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Risk factor analysis for "diagnosis not reached" results from bovine samples submitted to British veterinary laboratories in 2013 to 2017

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Abbreviations:

APHA: Animal and Plant Health Agency. DNR: Diagnosis not reached. DR: Diagnosis reached. SAC: Scottish Agricultural College. SRUC: Scotland's Rural College. VPF: Veterinary Post-mortem Facilities. VIO: Veterinary Investigation Officer. VIDA: Veterinary Investigation Diagnosis Analysis.

ABSTRACT

Routine diagnostic data from laboratories are an important source of information for passive animal health surveillance. In Great Britain, the Veterinary Investigation Diagnosis Analysis (VIDA) database includes records of diagnostic submissions made to a nationwide network of 28 veterinary post-mortem facilities (VPFs). Data on "diagnosis not reached" (DNR), i.e. where submissions do not lead to a confirmed diagnosis, are analysed quarterly to look for unexpectedly high incidences of DNRs which could indicate the presence of a new or emerging disease in British livestock populations. The objective of the present study was to provide a better understanding about the reasons of DNR occurrence and to inform improvements of the coverage and reporting of this kind of surveillance data.

A subset of the VIDA database comprising diagnostic submissions from cattle received from 2013 to 2017 (122,444 records) was analysed. A mixed-effects multivariable logistic regression model, accounting for clustering by farm and county, was used to investigate associations between potential predictors and DNR. The variables included in the model were: VPF identity, animal sex, age, production purpose, main presenting sign of the animal from which the sample was obtained, and sample submission type.

The variable that showed the strongest association with DNR was the main presenting sign of the animal, followed by submission type, VPF identity, animal age, sex, and production purpose, in that order. Submissions from animals with abortion as the main clinical sign had the highest odds ratio (OR 21.6, 95 % confidence interval [CI] 19.6-23.9, with mastitis taken as the baseline). Submissions where neither carcasses (i.e. a whole dead animal provided for post-mortem examination) nor foetuses (i.e. an

unborn dead animal) were provided had approximately 12 times the odds of being DNR, compared to submissions of a carcass (OR 11.6, 95 % CI 10.7-12.5). In addition, submission type and main presenting sign can be considered as important confounders in the association between the other predictors and DNR.

This study has helped characterise DNR occurrence and suggests some possible improvements that could be made to the passive surveillance system investigated, such as encouraging greater carcase submission, accounting for identified issues when interpreting increased occurrence of DNR and further investigating reduced submissions or greater DNR occurrence in some geographical regions.

Word count: 366

Keywords: Submission; Cattle; Diagnosis not reached; Passive surveillance.

INTRODUCTION

Animal health surveillance – the ongoing systematic collection, analysis, and interpretation of data and the dissemination of information to those who need to know in order to take action – is intended to ascertain the presence or distribution of health hazards. It is necessary for the planning, implementation, and evaluation of the different interventions designed to mitigate risks (Hoinville et al., 2013). Surveillance can be classified as active or passive, depending on the means by which data are collected. Active surveillance is designed and initiated by the competent bodies, i.e. the primary users of the data; and passive surveillance uses data collected for other purposes or by other people, such as disease notifications or laboratory records, among others (Food and Agriculture Organization, 2014).

Passive surveillance constitutes the core activity for detecting new or emerging diseases (Doherr and Audige, 2001; Rodriguez-Prieto et al., 2015). Nevertheless, passive surveillance has limitations because disease reporting (or suspicion thereof) depends on a wide range of factors including clinical presentation of the disease, willingness of farmers and/or veterinarians to submit samples for laboratory confirmation, and the value of the animal/s affected (APHA, 2016). As a result, several initiatives have been implemented in an effort to improve its performance (Dórea et al., 2011).

One of these initiatives is based on the analysis of submissions associated with diagnosis not reached (DNR). This can be considered as a type of syndromic surveillance (Dórea et al., 2011), where clinical signs and laboratory results constitute the data. The contribution of this form of surveillance has been demonstrated in different scenarios such as detecting a disease outbreak in pig populations (O'Sullivan et al., 2012b) and in determining that some unexplained epidemiological events were not new diseases (Gibbens et al., 2008).

In Great Britain (GB), DNR events are analysed quarterly to detect abnormal patterns based on main presenting signs and body systems affected. Both trends are compared over time to detect new and re-emerging diseases (APHA, 2018). However, two prior internal reports at the Animal and Plant Health Agency (APHA) identified a need for further epidemiological analysis, to allow for a better understanding of DNR occurrence, and thus to get a better management of the passive surveillance approach (APHA, personal communication). Therefore, this study aimed to investigate potential risk factors for a better characterisation of DNR submissions. Such information could help to improve this passive surveillance system.

MATERIALS AND METHODS

The analysis was carried out using data on cattle submissions from the Veterinary Investigation Diagnosis Analysis (VIDA) database. This collects diagnostic data from: i) Veterinary Investigation Centres of the APHA, ii) the APHA's post-mortem examination providers, iii) the Scotland's Rural College (formerly Scottish Agricultural College [SAC]) disease surveillance centres and iv) the SAC for Post Mortem Examination (SACPME), which are all hereafter referred to as veterinary post-mortem facilities (VPFs). Submissions are made by veterinary practitioners on behalf of farmers, and the diagnostic service is partly subsidised by the Department for Environment, Food and Rural Affairs (DEFRA).

Cattle were selected as the study species for two reasons. Firstly, a pilot study conducted in 2005 on cattle data from England and Wales highlighted several quality issues in the dataset that limited its epidemiological value at that time. Several improvements have been implemented since then, such as changes in the recording of data and an effort to avoid missing data by informing veterinary practitioners about the need for data collection (Hyder et al., 2011). We therefore took advantage of an opportunity to incorporate data from GB and over a longer period of time, to assess the impact of the aforementioned improvements. Secondly, data available on surveillance of cattle populations tend to be much more complete than for other species (APHA, personal communication). Probably because this species attracts the greatest amount of surveillance expenditure (Drewe et al., 2014), and because there has been a need to improve surveillance for detecting new diseases, especially after the unexpected outbreak of the bovine spongiform encephalopathy (BSE) in the 1980s (Gibbens et al., 2008).

Study population

The study population comprised all those GB cattle farms where samples had been submitted for diagnostic purposes to VPFs during the study period.

An approximate estimation of the total number of herds in GB was obtained from the Cattle Tracing System (CTS), which records data on cattle births, deaths and movements. The annual number of herds was 76,043 in 2013, 75,249 in 2014, 74,334 in 2015, 73,253 in 2016 and 69,326 in 2017. The percentage of these farms that used the passive surveillance system during the study period was considered as an indication of the participation in the system per year. This percentage was estimated as the annual number of farms making at least one submission to any of the VPFs, divided by the total farm population estimated for the same year.

Data source and data handling

An extract of VIDA data containing all relevant information on those samples submitted for diagnostic purposes from GB cattle herds during the period 2013 to 2017 was collected and anonymized using the protocols of APHA. The dataset consisted of 27 variables describing 122,444 submissions. A submission was defined as a sample (or group of samples) from one or more animals, which was collected for the same diagnostic purpose at the same time and from the same farm. Thus, a submission consisted of one data line that could represent one or more animals/samples.

Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and R Version 3.5.0 (The R Foundation for Statistical Computing) were used for data handling and analysis.

Data formatting was carried out before analysis. Variables with a substantial proportion of missing values (≥ 50% of the data lines) were omitted from further analyses. The decision to exclude variables was not based on a threshold but on the type of variables, which made them difficult to handle using missing data methods and the lack of auxiliary variables which can help in the use of those methods. They were mainly quantitative: “age in days”, “number affected in the group”, “number affected dead”, “total number of animals affected”, “number of animals affected in the herd” and “duration of illness”. When one or more variables addressed similar questions, the most accurate one was selected following the advice of APHA staff (e.g. “main presenting sign” instead of “syndrome”).

For age and sex variables, a “mixed” category reflected a mixture of animals with different age or sex included in the submission. For variable production purpose the category “other” included the following: pet, captive, zoological and wild.

Furthermore, in some variables (sex, age and purpose) some of the categories were insufficiently defined (e.g. “none”, “notapp”, “unknown”, “na”), making them difficult to interpret. Missing values were present too. These undefined categories were analysed to identify patterns of their occurrence and to establish whether their exclusion could bias the results. Since no evidence of patterns was found, these undefined categories were evenly distributed throughout DNR and DR (diagnosis reached) records, they were grouped together under the “unknown” label. It would have been difficult to draw biological conclusions about a possible association between

DNR and the "unknown" category. Thus, records classified as "unknown" were excluded and omitted from both the general and the univariable analysis on a per variable basis, except for "main presenting sign", as this reflected a submission with an unclear symptomatology whose potential relationship with DNR was particularly interesting to explore.

The final set of variables considered as potential predictors for the occurrence of DNR, based on their biological plausibility, were: "main presenting sign", "VPF", "year" (in which submission was received), "submission type", "number in submission" (number of animals contributing to that submission), "age category", "sex", "production purpose" and "region".

A submission was classified as DNR if a diagnosis was not reached despite reasonable testing (i.e. those submissions investigated at a level that a diagnosis would be expected to be achieved); or if limited testing had been carried out. For example, due to insufficient sample volume or because not all the range of tests available for the diagnosis of a particular condition was required by the submitting veterinarian. When a submission is categorised as DNR, Veterinary Investigation Officers (VIOs) are required to select a reason from a picklist (Hoinville et al., 2008) and this information is recorded in the variable "opinion of the VIO on why no diagnosis was reached". This variable was investigated, and an initial descriptive analysis was carried out to explore the reasons for DNR. Otherwise a submission was classified as DR. Other categories within the DR variable such as "na", "none" or missing values were excluded from the analysis.

Data analysis

Summary statistics were calculated for each variable and an initial descriptive analysis of the dataset was performed.

To account for the clustering of the submissions at farm level and for spatial clustering, a mixed-effects model was set to reduce bias in standard error, confidence intervals (CIs) and p-values. Farm and county were modelled as random effects.

A univariable mixed-effects logistic regression was conducted between DNR and the predictors of interest. For each variable, the category selected as reference was the one with the lowest frequency of association with DNR.

Variables with a likelihood ratio test (LRT) of $p<0.2$ on the univariable test were taken forward into the multivariable analysis. In the case of "year" (the only numerical variable further investigated), the LRT and the goodness of fit metric, Akaike's Information Criterion (AIC), were used to check for linear trend.

The full model explored in the analysis was:

$$\mathbf{y = \beta_0 + \beta_1(VPF) + \beta_2(Presenting\ sign) + \beta_3(Submission\ type) + \beta_4(Age\ category) + \beta_5(Sex) + \beta_6(Production\ purpose) + \beta_7(Year) + \beta_8(Region) + u_{farm} + u_{county} + \varepsilon}$$

where y was the outcome variable, β_0 was the intercept. β_1, β_2, β_3, etc. were the coefficients of the corresponding explanatory variable, u_{farm} and u_{county} were the random effects at farm and county level respectively, and ε was the random variation at submission level.

In all variables except "number in submission", statistical evidence of association was found, so all were included initially in the model. Using a backwards stepwise procedure, variables were excluded from the final model using a statistical significance of 5% in the LRT and the goodness of fit metric (AIC) and/or when removing the variable did not alter the odds ratio (OR) of the other variables by more than 20%. The analysis was then repeated using a forward selection, starting with the variable with the lowest AIC. It was not possible to fit any biologically plausible interaction into the final model due to data limitations.

LRT and goodness of fit metrics were also used to compare the mixed-effects models and the standard logistic regression model. The Wald tests were used to examine the significance (p value<0.05) of the variables retained in the final model, particularly for those with multiple categories (such as VPF), and a variance inflation factor was computed to assess collinearity among the predictor variables (Dohoo et al., 2009) in the final model.

Sensitivity analyses considering the initially excluded records with missing data

Sensitivity analyses including other potential scenarios was carried out to determine whether the addition of records with missing data would have affected the model. The two sets of analyses were made using: 1) the “unknown” categories for the predictors “age category” (17,015 submissions), “sex” (16,392 submissions) and “production purpose” (10,093 submissions) in the full model; and 2) the categories “na”, “none” or missing values included in the DR variable, as if they were DNR submissions in the full model (2,175 submissions).

RESULTS

Study population

A total of 28,870 farms submitted samples during the study period. The median number of submissions per farm was 2 (minimum: 1; maximum: 1,213 submissions per farm). An analysis of the size of the herd and number of submissions in those farms where herd size was available, revealed evidence of a positive correlation between both variables (p-value <0.0001). The Pearson's correlation coefficient was 0.30, thus the strength of the correlation found was weak.

Of the 122,444 submission records in the dataset, 120,269 had either a record of DR or DNR and these were taken forward to the full analysis. The proportion of farms that submitted samples for laboratory diagnosis during the study period gradually reduced from 19.5% (14,829/76,043) in 2013 to 17.0% (12,826/75,249) in 2014, 14.3% (10,611/74,334) in 2015, 13.0% (9,544/73,253) in 2016, and 11.9% (8255/69,326) in 2017. Whereas the proportion of DNR submissions was very similar during the study period: 62.3% (21,037/33,761) in 2013, 64.2% (18,285/28,469) in 2014, 64.6% (14,411/22,309) in 2015, 63.7% (12,296/19,294) in 2016 and 61.7% (10,135/16,436) in 2017.

Descriptive statistics and univariable analysis

Table 1 shows summary statistics and results from the univariable analysis, including crude OR of 113,267 submissions in the variables "VPF", "submission type", "main presenting sign" and "year received grouped" (excluding missing values and 'unknown' answers for "farm identifier" and "county"), of which 71,750 (63.3%) were DNR. In the rest of the variables, the number of submissions varied due to the presence of missing data.

The VPF with the highest percentage of DNR submissions was “Winchester” (76.0%, 1,714 submissions). Regarding clinical signs, the highest percentage of DNR submissions was “abortion” (83.7%, 14,792 submissions). The type of sample with the lowest percentage of DNR was “carcass” (18.3%, 1,494 submissions) while “other” was the category with the highest number of DNR submissions (67.2%, 100,529). A complete description of these and the other variables is shown in Table 1.

In the univariable analysis, evidence of association was found between all of the variables explored and DNR ($p < 0.001$, Table 1), except for the “number in submission” variable (p-value 0.583), and were added into the initial multivariable model. The use of a categorical variable for “number in submissions” was also tested but the results were inconclusive. In the case of “year”, a LRT was performed to check for a linear trend and the goodness of fit metric was explored, but it was not the best fit for the variable. Furthermore, a categorical variable for different groups of years was created and tested, providing a better model fit than a variable with categories for each year.

Information about why no diagnosis was reached

The analysis of the variable “Veterinary Investigation Officer’s opinion on why no diagnosis was reached” (Figure 1) suggested that the majority of DNR submissions was caused due to “complete diagnostic package not requested” (23,901 submissions) followed by “incomplete sample range submitted” (21,521). “Other” (10,630 submissions) included a wide range of reasons such as insufficient animals tested, inappropriate disease phase or unsuitable sample(s) provided.

Multivariable analysis

The multivariable model contained 81,191 submissions with no missing data in any of the variables included. In the mixed-effects logistic regression model (which included "VPF", "main presenting sign", "submission type", "age category", "sex", "production purpose", "year" and "region"), a high variable inflation factor was found in "VPF" and "region" indicating a high degree of collinearity. So "region" was omitted from the final model, since the AIC was better in the model with VPF. A better fit was obtained with the model including both "farm" and "county" as random effects in comparison to a fixed effect model, or with the model including only one of the variables, i.e. "farm" or "county" as random effect.

Table 2 shows the results from the mixed-effects logistic regression model. After adjusting for the rest of predictors, there was no evidence of an association between year and the outcome. No effect on the estimates of the other variables was found. Furthermore, the model without year of submission provided a better fit to the data and year was not associated with the outcome, so year was excluded from the full model. Evidence of association was found between the rest of the variables and DNR ($p<0.05$). "Main presenting sign" had the highest estimated OR of all the variables studied. Those submissions with "abortion" as the main clinical sign recorded had a significantly higher risk of being DNR compared to "mastitis" (the condition with the lowest percentage of DNR submissions which was set as the baseline) (OR= 21.6, 95 % CI 19.6-23.9).

"Submission type" was the variable with the second highest OR value, "other" and "foetus" had a higher risk of DNR compared to "carcass" (OR=11.6, 95 % CI 10.7-12.5

and OR=1.45, 95 % CI 1.28-1.64 respectively). “VPF” was very important too, and the highest risk of DNR was in those samples submitted to the SACPME (OR 6.32, 95 % CI 3.47-11.49) as compared to samples submitted to Bristol University. Submissions from post-weaned calves had around three times the odds of being DNR as compared to neonatal animals (OR 2.82, 95 % CI 2.58-3.09).

Samples from males (OR 1.46, 95 % CI 1.35-1.59) and females (OR 1.29, 95 % CI 1.20-1.39) had increased odds compared to submissions from a mixture of sexes. Submissions from non-commercial cattle had the highest risk of DNR compared to beef fattener (OR 2.00, 95 % CI 1.12-3.63).

Regarding confounding, “submission type” was the strongest confounder in the association of the rest of the variables with the outcome, followed by “main presenting sign”. Removing any of those two variables altered the OR estimations for the other risk factors by more than 20%.

The two sensitivity scenarios explored with the full model showed minimal effect in the OR estimations of the other risk factors (and categories). No changes in the direction of the OR values or in the statistical significance of the variables and categories were found.

DISCUSSION

The aim of this study was to investigate potential predictors for DNR in cattle diagnostic submissions to the British VPFs. The multivariable mixed-effects model identified “main presenting sign”, “type of submission”, “VPF”, “sex”, “age” and “production purpose” as predictors for DNR. Some of the associations reported in this analysis, especially in the case of “main presenting sign” and “submission type” were very strong (Table 2). For others, such as “production purpose” and “sex”, although still statistically significant there were weaker associations. However, as some of the categories included in those variables are quite common in the cattle population, they are important determinants for a DNR submission in cattle.

Main findings: Significance of predictors for DNR

In the final model, submissions from cattle that had aborted had the highest risk of DNR. The results are consistent with those obtained in the previous pilot study, which found that reproductive signs showed the strongest association with DNR.

Different issues can lead to a higher DNR risk in abortions submissions. Some of these could be: the lack of diagnostic tests for determining causal factors (Hoinville et al., 2008); the existence of a proportion of abortions due to non-infectious causes for which there are no diagnostic tests; and the eventual delay between the event leading to the abortion (either by expulsion of the foetus or by other means) and its detection. This can result in a lower proportion of bovine abortions being detected in a timely way. Furthermore, in these cases, the foetus is more likely to be autolysed (APHA, personal communication) and as placentas are rarely submitted from bovine abortions (SRUC, personal communication), these two factors can reduce the chance of achieving a diagnosis.

Perhaps unsurprisingly, “carcass” was the submission type with the lowest risk of DNR. A whole carcass submission makes it is easier to reach a diagnosis compared to “foetus” or “other”, maybe due to the more extensive range of samples that can be collected. Moreover, in some occasions, other types of samples submitted are not the most appropriate for reaching a diagnosis (APHA, personal communication). A similar finding was reported in a previous study (Hyder et al., 2011).

“Age”, “sex” and “production purpose” were also significant predictors for DNR (Table 2). Furthermore, most of the VPFs were significantly associated with DNR. Various explanations have been proffered for the association between different VPFs and DNR e.g. differences in the underlying population (such as main breed present on the farm), or type of samples (carcass, foetus or other type of sample) received by different laboratories. In this respect, distances from farm to VPFs might imply a major constraint, although there is a free carcass collection service. However, the association is still not well understood and requires further investigation (Hyder et al., 2011).

The variable “number in submission” which, in theory, reflects the number of animals contributing to the submission, was not associated with the outcome. This finding could be due to the fact that there is not an association in the data, or because in some submissions the variable could represent not the number of animals but the number of samples coming from the same animal (APHA, personal communication). The high number of missing values and the presence of outliers in this variable may also have impacted on the finding of no association. The missing values in “number in submission” were evenly distributed throughout the categories of the other variables.

Information from descriptive analysis and considerations on surveillance coverage

Our estimations of the proportions of farms participating in passive surveillance varied between 11.9% and 19.5% per year. The steady decrease in participation over the study period may reflect the use of other VPFs by veterinary practitioners (APHA, 2018), lower occurrence of disease, or simply that fewer samples were submitted. All those factors that might compromise the performance of the system to detect novel diseases.

A lack of economic resources can also play an important role in the lack of submissions to VPFs (O'Sullivan et al., 2012a) and in DNR occurrence. It could be an explanation of why only one specific test was requested by veterinary practitioners in 33.5% of the DNR submissions. This factor could lead to a higher DNR occurrence in more deprived farming communities (Dolors Bertran, 2004). It is also possible that although the geographical distribution of the 28 VPFs covers the whole area of study, some blind spots may have occurred where access to service or transport was more difficult at that time, influencing the participation in the surveillance system.

Our results concur with the findings in several other studies worldwide (Amezcua et al., 2010; Dohoo et al., 2009; Santman-Berends et al., 2016). In all of them, geographical coverage and participation are areas where further efforts are still required. Nevertheless, syndromic surveillance initiatives are considered to be a promising direction to pursue for detection of potential novel and/or emerging diseases in human and animals, where access to VPFs services are limited (Dupuy et al., 2013; Stärk and Nevel, 2009).

Limitations

An analysis of only completed records was performed, which meant around 32% of the records were excluded from the final model. It has been argued that when the reason for missing data in predictors was unrelated to the outcome, as in this instance as the information recorded for each submission was obtained before the DNR status was known, it should not cause biased results. Evidence for this was demonstrated in the two sensitivity scenarios we explored. The main concern in the present study was related to the loss of precision and power due to the exclusion of those records with unknown values. But considering the high number of submissions included, it may not have substantially impacted upon statistical power (Allison, 2000; Sterne et al., 2009; Steyerberg and van Veen, 2007).

It was not possible to explore any biologically plausible interaction due to the characteristics of the dataset. For instance, a particularly relevant and biologically plausible interaction might be present between "main presenting sign" and "submission type". However, in some submissions where multiple disease events were occurring on the farm, only one main presenting sign was recorded. In other situations, different submissions were sent from the same farm at the same time, for different problems, but the same main presenting sign was recorded by the practitioner in all of them.

Caution needs to be applied when attempting to extrapolate these findings to the whole population of cattle farms in GB or to other countries, since this is a passive surveillance system with voluntary notification (i.e. non-random sampling). The use of passive surveillance data in epidemiological studies has a large number of well-known limitations and biases. This type of study can exhibit a lack of sensitivity, with under-reporting being a major weakness, since not all the diseased animals are submitted for laboratory analysis because this depends on a wide range of factors (APHA, 2016; Gilbert et al., 2014). Laboratory resources and other constraints on the rate at which specimens are submitted (which may be specific to individual laboratories) may also cause a bias in reaching a diagnosis (Hoinville et al., 2008) and the present approach is unable to detect changes in syndromes for which there are few submissions (Gibbens et al., 2008). Furthermore, other factors not explored such as herd size, frequency of veterinary contact, and proximity to diagnostic laboratories could play a key role at a national interpretation level (Dolors Bertran, 2004; Gates et al., 2015; Watson et al., 2008). However, the combination of syndromic surveillance and laboratory diagnosis (as in this study) permits the use of data, which otherwise would be lost or discarded for surveillance purposes.

Perspectives and potential improvements

This study permitted an evaluation of the different predictors analysed to identify potential DNR submissions. The findings could help inform VPFs and other stakeholders working in surveillance once the identification of the limitations and the reasons for these are detected in the surveillance system. Support could also be given for initiatives to fill potential gaps in the system. For example, for the detection of the reasons why some VPFs had a higher risk of DNR and for amending these risks where possible. Likewise, for carrying out a revision of submission protocols established for each condition such as the type and number of samples required. Those submissions at a higher risk of DNR may be targeted.

An epidemiological profile of DNR submissions has been established, which can help to discriminate the presence of aberrant profiles, and which will give warning of the potential presence of new diseases in cattle. Thus, for example, if the quarterly analysis shows an unusual proportion of DNR results from those submissions with 'respiratory' as the main presenting sign, additional variables could be included (such as VPFs, age, and purpose) to further characterise those submissions before proceeding with a further investigation of the apparent increase in those DNR submissions.

Some suggestions of how to improve the use of this type of surveillance data would include:

1. emphasising the importance of collecting all the information on the variables included in the file at all levels of the surveillance system and as many submissions as disease events required to be explored. This would allow the use of numerical variables that were not included due to outliers and missing values. A way to overcome this problem is the implementation of an IT system where forms accompanying samples can be printed out and submitted only after all fields have been filled. Such a service has been implemented by the APHA in 2018, in the Animal Disease Testing Service, so it may be worthwhile evaluating its performance;
2. exploring distance from farms to VPFs. It may be that greater distances are related to sending other types of samples instead of carcasses, although there is a free carcass collection service for some of the VPFs in England and Wales;
3. encouraging communication between practitioners and VIOs so appropriate sample selection is discussed before submission;
4. including a spatiotemporal analysis for a more comprehensive characterization of the usefulness of surveillance data. A form of this analysis using SaTScan has been applied before (APHA, 2012; Hyder et al., 2011) but further efforts are still needed;

5. exploring the reasons why farm participation in the system is gradually decreasing;
6. incentivising the submission of carcasses if there are dead animals, especially when there are not specific signs, or a rare condition is suspected; thus emphasizing the importance among the vet practitioners of requesting a complete diagnostic package when submitting sample(s); and
7. creating additional categories for submission type, to enable "other" to be differentiated for a better understanding of DNR submissions.

Conclusion

The results of this study support the importance of DNR analysis as a way of improving the use and value of data obtained from a passive surveillance system. The output can serve as a baseline for future epidemiological analyses using the same methodology developed here, or other approaches, to evaluate system performance. In addition, the results highlight the need for more highly detailed epidemiological data collection by diagnostic laboratories at the time of submission. This could help to optimise resources through more complete use of the information.

DECLARATION

Acknowledgements

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Availability of data and materials

Data used in the study can be available upon request to the Animal and Plant Health Agency.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

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Ethical approval

The study was approved by Ethics Board of the Royal Veterinary College (SR2018-1602).

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REFERENCES

Allison, P.D., 2000. Multiple Imputation for Missing Data: A Cautionary Tale. Sociol. Methods Res. 28, 301–309.

Amezcua, M. del R., Pearl, D.L., Friendship, R.M., McNab, W.B., 2010. Evaluation of a veterinary-based syndromic surveillance system implemented for swine. Can. J. Vet. Res. Can. Rech. Vet. 74, 241–250.

APHA, 2018. GB Emerging Threats Quartely Report, Cattle Disease. https://doi.org/https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/717210/pub-survrep-c0118.pdf (Accessed 20/08/2018)

APHA, 2016. Veterinary Investigation Surveillance Report 2009-2016. https://doi.org/https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/702853/vida16.pdf (Accessed 20/08/2018)

APHA, 2012. Investigation of factors which improve the effectiveness of early outbreak detection methods for scanning surveillance data. https://doi.org/http://sciencesearch.defra.gov.uk/Document.aspx?Document=11120_FinalreportforSE4301May2013.doc (Accessed 20/08/2018)

Doherr, M.G., Audige, L., 2001. Monitoring and surveillance for rare health-related events: a review from the veterinary perspective. Philos. Trans. R. Soc. London B Biol. Sci. 356, 1097–1106. https://doi.org/10.1098/rstb.2001.0898

Dohoo, I., Martin, S., Stryhn, S., 2009. Veterinary Epidemiology Research, second ed. AVC Inc., Charlottetown.

Dolors Bertran, M., 2004. Analysis of diagnosis not reached data from scaning surveillance. MSc Veterinary Epidemiology project report. London.

Dórea, F.C., Sanchez, J., Revie, C.W., 2011. Veterinary syndromic surveillance:

Current initiatives and potential for development. Prev. Vet. Med. 101, 1–17. https://doi.org/10.1016/j.prevetmed.2011.05.004

Drewe, J.A., Häsler, B., Rushton, J., Stärk, K.D.C., 2014. Assessing the expenditure distribution of animal health surveillance: the case of Great Britain. Vet. Rec. 174, 16. https://doi.org/http://dx.doi.org/10.1136/vr.101846

Dupuy, C., Bronner, A., Watson, E., Wuyckhuise-Sjouke, L., Reist, M., Fouillet, A., Calavas, D., Hendrikx, P., Perrin, J.-B., 2013. Inventory of veterinary syndromic surveillance initiatives in Europe (Triple-S project): Current situation and perspectives. Prev. Vet. Med. 111, 220–229. https://doi.org/10.1016/j.prevetmed.2013.06.005

Food and Agriculture Organization, 2014. Risk-based disease surveillance - A manual for veterinarians on the design and analysis of surveillance for demonstration of freedom from disease. FAO Animal Production and Health Manual No.17. Rome, Italy. https://doi.org/http://www.fao.org/3/a-i4205e.pdf (Accessed 20/08/2018)

Gates, M.C., Holmstrom, L.K., Biggers, K.E., Beckham, T.R., 2015. Integrating Novel Data Streams to Support Biosurveillance in Commercial Livestock Production Systems in Developed Countries: Challenges and Opportunities. Front. Public Heal. 3, 74. https://doi.org/10.3389/fpubh.2015.00074

Gibbens, J.C., Robertson, S., Willmington, J., Milnes, A., Ryan, J.B.M., Wilesmith, J.W., Cook, A.J.C., David, G.P., 2008. Use of laboratory data to reduce the time taken to detect new diseases: VIDA to FarmFile. Vet. Rec. 162, 771–776. https://doi.org/10.1136/vr.162.24.771

Gilbert, W.H., Häsler, B.N., Rushton, J., 2014. Influences of farmer and veterinarian behaviour on emerging disease surveillance in England and Wales. Epidemiol. Infect. 142, 172–186. https://doi.org/10.1017/S0950268813000484

Hoinville, L., Hyder, K., Willmington, J., 2008. Analysis of DNR data at VLA: A review A report of work conducted in task 2.2.1 of ED1039 during 2007/08. Veterinary Laboratories Agency.

Hoinville, L.J., Alban, L., Drewe, J.A., Gibbens, J.C., Gustafson, L., Häsler, B., Saegerman, C., Salman, M., Stärk, K.D.C., 2013. Proposed terms and concepts for describing and evaluating animal-health surveillance systems. Prev. Vet. Med. 112, 1–12. https://doi.org/10.1016/J.PREVETMED.2013.06.006

Hyder, K., Vidal-Diez, A., Lawes, J., Sayers, A.R., Milnes, A., Hoinville, L., Cook, A.J.C., 2011. Use of spatiotemporal analysis of laboratory submission data to identify potential outbreaks of new or emerging diseases in cattle in Great Britain. BMC Vet. Res. 7, 14. https://doi.org/10.1186/1746-6148-7-14

O'Sullivan, T., Friendship, R., Pearl, D.L., McEwen, B., Ker, A., Dewey, C., 2012a. The association between submission counts to a veterinary diagnostic laboratory and the economic and disease challenges of the Ontario swine industry from 1998 to 2009. Prev. Vet. Med. 106, 275–283. https://doi.org/10.1016/J.PREVETMED.2012.03.005

O'Sullivan, T., Friendship, R.M., Pearl, D.L., McEwen, B., Dewey, C.E., 2012b. Identifying an outbreak of a novel swine disease using test requests for porcine reproductive and respiratory syndrome as a syndromic surveillance tool. BMC Vet. Res. 8, 192. https://doi.org/10.1186/1746-6148-8-192

Rodriguez-Prieto, V., Vicente-Rubiano, M., Sanchez-Matomoros, A., Rubio-Guerri, C., Melero, M., Martinez-Lopez, B., Martinez-Aviles, M., Hoinville, L., Vergne, T., Comin, A., Schauer, B., Dorea, F., Pfeiffer, D.U., Sanchez-Vizcaino, J.M., 2015. Systematic review of surveillance systems and methods for early detection of exotic, new and re-emerging diseases in animal populations. Epidemiol. Infect. 143, 2018–2042. https://doi.org/10.1017/S095026881400212X

Santman-Berends, I.M.G.A., Brouwer-Middelesch, H., Van Wuijckhuise, L., de Bont-Smolenaars, N.G., Van Schaik, G., 2016. Surveillance of cattle health in the Netherlands: Monitoring trends and developments using routinely collected cattle census data. Prev. Vet. Med. 134, 103–112. https://doi.org/10.1016/j.prevetmed.2016.10.002

Stärk, K.D., Nevel, A., 2009. Strengths, weaknesses, opportunities and threats of the pig health monitoring systems used in England. Vet. Rec. 165, 461–465. https://doi.org/10.1136/vr.165.16.461

Sterne, J.A.C., White, I.R., Carlin, J.B., Spratt, M., Royston, P., Kenward, M.G., Wood, A.M., Carpenter, J.R., 2009. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ 338: b2393. https://doi.org/10.1136/bmj.b2393

Steyerberg, E.W., van Veen, M., 2007. Imputation is beneficial for handling missing data in predictive models. J. Clin. Epidemiol. 60, 979. https://doi.org/10.1016/J.JCLINEPI.2007.03.003

Watson, E.N., David, G.P., Cook, A.J.C., 2008. Review of diagnostic laboratory submissions of adult cattle found dead in England and Wales in 2004. Vet. Rec. 163, 531–535. https://doi.org/10.1136/vr.163.18.531

Table 1 – Results of descriptive statistics and of univariable mixed-effects logistic regression model between selected variables and DNR of 113,267 cattle submissions in GB for the period from 1 January 2013 to 31 December 2017. County and farm were modelled as random effects. Only variables with a likelihood ratio test (LRT) of p<0.2 are reported.

Variable	Number of submissions	% of DNR	OR (95 % CI)	Wald p-value	LRT p-value
VPF					<0.001
Bristol University, PME	427	19.2	1.00		
Aberdeen, SAC	349	56.4	7.80 (5.57-10.91)	<0.001	
Aberystwyth, APHA	3,367	55.5	12.63 (8.73-18.28)	<0.001	
Auchincruive, SRUC	4,422	50.5	9.93 (7.34-13.45)	<0.001	
Bury St Edmunds, APHA	3,200	74.9	14.58 (11.00-19.31)	<0.001	
Carmarthen, APHA	10,322	61.0	8.43 (6.45-11.02)	<0.001	
Dumfries, SRUC	11,201	58.5	13.09 (9.95-17.23)	<0.001	
Edinburgh, SRUC	3,450	62.8	12.33 (9.40-16.18)	<0.001	
Inverness, SRUC	1,623	61.1	15.00 (10.62-21.18)	<0.001	
Langford, APHA	6,061	65.4	7.63 (5.93-9.82)	<0.001	
Liverpool University, PME	217	22.1	1.24 (0.85-1.96)	0.191	
Luddington, APHA	1,141	69.9	11.27 (8.45-15.04)	<0.001	
Newcastle, APHA	333	63.7	10.79 (7.57-15.39)	<0.001	
Penrith, APHA	1,0740	59.8	9.81 (7.50-12.84)	<0.001	
Perth, SRUC	2,819	57.0	9.69 (6.93-13.54)	<0.001	
Preston, APHA	492	54.5	6.47 (4.70-8.89)	<0.001	
Royal Veterinary College, PME	114	29.8	1.30 (0.96-2.68)	0.085	
SACPME	151	62.9	9.78 (6.35-15.04)	<0.001	
Shrewsbury, APHA	24,465	66.5	10.08 (7.83-12.97)	<0.001	
Starcross, APHA	10,212	69.0	9.55 (7.34-12.42)	<0.001	
St. Boswells, SRUC	3,722	68.6	15.02 (11.46-19.68)	<0.001	
Sutton Bonington, APHA	3,797	68.9	11.68 (8.89-15.34)	<0.001	
Thirsk, APHA	4,722	65.0	9.19 (6.93-12.17)	<0.001	
Thurso, SRUC	1,922	65.0	13.57 (9.47-19.46)	<0.001	
Truro, APHA	1276	68.6	12.48 (9.09-17.15)	<0.001	
University of Aberystwyth, PME	201	30.8	2.64 (1.77-3.94)	<0.001	
University of Surrey, APHA	263	33.1	1.94 (1.34-2.81)	<0.001	
Winchester, APHA	2258	76.0	10.47 (7.88-13.90)	<0.001	
Submission type					<0.001
Carcass	8,181	18.3	1.00		
Foetus	4,557	58.4	6.57 (6.04-7.12)	<0.001	
Other	100,529	67.2	9.14 (8.62-9.69)	<0.001	
Main presenting sign					<0.001
Mastitis	4,043	35.0	1.00		
Abortion	17,677	83.7	8.99(8.32-9.71)	<0.001	
Diarrhoea	31,423	53.8	2.13(1.98-2.28)	<0.001	
Other	25,412	61.2	2.87 (2.67-3.08)	<0.001	
Respiratory	7,744	68.3	3.90 (3.60-4.24)	<0.001	
Unknown	10,416	66.0	3.46 (3.20-3.74)	<0.001	
Wasting	16,552	65.9	3.57 (3.32-3.85)	<0.001	
Year received grouped					<0.001
2013	32,227	62.3	1.00		
2014-2015	48,041	64.4	1.09 (1.06-1.12)	0.001	
2016-2017	32,999	62.8	1.01 (0.98-1.05)	0.452	
Age category					<0.001
n=96,252 (missing values and not applicable records 17,015)					
Neonatal	5,910	40.7	1.00		
Adult	66,948	67.3	2.65 (2.51-2.79)	<0.001	
Mixed*	2,056	62.2	2.05 (1.85-2.28)	<0.001	
Postweaned	10,215	66.0	2.38 (2.22-2.54)	<0.001	
Preweaned	11,123	48.0	1.22 (1.14-1.30)	<0.001	
Sex					<0.001
n=96,875 (missing values and not applicable records 16,392)					
Mixed*	5,139	56.6	1.00		
Castrate	1,401	60.0	1.16 (1.02-1.31)	0.020	
Female	77,789	65.3	1.49 (1.40-1.58)	<0.001	
Male	12,546	57.3	1.11 (1.04-1.18)	0.003	
Production purpose					<0.001
n=103,174 (missing values and not applicable records 10,093)					
Finisher	4,873	57.6	1.00		
Milk	60,779	64.4	1.30 (1.22-1.38)	<0.001	
Other[#]	83	69.9	1.69 (1.05-2.70)	0.030	
Rearing	2,686	60.8	1.08 (0.98-1.19)	0.124	
Suckler	34,753	62.2	1.23 (1.16-1.31)	<0.001	
Region					<0.001
n=113,252 (missing values and not applicable records 15)					
Scotland	25,173	55.5	1.00		
East Midlands	4,554	69.1	1.72 (1.18-1.42)	<0.001	
East of England	2,307	73.3	2.05 (1.43-2.06)	<0.001	
London	24	62.5	1.59 (0.63-4.00)	0.322	
North East	2,826	66.0	1.36 (1.09-1.68)	0.006	

Variable	Number of submissions	% of DNR	OR (95 % CI)	Wald p-value	LRT p-value
North West	15,483	61.3	1.38 (1.15-1.65)	<0.001	
South East	4,873	74.4	2.30 (1.97-2.70)	<0.001	
South West	23,770	68.0	1.64 (1.41-1.90)	<0.001	
Wales	17,055	61.5	1.19 (1.02-1.38)	0.023	
West Midlands	11,792	65.2	1.58 (1.33-1.87)	<0.001	
Yorkshire and the Humber	5,395	66.7	1.64 (1.34-2.01)	<0.001	

DNR: Diagnosis not reached. OR: Odds Ratio. VPF: Veterinary post-mortem facility. PME: Post-mortem examination provider. APHA: Animal and Plant Health Agency. SRUC: Scotland's Rural College. SACPME: Scottish Agricultural College for Post-mortem Examination. LRT: Likelihood Ratio Test.

*"Mixed" categories in "Age" and "Sex" mean a mixture of animals with different age and sex that are included in the submission.

#"Other" included the following enterprises under the animals were being kept: pet, captive, zoological and wild.

Table 2 –Results from a mixed-effects multivariable logistic regression model between selected variables and DNR of 81,191 cattle submissions in GB for the period from 1 January 2013 to 31 December 2017. County and farm were modelled as random effects.

Variable	***Categories***	***Adjusted OR***	***95 % CI***	***p-value (WALD tests)***
VPF	Bristol University, PME	1.00		
	Aberdeen, SRUC	1.34	0.89-2.02	0.156
	Aberystwyth, APHA	1.75	1.24-2.46	0.001
	Auchincruive, SRUC	1.49	1.08-2.06	0.014
	Bury St Edmunds, APHA	2.65	1.92-3.65	<0.001
	Carmarthen, APHA	1.52	1.12-2.07	0.008
	Dumfries, SRUC	1.82	1.33-2.49	<0.001
	Edinburgh, SRUC	1.63	1.18-2.25	0.003
	Inverness, SRUC	2.09	1.48-2.95	<0.001
	Langford, APHA	1.47	1.08-1.99	0.014
	Liverpool University, PME	0.98	0.60-1.59	0.922
	Luddington, APHA	1.89	1.68-3.32	<0.001
	Newcastle, APHA	2.36	1.38-3.17	<0.001
	Penrith, APHA	2.09	1.14-2.11	0.005
	Perth, SRUC	1.55	1.50-2.96	<0.001
	Preston, APHA	2.11	0.95-2.02	0.087
	Royal Veterinary College, PME	1.39	1.09-3.28	0.024
	SACPME	6.32	3.47-11.49	<0.001
	Shrewsbury, APHA	1.75	1.29-2.37	<0.001
	Starcross, APHA	2.05	1.50-2.78	<0.001
	St. Boswells, SRUC	2.56	1.86-3.53	<0.001
	Sutton Bonington, APHA	2.04	1.49-2.80	<0.001
	Thirsk, APHA	2.03	1.48-2.78	<0.001
	Thurso, SRUC	2.32	1.61-3.34	<0.001
	Truro, APHA	2.25	1.58-3.19	<0.001
	University of Aberystwyth, PME	1.96	1.24-3.11	0.004
	University of Surrey, APHA	3.07	2.01-4.68	<0.001
	Winchester, APHA	2.86	2.06-3.96	<0.001
Submission type	Carcass	1.00		
	Foetus	1.45	1.28-1.64	<0.001
	Other	11.57	10.68-12.53	<0.001
Main presenting sign	Mastitis	1.00		
	Abortion	21.61	19.55-23.89	<0.001
	Diarrhoea	2.44	2.25-2.64	<0.001
	Other	5.26	4.85-5.71	<0.001
	Respiratory	5.58	5.06-6.16	<0.001
	Unknown	4.16	3.78-4.58	<0.001
	Wasting	3.45	3.18-3.75	<0.001

Variable	*Categories*	*Adjusted OR*	*95 % CI*	*p-value (WALD tests)*
Age category	Neonatal	1.00		
	Adult	2.07	1.92-2.23	<0.001
	Mixed*	1.70	1.49-1.94	<0.001
	Postweaned	2.82	2.58-3.09	<0.001
	Preweaned	1.66	1.52-1.81	<0.001
Sex	Mixed*	1.00		
	Castrate	1.13	0.97-1.31	0.130
	Female	1.29	1.20-1.39	<0.001
	Male	1.46	1.35-1.59	<0.001
Production purpose	Finisher	1.00		
	Milk	1.22	1.12-1.33	<0.001
	Other#	2.00	1.12-3.63	0.023
	Rearing	1.07	0.94-1.22	0.278
	Suckler	1.16	1.06-1.26	0.001

DNR: Diagnosis not reached. OR: Odds Ratio. VPF: Veterinary post-mortem facility. PME: Post-mortem Examination Provider. APHA: Animal and Plant Health Agency. SRUC: Scotland's Rural College. SACPME: Scottish Agricultural College for Post-mortem Examination Provider. LRT: Likelihood Ratio Test.

*"Mixed" categories in "Age" and "Sex" mean a mixture of animals with different age and sex that are included in the submission.

#"Other" included the following enterprises under the animals were being kept: pet, captive, zoological and wild.

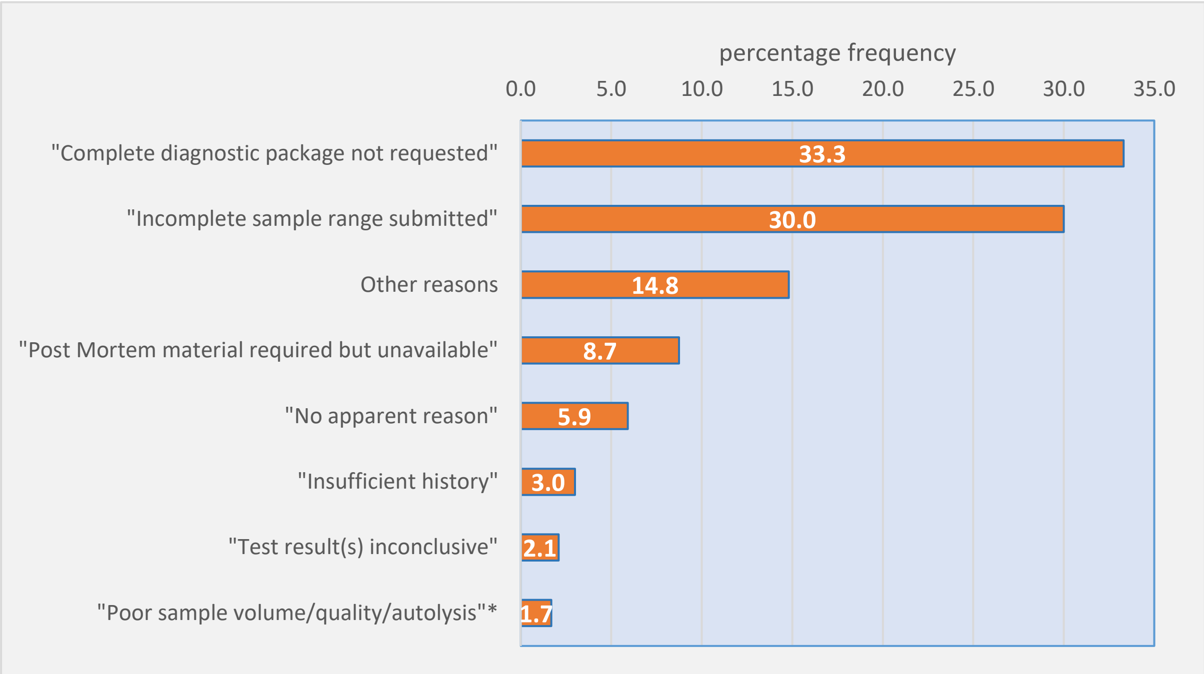

Figure 1 Relative frequency distribution of Veterinary Investigation Officers' opinions on why no diagnosis was reached on 71,750 DNR submissions. The "Poor sample volume/quality/autolysis" category refers to those situations where the type of sample submitted was appropriate but it was not possible to reach a diagnosis because of the poor condition of a sample (for example, insufficient sample volume collected, poor sample quality, such as inappropriate collection tube used for blood sampling, or autolysis of the sample)