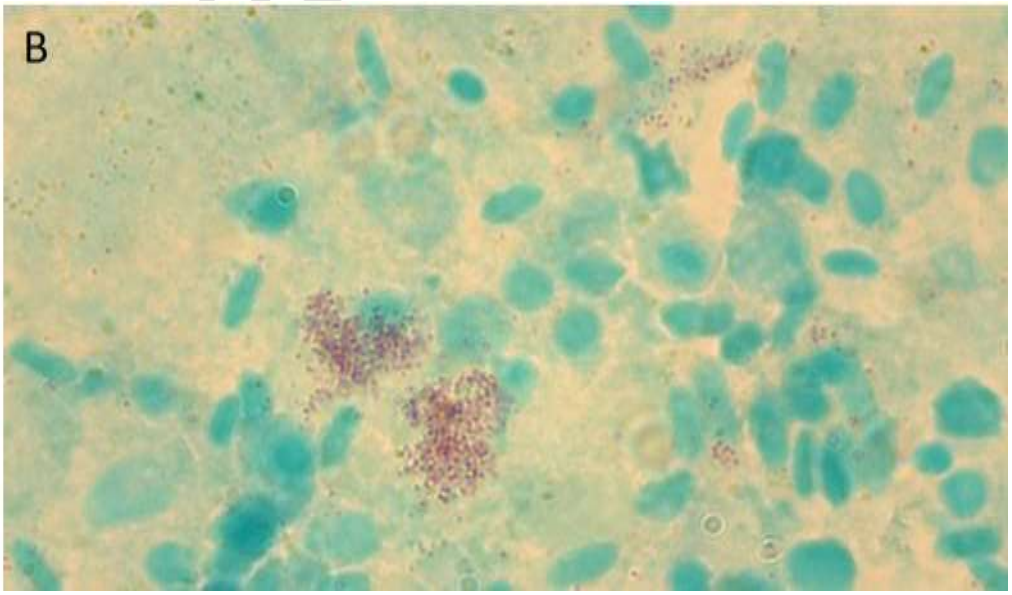


Photo B: Impression smear of spleen (Modified Ziehl-Neelsen stain)

I. What is your diagnosis?

**a. Chlamydiosis**

b. Colibacillosis

c. Mycobacteriosis

d. Aspergillosis

e. Adenovirus infection

II. The aetiological agent

a. grows on conventional culture media

b. is an acid-fast bacterium

c. produces intranuclear inclusions in hepatocytes

d. causes granulomatous lesions

**e. is an obligate intracellular pathogen**

III. What is the most sensitive and specific method to confirm the diagnosis?

a. Culture

b. Immunohistochemistry

c. Electron microscopy

**d. PCR**

e. Serology

IV. What would be the suggested disease management in this small flock of canaries ?

a. No specific measures, self-limiting disease

b. Culling of clinically affected or confirmed birds

**c. Isolation of sick or confirmed birds and antimicrobial treatment of up to 45 days**

d. Whole-flock treatment with tetracycline antibiotics

e. Flock vaccination

3. **Case summary:** A mare has been mated with a stallion in March and April, but in May the mare has come back into heat again. The owner contacts the local veterinarian to get advice on how to get the mare in foal. The veterinarian notices a mild muco-purulent discharge from the vulva. Cytology (1000x microscopy) of a hematoxylin and eosin stained smear from the vulval discharge is shown in figure 1. Culture of the same specimen in an external laboratory results in the report of a “heavy, pure culture of *Escherichia coli*" with the accompanying antibiogram shown in Table 1.

Figure 1. Cytology.

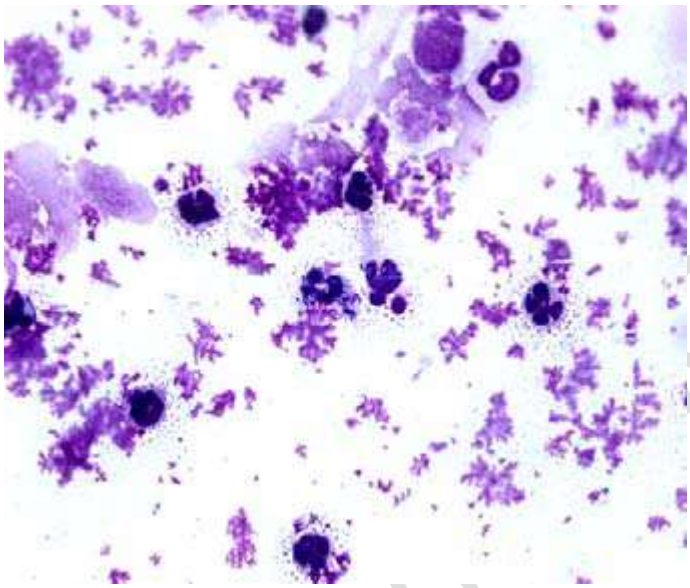


Table 1. Antibiogram.

| **Antibiotic** | **Interpretation** (S = susceptible, R = resistant) |
|---|---|
| Amikacin | S |
| Ampicillin | R |
| Cefazolin | R |
| Cefpodoxime | R |
| Ceftiofur | R |
| Chloramphenicol | S |
| Enrofloxacin | R |
| Gentamicin | R |
| Rifampicin | R |

| | |
|---|---|
| Tetracycline | R |
| Trimethoprim/Sulphamethoxazole | R |

I. For a horse with the above clinical signs, choose from among the following options the most appropriate sampling strategy for collecting a diagnostic specimen for cytology and culture

a. Take a sample of the thick discharge
b. Roll a cotton swab against the inner wall of the vagina
**c. Perform a uterine lavage with a small volume of saline and use that as a sample**
d. Take an endometrial biopsy
e. Treat with antimicrobials and re-sample 10 days later

II. Which of the following bacteria is **not** typically associated with endometritis in mares?

**a. *Streptococcus equi* subspecies *equi***
*b. Pseudomonas aeruginosa*
*c. Taylorella equigenitalis*
*d. Klebsiella pneumoniae*
*e. Streptococcus equi* subspecies *zooepidemicus*

III. On what basis can the laboratory suspect that the *E. coli* isolate is an ESBL-producer ?

a. Because the strain is resistant to most cephalosporins and fluoroquinolones
**b. Because the strain is resistant to cefpodoxime**
c. Because the strain is resistant to cefazolin
d. Because the strain is susceptible to amikacin but resistant to gentamicin
e. Because the strain is resistant to trimethoprim/sulphamethoxazole.

IV. Which of the following statements is correct?

a. Local antimicrobial administration is most suitable for treatment of endometritis and the antibiogram is well suited to guide this mode of administration

**b. Local antimicrobial administration is most suitable for treatment of endometritis but the antibiogram is not intended to guide this mode of administration**

c. Systemic antimicrobial administration is most suitable for treatment of endometritis and the antibiogram is well suited to guide this mode of administration

d. Systemic antimicrobial administration is most suitable for treatment of endometritis but the antibiogram is not intended to guide this mode of administration

e. Both local and systemic antimicrobial administration should be used.

4. **Case summary**: A wild boar destined for human consumption underwent a full veterinary meat inspection. Gross examination revealed left scrotal enlargement and a tissue sample was send to a diagnostic laboratory for analysis. Photo A illustrates key findings from this case.

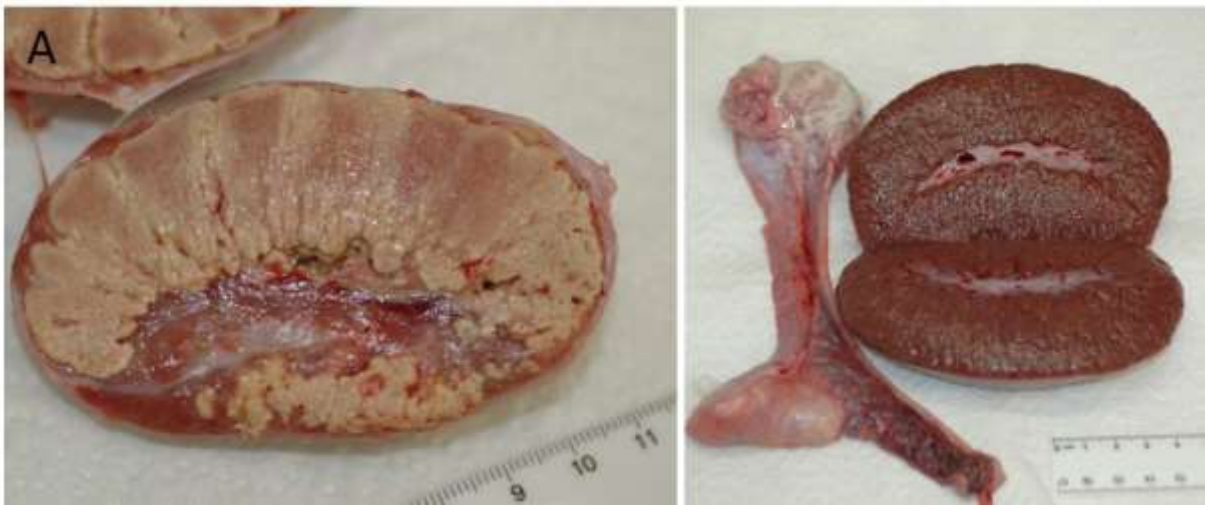



Photo A: Cross section of the left and right testis of an adult wild boar [© G. Wibbelt, Leibniz-IZW]

I. What is your suspected diagnosis based on macroscopic findings?

a. Chlamydiosis due to *Chlamydia suis*

b. Leptospirosis due to *Leptospira interrogans* serovar Pomona
c. **Brucellosis due to *Brucella suis***
d. Porcine rubulavirus infection, family *Paramyxoviridae*
e. Mycobacteriosis due to *Mycobacterium bovis*

II. How would you <u>confirm</u> your suspicion?
a. Stamp's modified Ziehl-Neelsen staining of impression smears
b. Ziehl-Neelsen staining of impression smears and isolation on egg-based media
c. Dark-field microscopy of deep scrapings from the tissue
d. **Bacterial isolation on conventional culture media and biotyping**
e. Isolation in cell culture and immunofluorescence staining of monolayers

III. The aetiological agent
**a. Is a small Gram-negative rod or coccobacilli**
b. Is an acid-fast rod-shaped bacterium
c. Is an enveloped RNA virus
d. Has a unique spiral-shaped cell morphology
e. Is an obligate intracellular pathogen

IV. What is the most effective control strategy for this disease in domestic pigs?
a. Removal of clinically affected pigs
b. Serological testing and removal of positive animals
c. Antimicrobial treatment of infected herds
**d. Whole-herd depopulation**
e. Whole-herd vaccination

5. **Case Summary**: a four months old cat was presented to the clinic with an alopecic lesion (3 cm long) on the right foreleg. The lesion was not pruritic but hyperaemic at the margins (Photo A). Vaccination and dewormings were all up to date. The owner had a very pruritic lesion on the forearm the size of a small coin. No other animal inhabited the house. Culture on Sabouraud's dextrose agar with chloramfenicol/cicloheximide yielded, after 8 days, a colony with a white and silky surface and lemon yellow colouration on the reverse side.

Photo A. lesion before and after hair shaving

I. Given the information you have been supplied with, what is your diagnosis:

a. *Malassezia* sp. dermatitis

***b.*** **Dermatophytosis due to *Microsporum canis***

**c.** Dermatophytosis due to *Tricophyton mentagrophytes* (var.*mentagrophytes*)

*d.* Infection with *Staphylococcus pseudointermedius*

e. Cryptococcosis

II. What other diagnostic test could have been useful for diagnostic purposes?

a. Skin scraping and smear stained with lactophenol cotton blue

b. Skin scraping and smear stained with Gram

**c. Wood 's lamp observation**

d. Blood serology

e. Treat with antimicrobials and re-assess one week later

III. If a preparation had have been made from the cultured isolate and observed under the microscope you would expect to see:

a. Yeast like forms with regular budding

b. Non-septate hyphae and sporangia

c. Grape-like clusters of cocci
d. Septate hyphae, abundant microconidia and little or no macroconidia
**e. Septate hyphae and multiple division, large, macroconidia**

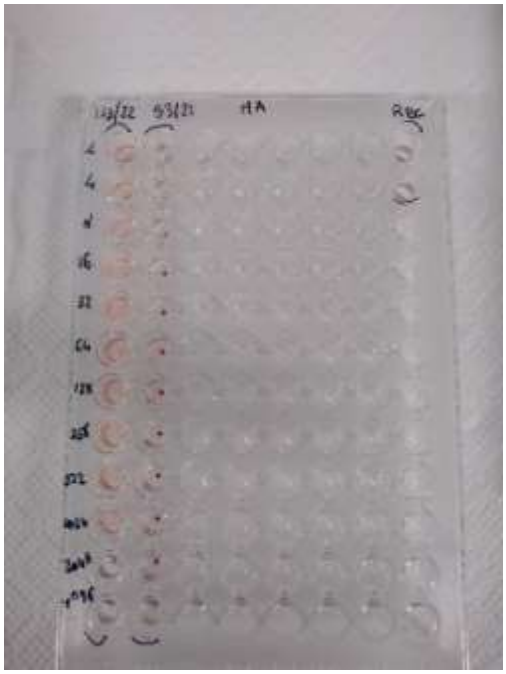
IV. Which treatment option is the most successful for this condition with few toxicity/adverse effects:

a. **itraconazole**
b. piperacillin
c. amphotericin b
d. colchicine
e. flucytosine

## Section B - Microbial identification[9]

**(each identification is worth 6 marks i.e. 90 marks for this part)**

1. This is a haemagglutination assay that was carried out on the feces of a 2.5-month-old pup with vomiting and diarrhea (sample ref. 123/22).



i. Which is the most likely pathogen that could be detected with this test?
Canine Parvovirus is the most likely cause which may be tested by this test.

ii. What is being measured using this test ?
The titre of haemagglutinating virus in the faecal sample. This assay is a virological method to quantify the presence of virus in serial dilutions of the sample

iii. Please read the titre and interpret the test .
The HA titre is 1/1024 (the highest dilution showing haemagglutination of porcine red blood cells).; The assay is positive in this individual with a fairly high titer indicating positivity to canine parvovirus.



[9] This part of the Qualifying Examination consists of 10 cases of microbial identification, all to be answered; 5 questions have been included in this mock exam.

2. This is a haemagglutination inhibition assay carried out on the serum of a 16-week-old pup recently vaccinated against canine parvovirus.


i. What is being measured with this assay and what is the purpose of the test?

The inhibition of haemagglutination assay is a serological test measuring antibody titers against haemagglutinating viruses, in this case canine parvovirus. It may be performed to determine the best time window to vaccinate a non-immunized pup or to detect seroconversion in vaccinated animals as in this case.

ii. What is the HI titre?

The HI titre at the time of testing is 1:1280.

iii. What is your interpretation of the test result ?
The pup has seroconverted against the vaccine. Titres higher than 1:80 are considered protective against positive natural challenge.

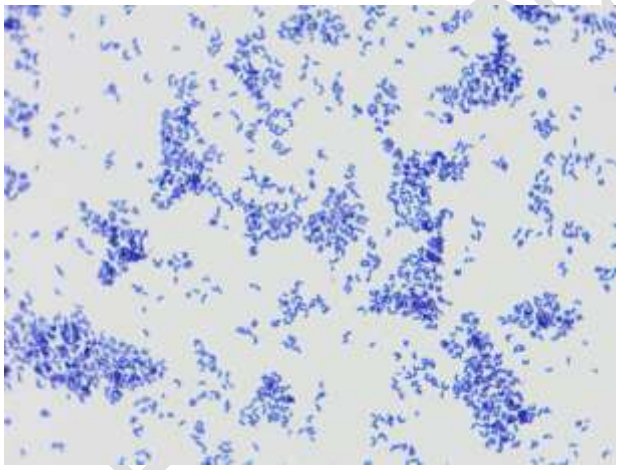
3. A recently calved dairy cow was depressed, restless, had an arched back and blood was seen in the urine. The farmer reported that the animal had been losing weight recently and that her production of milk had been lower than expected. A rectal examination revealed enlarged kidneys and ureters.

Examine the materials presented. Identify the pathogen(s), giving reasons for your answer. Indicate the most appropriate additional tests that may be used to identify the pathogen more fully.

*Materials provided to the candidate.*

*Corynebacterium renale,* culture on blood agar. Gram stained smear.

Blood agar



Gram-stain

4. A recently purchased stallion covered 10 mares. About 4 days after service all the mares developed a copious mucopurulent discharge. None showed signs of systemic disease and the stallion had no discharge from the prepuce.

Examine the materials presented.  Identify the pathogen(s), giving reasons for your answer. Indicate most appropriate additional tests that may be used to identify the pathogen more fully.

*Materials provided to the candidate.*

*Taylorella equigenitalis,* culture on Chocolate agar.

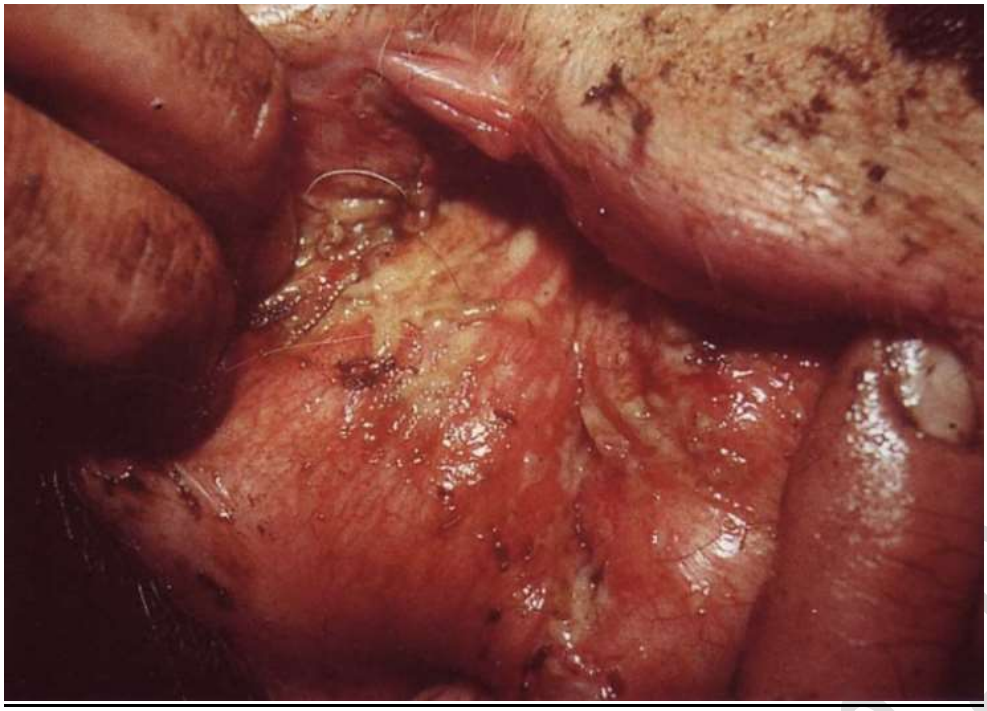
Chocolate agar

5. A farmer called you to several cows that appeared uneasy, constantly shuffled their hind legs and held their tail-heads high.  Clinical examination revealed that the vaginas of the cows were red and inflamed with small yellow pustules.

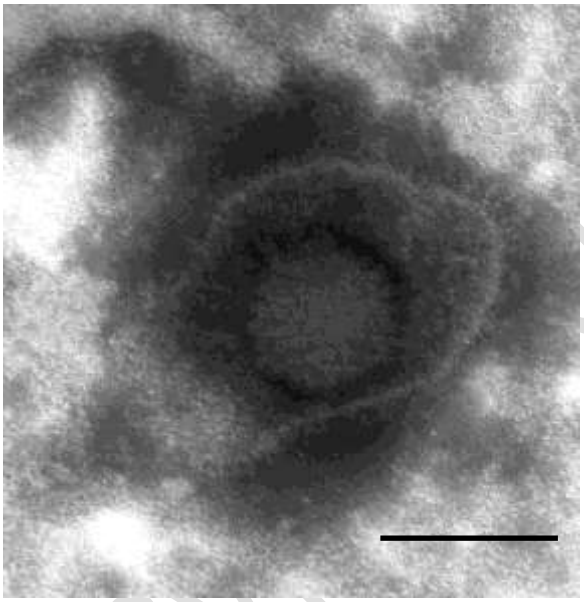
Examine the materials presented.  Identify the pathogen(s), giving reasons for your answer. Indicate the most appropriate additional tests that may be used to identify the pathogen more fully.

*Materials provided to the candidate.*

Infectious pustular vulvovaginitis photographs (seee below)



Vulva of affected cow.



Electron micrograph. Bar = 100 nm

**List of materials to be provided to candidate**

1. HA plate

2. HAI plate

3. *Corynebacterium renale,* culture on blood agar. Gram stained smear.

4. *Taylorella equigenitalis,* culture on Chocolate agar.

5. Infectious pustular vulvovaginitis photographs