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Authors(s)	Carroll, Rebecca I., Forbes, Andrew, Graham, David A., Messam, Locksley L. McV.
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A protocol to identify and minimize selection and information bias in abattoir surveys estimating prevalence, using *Fasciola hepatica* as an example.

Rebecca I. Carroll^{1,2}, Andrew Forbes³, David A. Graham², Locksley L. McV. Messam¹

¹Herd Health and Animal Husbandry, School of Veterinary Medicine, University College Dublin, Dublin, Ireland.

²Animal Health Ireland, 4-5 The Archways, Carrick on Shannon, Co. Leitrim, N41 WN27

³Scottish Centre for Production Animal Health and Food Safety, School of Veterinary Medicine, University of Glasgow, G61 1QH

*Corresponding author: rcarroll@animalhealthireland.ie; University College Dublin, Belfield, Dublin 4, Ireland. (Rebecca Carroll)

Highlights

- Selection and information bias often occur in abattoir surveys estimating prevalence.
- Presentation of a protocol to identify and minimize biases in abattoir surveys.
- Simple sensitivity analyses to quantify uncertainty due to biases.
- Description of abattoir, study and target populations and their relationships.

Abstract

Abattoir surveys and findings from post-mortem meat inspection are commonly used to estimate infection or disease prevalence in farm animal populations. However, the function of an abattoir is to slaughter animals for human consumption, and the collection of information on animal health for research purposes is a secondary objective. This can result in methodological shortcomings leading to biased prevalence estimates. Selection bias can occur when the study population as obtained from the abattoir is not an accurate representation of the target population. Virtually all of the tests used in abattoir surveys to detect infections or diseases that impact animal health are imperfect, leading to errors in identifying the outcome of interest and consequently, information bias. Examination of abattoir surveys estimating prevalence in the literature, reveals shortcomings in the methods used in these studies. While the STROBE-Vet statement provides clear guidance on the reporting of observational research, we have not found any guidelines in the literature advising researchers on how to conduct abattoir surveys. This paper presents a protocol in two flowcharts to help researchers (regardless of their background in epidemiology), to identify, and where possible, minimize biases in abattoir surveys estimating prevalence. Flowchart 1 examines the identification of the target population and the appropriate study population while Flowchart 2 guides the researcher

41 in identifying and where possible, correcting potential sources of outcome misclassification. Examples
42 of simple sensitivity analyses are also presented which approximate the likely uncertainty in
43 prevalence estimates due to systematic errors. Finally, the researcher is directed to outline any
44 limitations of the study in the discussion section of the paper. This protocol makes it easier to conduct
45 an abattoir survey using **sound** methods, identifying and, where possible, minimizing biases.

46

47 Keywords

48

49 Abattoir, prevalence, bias

50

1. Introduction

Abattoir surveys and findings from meat inspection are often used to estimate the prevalence of infection or disease. Examples include *Fasciola hepatica* (Bellet et al., 2016; Borji et al., 2012; Byrne et al., 2016), *Calicophoron daubneyi* (Toolan et al., 2015), *Taenia saginata* (Dorny and Praet, 2007), bovine respiratory disease complex (Rezac et al., 2015) and ovine pulmonary adenocarcinoma (Cousens et al., 2015). Abattoir records allow researchers to inexpensively gather data on large samples and provide information on the health status of farm animal populations (Alton et al., 2015; Rezac et al., 2015). However, as the function of an abattoir is primarily to slaughter animals for human consumption, these data are not primarily collected for research purposes, creating several potential methodological short-comings in their use.

Firstly, when used to estimate prevalence, abattoir populations are often taken to be representative of the target population (Durr et al., 2005), but this is not necessarily the case. The study population is the population which provides the data for analysis and includes animals from the abattoir population. It should not be assumed that these two populations are necessarily identical. Non-random errors in prevalence estimates occur when the study population is not representative of the target population (Delgado-Rodriguez, 2004). In order to judge whether prevalence estimates will be valid, it is first necessary to identify the target population and assess the means by which the animals are sampled to finally evaluate the extent to which a study population is an acceptable representation of this population.

Secondly, the primary purpose of meat inspection is to reduce the risk of foodborne health hazards. Surveillance for animal infections or diseases is a secondary objective (Edwards et al., 1997). Operational factors such as the high throughput in meat factories limiting the time available for recording (Rezac et al., 2015) may mean that meat inspection has imperfect sensitivity and specificity for detecting infections or diseases which impact animal health. This may be compounded by disease-

related factors, including the visibility, severity and distribution of lesions (Dorny and Praet, 2007 Aylate et al., 2013). In both cases, these factors can lead to misclassification of infection or disease status.

The goal of this paper is to present a step by step approach to guide researchers in the estimation of prevalence from an abattoir survey. We use, as examples, abattoir surveys estimating the prevalence of *F. hepatica* infection in the Republic of Ireland. Throughout the paper, “*F. hepatica* infection” is used to mean lifetime *F. hepatica* infection.

2. Populations to consider while conducting an abattoir survey

We define the target population as the group of animals of interest (Pfeiffer, 2010). The abattoir population is the group of animals, in the abattoir, for which meat inspection information is available and from which the study population is drawn. The abattoir population may include only animals from the target population (Figure 1a) or may also include animals that come from outside of the target population (Figure 1b). The study population is that subset of animals from the target population, which ultimately provides data for analysis (Dohoo et al., 2010). The abattoir population may provide all the animals (Figures 1a and 1b) or may supply some of the animals that will form the study population. If the abattoir population does not provide all the animals that should be considered as part of the study population, it will be necessary to include, hypothetically, animals from the target population for which no meat inspection data is available (Figure 1c). This might be because during the study period, some subcategories of target population animals are sent to other abattoirs or are not slaughtered, thus remaining on the farm. This results in subcategories of animals from the target population either not being proportionally represented or represented at all in the abattoir population. In this situation, a “best guess” is used to estimate a prevalence range of the infection in

these animals and a sensitivity analysis is used to estimate the resulting uncertainty. A sensitivity analysis is a form of quantitative bias analysis which allows a researcher to approximate the likely uncertainty in their estimates due to systematic errors (Lash et al., 2014). Bias analysis should be implemented when the report of an association goes beyond description and is essential when actions or policy recommendations are made based on study findings (Lash et al., 2014).

3. Protocol Development

We designed question-driven flowcharts to reflect generally accepted epidemiological principles aimed at avoiding selection and information bias (Dohoo et al., 2010; Gordis, 2009; Pfeiffer, 2010; Rothman et al., 2008; Szklo and Nieto, 2000) and generally agreed guidelines on reporting observational research (Elm et al., 2007; Sargeant et al., 2016a, 2016b, 2016c, 2016d, 2016e). We used process flowcharts (<http://asq.org/learn-about-quality/process-analysis-tools/overview/flowchart.html>) (Figures 2 and 3) because they allow a researcher to logically apply the questions to the process of estimating prevalence. To denote the steps and actions required at each stage, we employed conventional flowchart shapes (<https://www.smartdraw.com/flowchart/flowchart-symbols.htm>). Closed, study design-related questions are posed, and the simplest and most straightforward scenarios follow from affirmative answers, allowing the researcher to continue down the main stem. Negative responses result in circumstances that are less than ideal, and the flowcharts branch, leading to instructions on how to deal with these situations.

4. Managing selection bias

4.1. Flowchart 1

Flowchart 1 (Figure 2) identifies both the target (Figure 2, Question 1) and study populations and distinguishes them from the abattoir population (Figure 2, Questions 2 to 6 and sensitivity analyses where required). Answering the first 6 questions allows the researcher to identify the target population animals present in the abattoir. If these animals comprise the whole study population, a sensitivity analysis is not required. If they do not, a sensitivity analysis is required and other animals from the target population must be taken into account to form the study population. At the end of Flowchart 1, the researcher can calculate the apparent prevalence and associated 95% confidence interval (Box 1, Equation 1) or an apparent prevalence range following a sensitivity analysis (Examples 1 and 2) for the target population. The apparent prevalence (AP) is the proportion of animals in the study population which are detected as infected (Pfeiffer, 2010), e.g. the proportion of animals which show evidence of *F. hepatica* infection at meat inspection and is given by:

$$AP = \text{Number of animals detected as infected in study population} / \text{Number of animals in study population} \quad (1)$$

4.1.1. Sensitivity analysis: example 1

We wish to estimate the prevalence of *F. hepatica* infection in cattle on a farm in Ireland. Assume that the farm (the target population) sends 100 cattle to slaughter during a 1 month period. Meat inspection results are available for the 50 animals slaughtered in abattoir D and 10 animals are found to have evidence of *F. hepatica* infection, yielding an abattoir prevalence of 0.20. The remaining 50 animals were sent to abattoir E and because no meat inspection information is available the prevalence of *F. hepatica* infection in these animals is unknown. Unlike the cattle sent to abattoir D,

the cattle sent to abattoir E were grazed on a part of the farm which has soil with poor drainage. While the prevalence of *F. hepatica* cannot be known in the animals which were slaughtered in abattoir E, recent work examining the herd level prevalence of *F. hepatica* in Irish dairy herds found that herds on farms with poorly drained soils were more likely to be exposed to *F. hepatica* (Selemetas et al., 2014). Thus for the purposes of this example, we make a “best guess” and assume the prevalence of *F. hepatica* in this group to be at least equal to that in their herd mates and may be up to twice as high (0.20-0.40). Therefore between 10 (0.2×50) and 20 (0.4×50) of the animals sent to abattoir E are expected to have liver lesions associated with *F. hepatica* infection. Using the above information we can calculate an apparent prevalence (AP) range for the target population. If the assumed prevalence for the animals sent to abattoir E is 0.20, the total number infected is 10 (from abattoir D) + 10 (from abattoir E) = 20. Hence the $AP = 20/100 = 0.20$. If the assumed prevalence for abattoir E is 0.40, the total number infected is 10 (from abattoir D) + 20 (from abattoir E) = 30. Hence the $AP = 30/100 = 0.30$. Therefore the AP range for *F. hepatica* infection on the farm (the target population) is from 0.20 to 0.30. This gives an AP for the target population that is up to 50% more than the abattoir prevalence.

4.1.2. Sensitivity analysis: example 2

We wish to estimate the prevalence of *F. hepatica* infection in a target population comprised of 18 to 24 month old heifers and steers bred for beef production, in a county in Ireland. From information obtained from the Department of Agriculture Food and the Marine’s Animal Identification and Movement system (official database which captures details on cattle origin, identity and movements in Ireland), there are 11,000 beef bred heifers and steers in the county. More rain falls in the western part of the county than the eastern region and a subgroup of 4000 of these cattle reside in the wetter western part of the county. This subgroup makes up approximately 36% of the target population ($4000/11000 = 0.36$).

In one month, 1000 cattle from the target population are supplied to abattoir D. From livers examined, 200 cattle are found to have evidence of *F. hepatica* infection, yielding an abattoir prevalence of 0.20. Only 100 animals from the subgroup from the western part of the county are sent to the abattoir giving a representation of 10% in the abattoir population ($100/1000 = 0.10$). Thus, these cattle are not proportionately represented in the study population. Evidence of *F. hepatica* infection is found in 25 (i.e. 25%) of these animals in the abattoir. A sensitivity analysis is conducted to due to the uncertainty in the prevalence in the subgroup from the western part of the county. A paper published in 2014 examining the herd level prevalence of *F. hepatica* in Irish dairy herds, found herds on farms with high rainfall levels were more likely to be exposed to *F. hepatica* (Selemetas et al., 2014). Based on this, it is likely that herds coming from the western part of the county will have a higher prevalence than those that don't. We assume that the prevalence of infection in the subgroup is between 0.25 (based on the proportion of infected subgroup animals in the abattoir) and 0.40 (a "best guess" of double the abattoir prevalence).

To get a more accurate estimate of the prevalence in the target population we have to calculate the prevalence assuming that the animals in the subgroup were included in a manner proportionate to their representation in the target population. Therefore, we calculate how many animals from the subgroup should be in the sample. Let x = total number of animals from the subgroup which should be in the abattoir population to give proportionate representation. Then, $x/(900+x) = 0.36$. Therefore $x = 506.25$. One hundred animals from the subgroup are already in the abattoir population, therefore $(506.25-100 =) 406.25$ animals are hypothetically added to the animals in the abattoir population to form the study population. The prevalence of *F. hepatica* is calculated for these animals based on the assumptions above: if the prevalence is assumed to be 0.25, the number of infected animals will be $0.25 \times 406.25 = 101.56$. If the prevalence is assumed to be 0.40 the number of infected animals will be $0.40 \times 406.25 = 162.50$. The AP range for *F. hepatica* infection in the target population is therefore $(101.56 + 200)/1406.25 = 0.21$ and $(162.50 + 200)/1406.25 = 0.26$, for prevalences of 0.25 and 0.40, respectively. The AP range between 0.21 and 0.26 gives an AP for the

target population that is between 5% and 30% greater than the abattoir prevalence of 0.20. We state, parenthetically, that if it were felt that the 100 animals were a good representation of the subgroup from the western part of the county, then the prevalence could be estimated by taking a weighted average of the 1000 animals in the abattoir and the 406.25 hypothetically added animals

$\left(\frac{(1000 \times 0.2) + (406.25 \times 0.25)}{1406.25} \right)$ and would be equal to 0.21 the lower bound of our prevalence range above.

5. Managing information bias

5.1. Flowchart 2

Flowchart 2 (Figure 3) guides the researcher through a series of questions intended to identify and correct potential sources of outcome misclassification by means of precisely identifying the outcome (Figure 3, Question 1), the post mortem inspection protocol used to determine the outcome (Figure 3, Question 2) and the protocol's sensitivity and specificity (Figure 3, Question 3 and 4). At the end of Flowchart 2, the researcher is directed to estimate the true prevalence (Equation 2) of the outcome and associated 95% confidence intervals (Box 1, Equations 3-5) or the true prevalence range (Example 3) for the target population. The true prevalence is the proportion of animals in the target population that are infected (Pfeiffer, 2010). The true prevalence (TP) is calculated using the apparent prevalence identified in Flowchart 1 along with the protocol sensitivity and specificity identified in Flowchart 2 and is given by:

$$TP = (AP + Sp - 1) / (Se + Sp - 1) \quad (2)$$

Where, AP = apparent prevalence, Se = sensitivity and Sp = specificity.

5.1.1. Sensitivity analysis: example 3

We wish to calculate the true prevalence of *F. hepatica* infection in the target population described in example 2. A validation study using a Bayesian non-gold standard approach has found the sensitivity and specificity of meat inspection for diagnosing *F. hepatica* infection to be 0.68 (95% probability interval: 0.61 - 0.75) and 0.88 (95% probability interval: 0.85 - 0.91), respectively (Mazeri et al., 2016). This study was conducted in a Scottish abattoir and it would be reasonable to expect that abattoirs in Ireland using the same protocol, as set down in Regulation (EC) No 854/2004, would have meat inspection sensitivity and specificity within the 95% probability intervals. We therefore assume that the meat inspection technique used in abattoir D has a sensitivity between 0.61 and 0.75 and a specificity between 0.85 and 0.91. The apparent prevalence range in the target population from example 2 is between 0.21 and 0.26. A range of TPs are then calculated corresponding to these apparent prevalence values and various combinations of sensitivity and specificity using Microsoft Excel. The results are presented in the Table.

For AP = 0.21, the TP is greater than the AP for a small minority of sensitivity and specificity combinations. For AP = 0.26, the TP is greater than AP for approximately one third of the possible combinations of sensitivity and specificity.

6. Discussion

Abattoir data provides an excellent resource for researchers estimating infection or disease prevalence in farm animal populations. Prevalence studies inform scientists and policy makers about the burden of disease, thereby helping to identify priorities in animal health policy and can also be used in economic models and to assess interventions (Harder, 2014). It is therefore essential that abattoir studies are conducted using sound methods in order to ensure unbiased prevalence estimates.

A search of the literature in PubMed for papers with full text available, using the terms “abattoir”, “prevalence” and “cattle”, for the period October 2015 to September 2016 found 40 papers (Figure 4). Of these, 15 were abattoir surveys with the stated or implied aim of estimating infection or disease prevalence in an animal population. The target population was explicitly identified in 2 papers (Chihai et al., 2015; Vipham et al., 2015) and briefly discussed in 1 other paper (Bellet et al., 2016). Two articles (Egbe et al., 2016; Sargison et al., 2016) provided, and 2 (Bellet et al., 2016; Byrne et al., 2016) discussed but did not provide, the sensitivity and specificity of the protocol used to identify the outcome. These results reveal methodological problems in most studies reviewed and highlight the need for guidance on study design for abattoir surveys estimating prevalence. While the recently published STROBE-Vet guidelines provides clear guidance on the reporting of observational research (Sargeant et al., 2016a, 2016b, 2016c, 2016d, 2016e), we have found no protocols in the literature to help researchers when conducting abattoir surveys. While both information and selection bias are discussed in major text books, examples given are invariably based on measures of association and not prevalence (Dohoo et al., 2010; Gordis, 2009; Rothman et al., 2008; Szklo and Nieto, 2000). This paper applies those principles to prevalence surveys helping researchers, particularly those without formal training in epidemiology, to minimize biases.

The procedures in Flowchart 1 are presented first to emphasise the major importance of population selection and to emphasize that identification of the target population is the first and most important action that should be undertaken when planning an abattoir survey. The population

structure of an abattoir will not always reflect the population structure on farms (Thrusfield, 1986). For instance, sick animals and animals that die on farms are not sent to abattoirs and abattoir populations generally consist of healthy animals which may be of uniform age. Recent examples in the literature demonstrate abattoir surveys used to derive an abattoir prevalence rather than a target population prevalence (Borji et al., 2012; Byrne et al., 2016; Taghadosi et al., 2016). However, abattoir populations may or may not include a representative sample of either the infected animals or the at risk population. For these reasons, it is vital to scrutinise the abattoir population in order to identify a study population which is representative of the target population. Defining the target population in terms of animal category, geographic location and the time period during which the animals were slaughtered allows the researcher to systematically compare it to the abattoir population and identify the correct study population.

It is only possible to estimate prevalence for categories of animals, represented in the abattoir, from the geographic location from which they originated and for the time frame during which they entered the abattoir. If there are no animals from the target population in the abattoir population, valid inferences on prevalence for this population cannot be made. Conversely, as highlighted in Question 4 in Flowchart 1, if there are animals from outside the target population in the abattoir population, these animals should be excluded from the study population. If these animals are included in the study population and have different risk factors for infection or disease than animals in the target population, their presence would result in a biased apparent prevalence.

It is essential that all subcategories of animals from the target population are proportionately included in the study population. This may be achieved by a study population derived from the abattoir population (Figure 1a and 1b) or it may be necessary to **hypothetically** add target population animals not present in the abattoir population to the study population in a sensitivity analysis (Figure 1c). Therefore, in situations where **subcategories** are not represented or are not proportionally represented, a sensitivity analysis is required and the study population will contain both animals from

the target population which were sent to the abattoir in question and, **hypothetically**, animals which were not sent to that abattoir (Figure 1c). As there is no meat inspection information giving a prevalence for these unrepresented animals, the sensitivity analysis takes them into account by making a “best guess” at an infection prevalence range and including them in the calculation in a proportional manner (**Examples 1 and 2**). This “best guess” should be transparently motivated and based on subject matter considerations. For instance, in Example 2 the underrepresented animals are from a part of the county with **more rainfall**, a risk factor for *F. hepatica* infection (Selemetas et al., 2014). As s with any sensitivity analysis, the point is not to present unquestionable scenarios but to present reasonable alternative possibilities. To the reader, these may either be acceptable or provoke discussion leading to more acceptable estimates. **For example, many studies show that rainfall or water logged soil is a risk factor for *F. hepatica* at the herd level (Olsen et al., 2015; Selemetas et al., 2014; Selemetas and de Waal, 2015), however, we have not found any studies which predict the impact of exposure to these factors on individual-level infection prevalence.**

Given that virtually all of the protocols used to detect infection or disease in abattoir surveys are imperfect (Aylate et al., 2013; Bonde et al., 2010; Dohoo, 2014), it is essential to acknowledge that information bias can affect prevalence estimates from abattoir surveys. Defining the outcome and the protocol used to identify the outcome are the two most important steps in minimising this type of bias. A precise outcome definition aids the researcher in distinguishing animals that have the outcome from those that do not. Arguably the definition itself is not as important as is being unambiguous. For instance, it is possible to define the outcome as “*F. hepatica* found in the liver during meat inspection” or “changes in the liver consistent with *F. hepatica* infection” or a combination of both. It is the responsibility of the researcher to unambiguously define the outcome they are measuring in the study. Similarly the meat inspection protocol should be defined and standardised.

It will not be possible to eliminate information bias if test sensitivity and specificity are unknown. This will occur where the meat inspection protocols have not been validated or where local conditions

are not consistent with previous protocol validation conditions. In these scenarios, one possibility is that the researcher will only be able to estimate the apparent prevalence. This should be acknowledged in the paper as the apparent prevalence does not take into account the impact of imperfect protocol sensitivity and specificity on the estimation of prevalence.

Alternatively expert opinion could be used to identify a likely range for the sensitivity and specificity of the test and this could be used to calculate the true prevalence. While this may be the best approach in the circumstances, there will still be uncertainty in these values and the researcher should conduct a sensitivity analysis to see the impact of this uncertainty on the true prevalence values obtained as this can have policy implications.

In example 3, for $AP = 0.21$, the true prevalence is greater than the apparent prevalence value for only two sensitivity and specificity combinations (shaded values). For $AP = 0.26$, the true prevalence is less than the apparent prevalence value in approximately one third of the possible combinations of sensitivity and specificity (shaded values). As the apparent prevalence depends on the sensitivity, specificity and true prevalence any given apparent prevalence value can result from a number of combinations of the three. This makes it difficult to determine the actual true prevalence if one does not know the sensitivity and specificity thus demonstrating the importance of sensitivity analyses. Both the under- and overestimation of true prevalence can have negative effects (Bonde et al., 2010). Underestimation can lead to a scenario where farmers, veterinarians and policy makers underestimate the impact of disease and potentially animals are not treated when they should be and overestimation can give rise to a situation where treatments are over used leading to increases in antimicrobial or anthelmintic resistance.

If there is no validation study available, it is not possible to use equation 4 (Box 1) to calculate the standard error of the true prevalence. One approach is to use equation 5 (Box 1). However this results in confidence intervals that are much narrower than they should be, giving a false impression of greater precision. In such cases, use of equation 5 should always be mentioned as a study limitation.

We acknowledge that aspects of an abattoir survey may not be modifiable by the time a researcher begins to plan a study. For example the abattoir population and the protocols used to identify infected animals may have already been determined. Nevertheless, the flowcharts may be used to highlight limitations in population selection and/or outcome misclassification. This will help the researcher to acknowledge these limitations when reporting on the survey. This in turn enables the reader to make an informed judgment on the study's quality.

All the examples in this paper are simple and we acknowledge that the reality is often more complex. It is assumed throughout the manuscript that a researcher has meat inspection information from one abattoir but this protocol can also be applied where information is available from multiple abattoirs. Abattoir surveys estimating the prevalence of *F. hepatica* are given as examples but the principles in the flowcharts can be applied to abattoir surveys for other infections or diseases. A single farm is used in Example 1 as this is the most basic unit for which prevalence is usually estimated in farm animals. It is likely that researchers will wish to calculate prevalence for larger target populations and the principles can be extrapolated to larger groups. While a single systematic error is highlighted in each example, it is possible that an abattoir survey could have multiple biases which affect prevalence estimates. An abattoir prevalence survey could have a target population which is not proportionally represented in the abattoir population and at the same time a protocol with an uncertain sensitivity and specificity. Nevertheless, for the purposes of this paper, the simplicity aids the communication of the concepts discussed.

Guidelines on the quality of reporting for observational studies exist and are supported by many veterinary journals (Sargeant et al., 2016a, 2016b, 2016c, 2016d, 2016e). While good quality reporting is vital in studies, it is important to distinguish transparent and quality reporting from the methodological quality of a study (Harder, 2014; O'Connor, 2010) as studies with similar reporting quality may vary in methodological quality (Huwiler-mu and Ju, 2008). We have attempted in this paper to encourage both sound methods and the transparent reporting of any study limitations. We

370 recommend that editors require researchers and authors to use sound practices in the design and
371 conduct of abattoir surveys both in terms of population selection and outcome identification and to
372 transparently report any limitations of the study. This will ensure that prevalence estimates are as
373 accurate as possible and that readers can reliably judge their merits. We believe the protocol
374 presented here can be used as a tool to guide researchers in the estimation of infection or disease
375 prevalence from an abattoir survey.

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485 Figure 1: Illustration of possible relationships between target, abattoir and study populations.

486

487 Figure 2: Flowchart 1: Steps guiding the identification of a study population from a target population
488 and the estimation of the apparent prevalence during an abattoir survey.

489

490 Figure 3: Flowchart 2: Steps guiding the estimation of true prevalence during an abattoir survey
491 by identifying and correcting for potential sources of misclassification.

492

493 Figure 4: inclusion criteria for literature review of abattoir surveys estimating infection or
494 disease prevalence

495

1a

1b

1c

Box 1: 95% Confidence interval calculations

A 95% confidence interval for the apparent prevalence (AP) is given by

$$95\% \text{ CI (AP)} = \text{AP} \pm (1.96 \times \text{S. E. (AP)}) \quad (1)$$

Where CI = confidence interval, S. E. = standard error

The S. E. (AP) is given by

$$\text{S. E. (AP)} = \sqrt{\text{AP}(1-\text{AP})/n} \quad (2)$$

Where n = number of animals in the study population

A 95% confidence interval for the true prevalence (TP) is given by

$$95\% \text{ CI (TP)} = \text{TP} \pm (1.96 \times \text{S.E. (TP)}) \quad (3)$$

When a validation study is available the standard error is then given by:

$$\text{S.E.(TP)} = \sqrt{[X + Y\text{TP}^2 + Z(1-\text{TP})^2] / J^2} \quad (\text{Greiner and Gardner, 2000}) \quad (4)$$

Where $X = \text{AP}(1 - \text{AP})/n$, $Y = \text{Se}(1 - \text{Se})/n_1$, $Z = \text{Sp}(1 - \text{Sp})/n_2$, $J = \text{Se} + \text{Sp} - 1$, Se = sensitivity, Sp = specificity, n = number of animals in the current study population, n_1 and n_2 are the numbers of infected (diseased) and non-infected (-diseased) animals in the original test validation study respectively.

If Se and Sp are known without error, then the S. E. is given by:

$$\text{S. E. (TP)} = \sqrt{\text{AP}(1-\text{AP})/nJ^2} \quad (\text{Greiner and Gardner, 2000}) \quad (5)$$

Table: Estimates of true prevalence of *F. hepatica* infection corresponding to combinations of sensitivity (0.61-0.75), specificity (0.85-0.91) and AP values of 0.21 and 0.26

Sensitivity	Specificity									
	Apparent Prevalence = 0.21					Apparent Prevalence = 0.26				
	0.85	0.87	0.88	0.89	0.91	0.85	0.87	0.88	0.89	0.91
0.61	0.13	0.17	0.18	0.20	0.23	0.24	0.27	0.29	0.31	0.33
0.63	0.13	0.16	0.18	0.19	0.22	0.23	0.26	0.27	0.30	0.31
0.65	0.12	0.15	0.17	0.19	0.21	0.22	0.25	0.26	0.28	0.30
0.67	0.12	0.15	0.16	0.18	0.21	0.21	0.24	0.25	0.26	0.29
0.68	0.11	0.15	0.16	0.18	0.20	0.20	0.23	0.25	0.26	0.28
0.69	0.11	0.14	0.16	0.17	0.20	0.20	0.23	0.25	0.26	0.28
0.71	0.11	0.14	0.15	0.17	0.19	0.20	0.22	0.24	0.25	0.27
0.73	0.10	0.13	0.15	0.16	0.19	0.19	0.22	0.23	0.24	0.27
0.75	0.10	0.13	0.14	0.16	0.18	0.18	0.21	0.22	0.23	0.25

Shaded cells indicate true prevalence values that are greater than the corresponding apparent prevalence values