Certifying the French population of *Crassostrea gigas* free from exotic diseases: a risk analysis approach

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Summary

Sample-size calculations in the context of surveys aimed at substantiating freedom from infection have been commonly undertaken on terrestrial animals over recent years, but not on aquatic animals. A recent model developed by Audigé and Beckett in 1999 can be used to plan and assess animal health surveys. The aim of this study was to adapt that model for marine aquaculture, in particular to help in planning surveys aimed at substantiating freedom from two exotic diseases, mikrocytosis and perkinsosis, in the French population of Crassostrea gigas. *As a first approach, farmed animals were targeted without dividing the French coast into different zones, since the movement and mixing of animals are so frequent that it would be very difficult to be representative of a single area or zone.*

To find the most appropriate sampling scheme, the model was run using @Risk with 1,000 iterations and Latin hypercube sampling for each simulation. Sixty samples from 30 animals within animal clusters were sufficient to detect a cluster prevalence of 10% with 90% confidence, or a prevalence of 20% with more than 95% confidence. Alternatively, 100 samples from 30 animals would be enough to detect 10% of infected clusters with more than 90% confidence.

A sensitivity analysis was conducted to attempt to distinguish between parameter uncertainty and variability. Uncertainty about the sensitivity of the diagnosis test (varying between 50% and 70%) had a major influence on the testing scheme at cluster level, but not much influence at the survey level. This model was very useful in assessing different sampling strategies. However, the model also requires enhancements, such as the availability of more accurate data to confirm the various assumptions made, and being able to take into account other factors, such as the results from past surveys, exchanges and movement of animals and environmental factors.

Keywords: Aquatic animal surveys – Certification – Crassostrea gigas – Molluscs – Oysters – Risk analysis – Sensitivity modelling – Stochastic modelling.

Introduction

Surveying the production of oysters (*Crassostrea gigas*) in France for animal health purposes is not new. Occasional pathological examinations over more than 20 years allow one to believe that France is probably free from some listed diseases, such as *Perkinsus marinus* or *Mikrocytos mackini*. Sampling in recent years has mainly been concerned with abnormal mortalities, and in this context samples have been taken twice a year for each zone (Thébault, 1999). These zones were originally defined for the study of *Bonamia* and *Marteilia* in *Ostrea edulis* (Thébault, 1999). To evaluate the quality of such sampling and to certify quantitatively that *Crassostrea gigas* production in France is free from exotic disease, it was necessary to study the sampling strategy of the French disease surveillance network.

The *International Aquatic Animal Health Code* of the Office International des Epizooties (OIE) (2001) and the corresponding *Diagnostic Manual for Aquatic Animal Diseases* (Office International des Epizooties, 2000) provide a sampling strategy to substantiate freedom from various infections in a particular zone. For molluscs, it is stated that at least three sampling points must be

selected and, whenever possible, one of the three samples must be sourced from natural beds. This must occur once a year for *P. marinus* and twice a year for *M. mackini*. The sample size must be maintained at 150 oysters to ensure the detection of pathogen carriers at a prevalence of 2% with a 95% confidence level (Office International des Epizooties, 1997). Several assumptions were made for these calculations, as follows:

– that the sampled population was infinite

– that the screening procedure used was perfect

– that a cluster level was not taken into account.

The first assumption is acceptable for marine molluscs, because sampling of 30 or 150 animals usually corresponds to sampling fractions of less than one to 1,000. The second assumption is probably not correct because the reference diagnosis test employed is histological analysis. The sensitivity of this test depends principally on the following factors:

– the quality of the slide

– the part of the body from which the sample is taken

– the level of infestation of the animals sampled

– the ability of the scientist to recognise an exotic agent.

A first evaluation of the detection of *Marteilia refringens* by histological examination, using Gibbs sampling (Poulliot and Gerbier, 2000), showed that its sensitivity was between 60% and 80%, while its specificity was more than 99.5% (Thébault *et al.*, unpublished data). Since *M. refringens* is an endemic disease in France, researchers expect less accurate results when testing for exotic diseases, especially for sensitivity.

The third assumption, i.e. not taking the cluster level into account, is believed to be inappropriate for French shellfish production, because animal management practices can have an important impact on the prevalence of infection within clusters. The authors believe that results from surveys conducted on natural beds cannot be interpreted in the same way as those from surveys on farmed animals. Thus, there was a need to adapt survey methodologies for wild and cultured animals. As farmed animals form the major part of commercial oyster production, and as they are more likely to undergo exchanges or movements, they were the target population of this study.

A zone was defined as a single hydrological area including several thousand leases, with different breeders and animal management practices. Each lease can be considered as a single cluster because animals are more similar within a cluster in terms of historical movements, animal origin and age, and animal management practices.

However, surveys of farmed *C. gigas* within a single zone are limited by the frequent movement and mixing of animals between zones, which makes sampling representative groups of animals difficult.

Over recent years, methods have been developed to help plan animal health surveys. FREECALC is a computer program for sample size calculations (Cameron and Baldock, 1998b), which takes into account the herd level and the results of the screening tests, and is applicable when the sampled population is infinite. Audigé and Beckett (1999) developed a stochastic simulation model which can be used to interpret animal health survey sampling to substantiate freedom from disease. This approach can easily be used to examine the effects of the variability and uncertainty of influencing parameters, such as screening test characteristics. An updated version of this model has been published recently (Audigé *et al.*, 2001). The pathogens *P. marinus* and *M. mackini* are listed by the OIE and are exotic to the French colonies of *C. gigas*. These pathogens can cause mass mortalities in oysters (Andrews, 1988; Farley *et al.*, 1988). The species *C. gigas* is less sensitive than *C. virginica*, but *C. gigas* can be a carrier for these two pathogens. Variation of infection prevalence is associated with variation of temperature and salinity (Bower *et al.*, 1997; Chu, 1996). Adults or juveniles of *Crassostrea gigas* seem more sensitive to these pathogens (Farley *et al.*, 1988), so this study was limited to oysters older than 12 months.

The French national surveillance network for these pathogens can support screening of between 900 and 2,000 animals each year, which limited the number of samples which could be analysed for a given survey. Typically, samples of 150 animals were taken in case of abnormal mortalities and, occasionally, samples of

30 animals per lease were removed for routine surveys.

The aim of this study was to assess if the existing surveillance network was sufficient, for both abnormal mortalities and routine surveys, to provide a sampling strategy to substantiate, with a certain level of confidence, that France was free from those two infections. In addition, the authors illustrate the use of stochastic modelling for the planning of aquatic disease surveys.

Materials and methods

In this study, the authors adapted the model presented by Audigé and Beckett (1999) to this specific problem. Variable inputs and outputs are described in Table I and the structure of the model is shown in Figure 1. The model was written in Microsoft Excel (Microsoft, Redmond, Washington, USA) and simulated using @Risk (Palisade, Newfield, New York, USA). For each simulation the authors used 1,000 iterations and Latin hypercube sampling.

As a first step the authors made the following assumptions.

– Each lease was considered as a cluster.

– The number of animals sampled per cluster was 30 animals for a routine survey and 150 animals in the case of abnormal mortalities, i.e. as currently performed.

– Since each lease contained several thousand adult oysters, it could be considered an infinite population. The Hypergeometric distribution used to estimate the number of infected animals which were expected to be sampled from infected clusters was replaced by a Binomial distribution.

– Two scenarios for the within-cluster prevalence of infection were compared, i.e. with 10% and 20%, respectively.

– Individual test sensitivity was modelled using a Betapert distribution with parameters set at: (minimum value = 0.5; most likely value = 0.7; maximum value = 0.8), while specificity was set at 0.999. The latter assumption was based on the results of several years of surveys, with no detection of targeted pathogens in several thousand animals sampled, as well as on expert opinion (F. Berthe, personal communication), and the results of histological examination for *M. refringens*. The authors believe that the choice of such a high specificity value is reasonable because histology is a direct examination of the disease agent itself (several parasites must be seen to give a positive test result), and different experts always confirmed suspicious cases.

– The number of clusters sampled was chosen as 30, 60 and 100, respectively, to remain below the analytical capacity of the laboratory network.

– The number of clusters of *C. gigas* was obtained from the official institute Direction des Pêches et des Cultures Marines. In 1999, there were approximately 30,840 leases.

– The cluster infection prevalence was considered as being 5%, 10% and 20% for three separate simulations, respectively.

– The cut-off number for individual animals returning positive results when tested was set at 1, when at least two animals were found positive in a cluster, and this cluster was considered as positive. This choice maximised herd-level test sensitivity compared with a higher cut-off number, but was also associated with reduced specificity. This was justifiable, however, because the specificity of the histological examination was assumed to be very high.

– Sensitivity and specificity distributions at cluster and survey level were modelled, using a Beta distribution.

In addition, the authors investigated the impact of the variability and uncertainty of the individual-animal test characteristics on the model outputs. This example illustrated the difficulty often encountered in obtaining accurate data on individual test characteristics.

A first sensitivity analysis was conducted on the number of positive individual-animal tests expected from infected clusters. The Spearman rank analysis performed in @Risk was used to identify the input parameters which most correlated with the output. Three consecutive simulations were conducted with the following specifications for the sensitivity of the individual-animal test, as follows:

– as specified above

– with a wider range of values, from 0.2 to 0.8, with a Betapert distribution of (0.2, 0.7, 0.8)

– with a uniform distribution from 0.5 to 0.8 instead of the Betapert distribution.

Table I

A model aimed at planning and assessing aquatic animal health surveys: description of model inputs and output

Description of variables	Notation	Formula used with @Risk
True individual test sensitivity	IndTSens	RiskPert (0.5, 0.7, 0.8)
True individual test specificity	IndTSpe	0.999
Estimated cluster size		50,000
Within-herd infection prevalence estimation	Prev	0.1 or 0.2
Number of animals sampled per herd	n	150 or 30
Number of infected animals expected in the infected cluster sample	inf	RiskBinomial (n, Prev)
Number of positive individual tests expected from infected clusters	pos	IF (inf > 0, RiskBinomial (inf, IndTSens), 0) + IF(n – inf,1 – IndTSp), 0)
Number of negative individual tests expected from non-infected clusters	neg	RiskBinomial (n,1 – IndTSp)
Number of simulations used to derive the probability distributions of 'pos' and 'neg'	Iter	
Number of simulations giving a value of 'pos' above a cut-off value C	Cpos	
Number of simulations giving a value of 'neg' below or equal to a cut-off value C	Cneg	
True cluster-level test sensitivity	CluTSens	RiskBeta (Cpos + 1, Iter – Cpos + 1)
True cluster-level test specificity	CluTSpe	RiskBeta (Cneg + 1, Iter – Cneg + 1)
Number of clusters in the population		30,840
Cluster infection prevalence	CluPrev	10% or 20%
Number of clusters sampled	N	30, 60 or 100
Number of infected clusters sampled if the country is infected	INF	RiskBinomial (N, CluPrev)
Number of positive clusters expected if the country is infected	POS	IF (INF > 0, RiskBinomial (INF, CluTSens), 0) + IF(N – INF > 0, Risk Binomial (N – INF, 1 – CluTSp), 0)
Number of positive clusters expected if the country is free from infection	NEG	RiskBinomial (N, 1 – CluTSp)
Number of simulations used to derive the probability distributions of 'pos' and 'neg'	ITER	
Number of simulations giving a value of 'pos' above a cut-off value x	XPOS	
Number of simulations giving a value of 'neg' below or equal to a cut-off value x	XNEG	
Survey sensitivity	ClutSens	RiskBeta (Cpos + 1, Iter – Cpos + 1)
Survey specificity	Clutspe	RiskBeta (Cneg + 1, Iter – Cneg + 1)

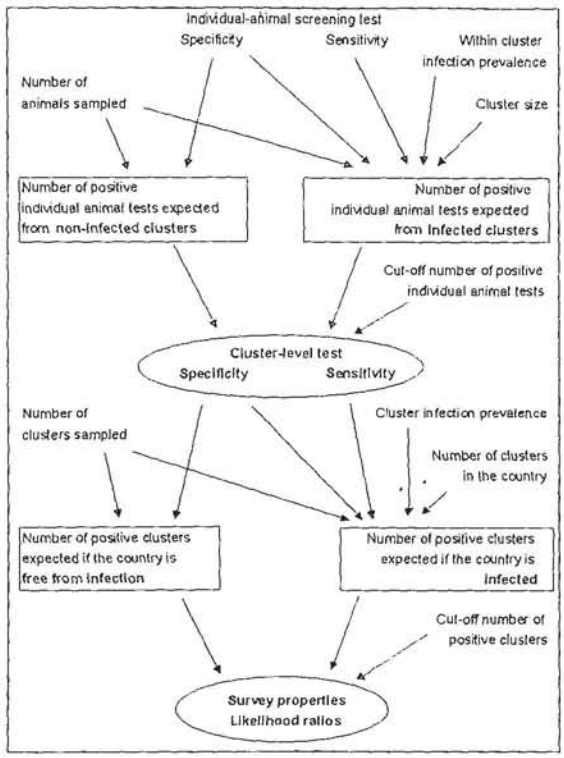

Figure 1
Structure of a model adapted to assist in planning and assessing aquatic animal health surveys
(with special authorisation from *Preventive Veterinary Medicine*, Elsevier)
(Audigé and Beckett, 1999)

A second sensitivity analysis was conducted to assess the effects of different values of individual sensitivity on the cluster and survey level results. The specificity of the individual test was assumed to be high, as specified above. Four simulations of 1,000 iterations each were conducted with sensitivity values of 0.5, 0.6, 0.7 and 0.8, respectively. Sampling of 60 clusters with 30 animals each and a prevalence of infection within herds and between herds of 10% were examined.

Results

Abnormal mortalities

Results are presented only at the cluster level. The output probability distributions of the expected numbers of individual-animal tests returning positive results from non-infected and infected clusters, considering the two levels of infection prevalence of 1% and 5%, are presented in Figure 2.

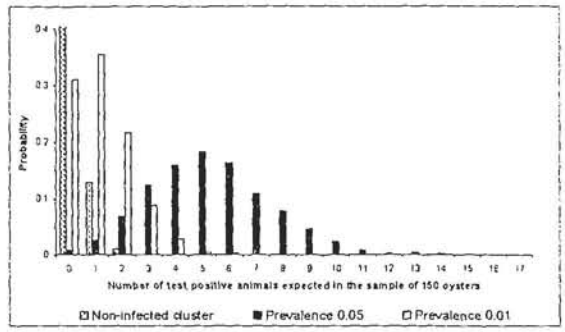

Figure 2
Surveying French populations of *Crassostrea gigas* for disease: probability distribution of the number of animals expected to give positive results from a sample of 150 oysters

In this simulation, using 1,000 iterations (309 iterations at the 1% prevalence level and 8 iterations at the 5% prevalence level) resulted in no oysters testing positive for the presence of the disease agents. At a prevalence of 2%, 103 iterations showed no oysters giving positive test results. The authors used these data to model a cluster-level test of sensitivity and specificity at 2%, using Beta distributions Beta (898, 104) and Beta (991, 11), respectively.

Routine survey of leases without abnormal mortality

Cluster-level testing (involving the sampling of 30 animals)

The output probability distributions for the expected numbers of positive individual-animal tests from non-infected and infected clusters, taking into account an infection prevalence of 10% within infected clusters, are given in Figure 3. In this simulation, also using 1,000 iterations, with a cut-off value of 1, one iteration showed a positive result from non-infected leases, and 369 iterations showed negative results from infected leases. The authors used these data to model a cluster-level test of sensitivity and specificity using Beta distributions Beta (632, 370) and Beta (1,000, 2), respectively.

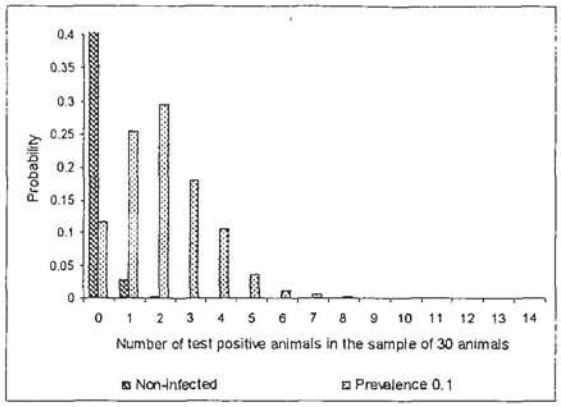

Figure 3
Surveying French populations of *Crassostrea gigas* for disease: probability distribution of the number of animals expected to give positive results from a sample of 30 oysters

Survey level: thirty samples of thirty animals

The output probability distributions of the expected numbers of positive clusters from a non-infected country versus those from an infected country, considering cluster infection prevalences at 10% and 20%, respectively, are given in Figure 4. In this simulation, using 1,000 iterations, with a cut-off value of 1, six iterations showed positive results from non-infected clusters, while 393 and 89 iterations showed negative results from infected clusters at 10% and 20% levels of prevalence, respectively. With these results, the survey sensitivity and specificity can be modelled using Beta distributions of Beta (995, 7) and Beta (608, 394) at the 10% cluster prevalence, respectively.

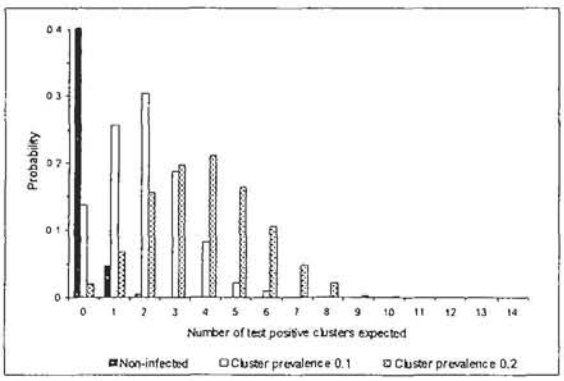

Figure 4
Surveying French populations of *Crassostrea gigas* for disease: survey results when 30 animals are sampled and 30 clusters are also sampled

Survey level: sixty samples of thirty animals

The output probability distributions of the expected numbers of positive clusters from non-infected and infected countries, at cluster prevalences of 10% and 20%, are given in Figure 5. In this simulation, using 1,000 iterations, with a cut-off value of 1, 8 iterations showed positive results from non-infected clusters, whereas 90 iterations showed negative results from infected clusters at a prevalence of 10%, and 8 iterations at a prevalence of 20%. The survey specificity can be estimated at the survey level by a Beta (993, 9) distribution, the survey sensitivity by a Beta (911, 91) distribution at a prevalence of 10%, and a Beta (993, 9) distribution at a prevalence of 20%.

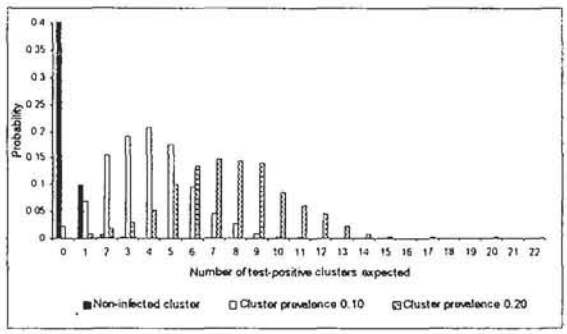

Figure 5
Surveying French populations of *Crassostrea gigas* for disease: survey results when 30 animals are sampled and 60 clusters are sampled

Survey level: one hundred samples of thirty animals

The output probability distributions of the expected numbers of positive clusters from the non-infected and infected country, at a cluster prevalence of 10% and 5%, are given in Figure 6. In this simulation, using 1,000 iterations, with a cut-off value of 1, 23 iterations showed a positive result from non-infected clusters. In addition, 147 iterations showed negative results from infected clusters at a prevalence of 5% and 12 iterations at a prevalence of 10%. The survey specificity can be estimated at the survey level by a Beta (978, 4) distribution, and the survey sensitivity with a Beta (989, 13) distribution at the 10% prevalence level, and a Beta (853, 148) distribution at 5% prevalence.

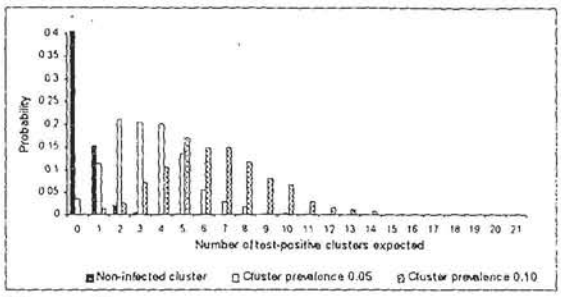

Figure 6
Surveying French populations of *Crassostrea gigas* for disease: survey results when 30 animals are sampled and 100 clusters are sampled

Descriptive statistics of Beta distribution data for survey-level sensitivity and specificity, in the different sampling schemes, with prevalence within and between clusters of 10%, are shown in Table II.

Sensitivity analysis at a prevalence of ten percent

The number of positive individual-animal tests expected from infected clusters was correlated with the sensitivity of individual-animal tests, using a Spearman rank correlation of 0.102. This correlation increased slightly if uncertainty about the sensitivity also increased, as shown by the use of either a uniform distribution (instead of the Betapert), or a wider range of possible values for the sensitivity, Spearman rank correlation of 0.229 and 0.233, respectively.

The number of iterations showing negative results from infected clusters at the cluster level, with a cut-off value of 1, varied with the sensitivity of individual-animal tests. For the four specified distributions of individual-animal tests, with a sensitivity of 0.5 to 0.8, the sensitivity at the cluster level varied from 528 to 300, respectively, using 1,000 iterations. The distribution of positive animals is shown in Figure 7. The number of iterations showing negative results from surveys conducted in an infected country, at the survey level, with a cut-off value of 1, varied with the sensitivity of individual-animal tests from 194 to 59. The distribution is shown in Figure 8.

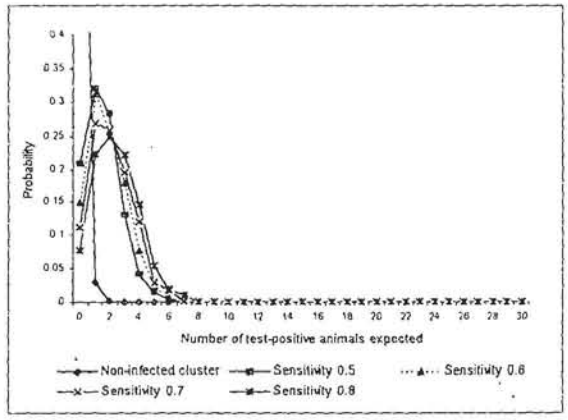

Figure 7
Surveying French populations of *Crassostrea gigas* for disease: uncertainty about sensitivity when disease prevalence within the cluster is 0.1

Table II
Sensitivity and specificity at the survey level with different sampling at the cluster level, with a prevalence level of 10% within clusters and between clusters: @RISK simulation

Name	Specificity 30	Sensitivity 30	Specificity 60	Sensitivity 60	Specificity 100	Sensitivity 100
Description	Beta (995, 7)	Beta (608, 394)	Beta (993, 9)	Beta (911, 91)	Beta (978, 24)	Beta (989, 13)
Minimum	0.98	0.56	0.98	0.88	0.95	0.97
Maximum	0.99	0.66	0.99	0.93	0.99	0.99
Mean	0.99	0.60	0.99	0.91	0.97	0.98
Standard deviation	2.62E-03	1.55E-02	2.97E-03	9.07E-03	4.85E–03	3.56E–03
Kurtosis	3.58	3.08	3.46	3.01	3.44	3.26
Mode	0.99	0.60	0.99	0.91	0.97	0.98
5% percentile	0.99	0.58	0.98	0.89	0.97	0.98
95% percentile	0.99	0.63	0.99	0.92	0.98	0.99

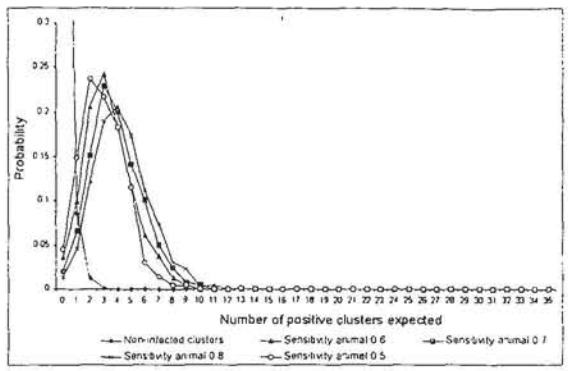

Figure 8
Surveying French populations of *Crassostrea gigas* for disease: uncertainty about animal sensitivity when disease prevalence in clusters is 0.1

Discussion

In this paper, the authors considered a novel approach for the planning and evaluation of a survey of infections in aquatic animals. The recently developed stochastic model presented by Audigé and Beckett (1999) was used. In the opinion of the authors, this model presents some advantages over alternative models, such as that of Cameron and Baldock (1998a, 1998b). An important enhancement is that the model of Audigé and Beckett (1999) allows one to use probability distributions for individual-level screening test characteristics, and within-cluster and cluster infection prevalences. This model also allows researchers to choose cut-off values at the cluster and survey level. The variation of infection prevalences derived from the use of histological examinations (i.e. the currently accepted gold standard test) in infected countries is not known for *M. mackini* and *P. marinus* in *C. gigas*. With more data this model could be amended, using more realistic distribution probabilities for within-cluster and cluster infection prevalences.

The choice of the cut-off values is associated with high cluster-level and survey sensitivities, while the specificity was very high. The individual-animal test specificity was derived from field experience, which is believed to be appropriate without validation data. Audigé *et al.* (1999) also used field data from previous surveys to assess individual-animal test specificity.

The authors acknowledge that, without validation data, the choice of sensitivity and specificity values was somewhat subjective. In addition, the detection of an endemic disease agent, such as *M. refringens* in France, differs from that of an exotic disease agent. Data concerning the characteristics of histological examination for the detection of *M. mackini* and *P. marinus* were missing, and therefore the authors relied on expert opinion. In the detection of *P. marinus*, the validity of histological examination depends on the following:

- staff training
- the level of infestation (number of parasites) by slide
- the life cycle stage of the parasite.

However, these data were not available. If validation data become available, the true sensitivity and specificity of the diagnosis test can be modelled by a Beta distribution (Vose, 1996).

The sensitivity analysis of the authors allowed assessment of the effects of uncertainty about the individual-animal test sensitivity for the cluster-level and survey sensitivity. However, the Spearman rank test relates the sensitivity specification to the number of test-positive animals expected, and not directly to the cluster-level sensitivity. The change in cluster-level sensitivity is reflected by the variation of the output distribution around the chosen cut-off point. Although difficult to quantify, the results of the authors show that a high level of uncertainty in individual-animal sensitivity is associated with a lower overall cluster-level sensitivity.

The impact of the uncertainty of the test sensitivity (as reflected in the distribution used) on the cluster-level sensitivity appears less important if the within-cluster prevalence considered is higher, or if the sample size is increased, since the authors obtained good values of sensitivity at the cluster and survey level. This uncertainty could be reduced through additional information (Hattis and Burmaster, 1994), using knowledge of the real sensitivity and specificity of these exotic diseases. Better information on the prevalence levels in infected countries would help to improve the accuracy of the input data.

In this study, the authors did not assess the impact of the individual-animal test specificity on the survey characteristics because they were confident that the specificity of the histological

examination was very high. However, it is recognised that this impact can be very high when there is uncertainty about the specificity value. Some positive test results in infected clusters are consequences of this lack of specificity. While test sensitivities at the cluster and survey level are known to increase with the lack of test specificity at the individual-animal level (Sanaa *et al.*, 1994), it is at the cost of lower cluster-level and survey specificity. Therefore, when increasing the sample size to account for a low test sensitivity or infection prevalence, whether at the cluster or survey level, the cut-off must be chosen to minimise the negative impact of low test specificity.

During sampling for abnormal mortalities, taking into account realistic values of sensitivity and specificity, sampling 150 animals per cluster does not appear to be sufficient to detect a prevalence of 2%, with a confidence level of 95%. In mollusc aquaculture, massive mortalities are rapid and, when the sample is feasible, it applies only to the few survivors. That is why one expects low prevalences in cases of abnormal mortalities. Sometimes one can sample not only at the focus of the massive mortality, but also at the periphery, where mortalities and prevalence could be different. It would sometimes be more useful to take different samples at different levels of mortality, to detect a higher within-cluster prevalence in those subgroups.

During sampling for a routine survey, 100 samples of 30 animals seem sufficient to detect a 10% cluster prevalence, with a 10% prevalence within infected clusters. It is more interesting to sample more clusters rather than animals inside the cluster, because the within-cluster prevalence could be different from the history of the individual oysters. However, the authors did not consider this in the model, because quantified data for this phenomenon were not available.

This paper addresses some important issues related to the sampling of cultured oysters, but by no means all of them. However, there are other factors to consider. For instance, the sampling strategy of leases, which would also take into account such factors as the relative part of production between areas in France. The number of leases in each area of France is known. If 40% of leases of production of *C. gigas* are located in one area, 40% of the samples should be taken in this area to describe the production. Other factors, such as the age of the animals and the frequency of sampling, should also be taken into account. These additional considerations would optimise the detection process as recommended by the OIE *Code*. In addition, factors such as the history of the surveillance of *C. gigas* (i.e. about 20 years of sampling), movements of oysters between European countries and surveillance efforts among European countries could be taken into account, using the approach presented by Audigé *et al.* (1999). For this latter approach, which is still under development, accurate data are currently not available. Other specific strategies must consider natural beds and hatcheries.

While few accurate data were available, the modelling approach used in this study attempts to mimic a realistic farming environment/ situation. As a result, it highlighted an important area where specific data are missing and, therefore, can help to support decision-making directed towards further studies and research on marine mollusc aquaculture.

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