Australian and New Zealand College of Veterinary Scientists

Fellowship Examination

June 2013

Veterinary Epidemiology

Paper 2

Perusal time: **Twenty (20)** minutes

Time allowed: **Four (4)** hours after perusal

Answer **ALL <u>FOUR (4)</u>** questions

Answer **<u>FOUR</u>** questions each worth 60 marks .....total 240 marks

Paper 2: Veterinary Epidemiology

Answer all four (4) questions

1. Paratuberculosis (Johne's disease), a chronic gastroenteritis of ruminants, is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and occurs worldwide. Antibody and organism detection tests are commonly used to detect infected animals by testing specimens such milk, serum and faeces. Field evaluation of the accuracy of tests to detect MAP in subclinically-infected animals follows similar principles regardless of whether the target species is sheep, goats, cattle or deer.

   Assume that you have been asked to evaluate the accuracy of a novel serum antibody enzyme-linked immunosorbent assay (ELISA) and a novel faecal real-time quantitative polymerase chain reaction (PCR) for detection of infection in subclinically-infected adult dairy cattle, defined as all eligible cows that are in or have completed at least their first lactation. In most herds, this will equate to cows greater than two years of age.

   Data from ELISA testing are reported as optical density values and from PCR as cycle threshold values. For the purposes of the question, assume that all herds in the source population are free of bovine tuberculosis and hence, cross-reactivity from *M. bovis* is not a relevant consideration in the source population of herds. Your study design should be prospective.

   Answer **all** parts of this question:

   a) Outline the most important features of a study to evaluate and compare the accuracy of the ELISA and PCR for the purpose of determining animal-level infection status of subclinically-infected adult cattle. *(36 marks)*

   b) List measures of test accuracy that you would typically use for reporting results of the study assuming that both tests will generate results on a continuous scale.

   (6 marks)

   c) Outline the main disadvantage of using a retrospective (versus prospective) design based on banked samples. *(4 marks)*

   d) Define the laboratory factors (other than accuracy) that should be considered to decide whether the test would have utility for use in a state-wide survey to estimate the prevalence of MAP at the herd and animal level. *(6 marks)*

   e) Briefly discuss all considerations that would be most important to a dairy herd owner who might voluntarily decide to use the test(s), either individually or in combination, to aid in culling decisions. *(8 marks)*

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2. In some randomised controlled trials (RCT) in animal health, the unit of allocation, and hence, analysis is the group (e.g. pen, litter, herd or flock). All units in the same group receive respective treatment and their responses tend to be correlated.

Assume that you are the research veterinarian for a large feed manufacturer and have designed and implemented a pilot RCT of a new high energy diet given to 32 sows (16 on new diet and 16 on the standard diet) during the last two weeks of gestation and then during lactation in a well-managed herd of 100 sows. Sows (parity ≥ 2) were randomly allocated to treatment. Sows were housed in individual stalls during the last two weeks of gestation and in crates from the day before farrowing until weaning. Creep feed was provided to newborn pigs and there was no cross-fostering of pigs among litters. For each litter, the number of piglets alive at birth and the number that survived until weaning at 28 days were recorded. The date of death was recorded for all dead piglets.

The primary objective of the trial was to determine whether the new sow diet significantly improved survival of piglets (compared with the standard diet) from birth to weaning at 28 days.

Answer **all** parts of this question:

a) For data analysis, would it be preferable to use the crude counts of dead piglets as the outcome or the proportion of dead piglets in each litter? Justify your answer. *(8 marks)*

b) Suppose the data from the trial could be cross-classified as follows in a table collapsed across litters.

Treatment	**Died**	**Survived**	**Total**
High energy diet	11	155	166
Standard diet	22	124	146
Total	33	279	312

State which statistical test could be used to analyse these data. *(4 marks)*

c) Explain why the test you chose in 2 b) would be inappropriate based on the underlying statistical assumptions. *(4 marks)*

d) Explain why would the test you chose in 2 b) would be inappropriate based on biological considerations. *(6 marks)*

e) List **two (2)** statistical tests that might be appropriate to analyse these data. What are the underlying assumptions of these tests and which test would you recommend be used? Justify your answer. *(15 marks)*

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f) The analysis of the 2x2 table in part 2 b) yielded a P value of 0.02. Would you expect the analysis using the approaches listed in part 2 e) to give a lower (<0.02) or higher (>0.02) P value? Justify your answer. *(6 marks)*

g) Define the data/information that would be necessary to allow you to do a simple benefit-cost analysis of the survival data. List **two (2)** assumptions that might be necessary to simplify your analysis. *(12 marks)*

h) Assume that weaning weight data were available for all pigs in the study. State how could this information be incorporated into the benefit-cost analysis. What is the outcome of this analysis, assuming:

- that pigs which are 1 kg heavier at weaning than litter mates are 3 kg heavier at market — this is equivalent to reaching market weights of 100 kg about five to seven days earlier
- daily feed costs of $0.80 per day and other variable costs of $0.20 per day average from weaning (28 days to market at about 150 days). *(5 marks)*

3. Reports about the Schmallenberg virus (SBV) outbreak in Europe were first received in mid-November 2011. The following is extracted from a ProMED post on 18 November 2011:

Since August 2011, farmers and veterinarians in North Rhine-Westphalia (Germany) and The Netherlands have reported clinical disease in cattle and suspected a new introduction of Bluetongue disease. The main clinical signs were fever and a significant drop of milk yield for several days, in some cases also diarrhea and abortions. Samples were submitted to the German national reference laboratory for bluetongue disease at the Friedrich-Loeffler-Institut, Insel Riems.

Diagnostic analyses excluded BTV, FMDV, BVDV, BHV-1, MCFV or exotic viruses like EHDV, Rift Valley fever virus or bovine ephemeral fever virus.

Therefore, 3 pooled samples from a farm with acute signs of the disease (fever greater than 40 C and milk drop of less than 50 percent) were investigated using metagenomic analysis with a Genome Sequencer FLX instrument (ROCHE). The analysis yielded 6 sequence fragments ... (which) *were related to genomic sequences of Shamonda-, Aino-, and Akabane-virus, viruses which are mainly transmitted by* Culicoides *spp. The virus was provisionally named as "Schmallenberg-virus," according to the location where the samples originated.*

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Subsequently, an extract from a ProMED report from Belgium on 16 January 2012:

Since the 1st detection (23 Dec 2011), an increasing number of farms with abortions, stillbirth, and congenital malformations have been observed in ruminants in Belgium. These included 13 cattle farms (all found negative), 34 sheep farms (of which 23 tested positive) and 2 negative goat farms. So far, suspect samples from 94 lambs, 17 calves and 5 newborn goats have been tested, among which 49 lambs proved positive. At the farm level, the average percentage of ewes giving birth to SBV-positive lambs is around 32 per cent, with even 3 of the 4 ewes in one outbreak.

From an early stage, it was clear that the outbreak was transboundary in nature. Ruminants (cattle, bison, sheep, goats) were affected in an increasing number of EU countries during late 2011 and throughout 2012. An intensive research effort commenced from late 2011, both epidemiological and laboratory-based, leading to confirmation of SBV virus, closely related to Akabane virus, as the cause of the outbreak. Clinical signs in adult cattle were generally transient, lasting approximately seven days, including fever, loss of appetite, up to 50% reduction in milk yield and, in rare cases, severe diarrhoea. Infection has also been associated with a range of congenital abnormalities in stillborn or newborn calves, lambs and kids, including severe arthrogryposis, torticollis, brachygnathia, hydrocephalus and other severe brain malformations.

Several diagnostic tests were available from early 2012, including:

- a PCR test, allowing direct detection of SBV virus in foetuses and neonates, and
- several serological tests for SBV exposure in adult animals.

Within the EU, the collection of animal health data is undertaken either at a national level or, in the case of Germany, at the level of the Länder (state).

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Given this background information, you are asked to answer **all** parts of a) and b), and c) below:

a) An urgent need for reporting guidelines during the SBV outbreak was identified from late 2011, to allow harmonised data collection across all EU countries. Provide an outline of such reporting guidelines, but **focusing solely on the data required to provide EU-level insights** into:

   i. the temporal and geographical distribution of SBV,

   ii. the magnitude of the SBV outbreak (both at the level of the herd/flock and the animal) and

   iii. herd-level risk factors associated with SBV infection.

   Limit your answer to reporting guidelines towards the development of a harmonised herd-level data set. *(16 marks)*

b) Describe how these data might be analysed and presented, to enable the above three questions (i, ii, iii) in part a) to be answered. Use a separate sub-heading for:

   - Temporal and spatial distribution *(10 marks)*
   - Magnitude of the outbreak, both at the animal and the herd/flock level *(10 marks)*
   - Herd-level risk factors *(10 marks)*

c) Briefly discuss the key epidemiological challenges to be addressed during data collection and data analysis and reporting? Use separate sub-headings for:

   - Data collection *(7 marks)*
   - Data analysis and reporting *(7 marks)*

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4. In recent years, several authors have highlighted the potential for interference of bovine tuberculosis (bTB; caused by *Mycobacterium bovis*) diagnosis due to co-infection with Johne's disease (caused by *M. avium* subspecies *paratuberculosis*, Map). A number of authors, including Barry et al., 2011[1], have highlighted the high level of cross reactivity between PPD (purified protein derivative) tuberculins of *M. bovis* and Map, with a negative impact on bTB test specificity. As yet, however, field results have been equivocal. As one example, Dunn et al. (2005[2]) reporting an increased likelihood of false-positive results on the single intradermal tuberculin test to cattle with positive (compared with negative) responses to Map, although this association was not significant.

   Further work is needed to clarify these results, as any interference with bTB diagnosis, as a consequence of Map exposure, will have important implications for countries, such as New Zealand, with ongoing bTB eradication programmes.

   An industry funding body has invited submission of research proposals. The submitted proposals will be evaluated by a committee with relevant industry and technical expertise, and evaluated in terms of the quality of research outcomes and the cost-effectiveness of the project.

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[1] Barry et al., 2011. The effect of *Mycobacterium avium* complex infections on routine *Mycobacterium bovis* diagnostic tests. *Vet Med Int* article ID 145092

[2] Dunn et al., 2005. Effects of positive results for *Mycobacterium avium* subsp *paratuberculosis* as determined by microbial culture of feces or antibody ELISA on results of caudal fold tuberculin test and interferon- assay for tuberculosis in cattle. *J Am Vet Med Assoc* 226, 429–435.

You are tasked by your research group to develop a detailed study design for **a case-control study to quantify the herd-level association between *Map* exposure and the presence of false-positive bTB reactors** (animals positive to the single intradermal test (SIT) but bacteriologically negative following detailed investigation). This study design will be submitted to the funding body in response to its call for submission of research proposals.

In your answer present the study design that you develop for submission to the funding body.

Include a detailed discussion of how you might resolve relevant epidemiological issues/challenges in the proposed case-control study.

As part of the study design, provide a broad overview of the analytical methods to be used, but do not consider this aspect in detail. *(60 marks)*

An additional comment:

A brief summary of bTB[3] and Johne's disease[4] in New Zealand is presented, which you may draw on, if you wish, in formulating your answer. However, you are under no obligation to do so.

[3] There is a national bTB eradication programme in New Zealand. In the programme, the detection of infected animals relies on both field and abattoir surveillance, the former using the single intradermal test (SIT) test, and the latter on inspection of all animals at slaughter. Infected herds are restricted from trading until infection has been cleared. The number of infected cattle and deer herds in New Zealand has reduced from over 1,700 in the mid '90s to fewer than 100 in 2011. Infection in cattle is now limited to defined geographical areas. Eradication is complicated by the maintenance of infection in the brushtail possum. Other wildlife species are also infected, including red deer, ferrets and feral pigs, but generally as spillover hosts.

[4] Johne's disease (JD) is endemic in cattle and sheep in New Zealand, and spreading in farmed deer populations. In 2007, it was estimated that herd-level prevalence of JD by ELISA and/or faecal culture ranged from 4.5% (95% CI 2.6-6.9) to 14.2% (95% CI 9.2-20.6) (Norton, 2007. The epidemiology of Johne's disease in New Zealand dairy herds. PhD thesis, Massey University)

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