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Abstract: Jaagsiekte sheep retrovirus (JSRV) is the causative agent of ovine pulmonary adenocarcinoma (OPA). JSRV infection is usually detected post-mortem by macroscopic and histological examination of lungs for lesions of OPA. Subsequently, the presence of JSRV may be confirmed using reverse-transcriptase polymerase chain reaction (RT-PCR) on tumour tissue. Our goal was to determine the most effective way of combining macroscopic and histological examination with reverse transcriptase PCR (RT-PCR) to detect JSRV infection post-mortem. Lungs of slaughtered sheep (n=369) with macroscopic lesions were examined macroscopically and histologically to identify lesions consistent with OPA, and subsequently subjected to RT-PCR for JSRV. Positive (Ppos) and negative (Pneg) agreements and Cohen's Kappa coefficient were calculated between RT-PCR and: 1) macroscopic examination; 2) histological examination; 3) macroscopic and histological examination combined in series, and; 4) in parallel. The highest Ppos was between macroscopic and histologic examination in parallel and RT-PCR (0.38). Conversely, Pneg for all combinations of RT-PCR and macroscopic and histological examinations was high (95-96%). All Kappa values were low (0.1-0.33). This indicates that macroscopic and histological examination combined in parallel is the most effective way to identify animals that should be tested using RT-PCR for JSRV. If a positive result is obtained on macroscopic examination and/or histological examination, RT-PCR should be carried out to ascertain the presence of JSRV. The high Pneg indicates that if a negative result is obtained on macroscopic and histological examination, RT-PCR testing is not merited, as the result is likely to be negative. This provides an evidence-base for the diagnosis of JSRV infection.

Highlights

Evidence-based guidelines for JSRV diagnosis using OPA lesions have been provided

Macroscopic + histologic exam combined in parallel: Used to select lungs for RT-PCR

If macroscopic and histologic exam are both negative, JSRV RT-PCR is not required

If macroscopic and/or histologic exam are positive, JSRV RT-PCR is recommended

Approach may be applied to efficiently detect JSRV infection in a flock

An approach to diagnosis of Jaagsiekte sheep retrovirus infection in sheep based on assessment of agreement between macroscopic examination, histopathologic examination and reverse-transcriptase polymerase chain reaction.

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Abstract

Jaagsiekte sheep retrovirus (JSRV) is the causative agent of ovine pulmonary adenocarcinoma (OPA). JSRV infection is usually detected post-mortem by macroscopic and histological examination of lungs for lesions of OPA. Subsequently, the presence of JSRV may be confirmed using reverse-transcriptase polymerase chain reaction (RT-PCR) on tumour tissue. Our goal was to determine the most effective way of combining macroscopic and histological examination with reverse transcriptase PCR (RT-PCR) to detect JSRV infection post-mortem. Lungs of slaughtered sheep (n=369) with macroscopic lesions were examined macroscopically and histologically to identify lesions consistent with OPA, and subsequently subjected to RT-PCR for JSRV. Positive (P_{pos}) and negative (P_{neg}) agreements and Cohen's Kappa coefficient were calculated between RT-PCR and: 1) macroscopic examination; 2) histological examination; 3) macroscopic and histological examination combined in series, and; 4) in parallel. The highest P_{pos} was between macroscopic and histologic examination in parallel and RT-PCR (0.38). Conversely, P_{neg} for all combinations of RT-PCR and macroscopic and histological examinations was high (95-96%). All Kappa values were low (0.1-0.33). This indicates that macroscopic and histological examination combined in parallel is the most effective way to identify animals that should be tested using RT-PCR for JSRV. If a positive result is obtained on macroscopic examination and/or histological examination, RT-PCR should be carried out to ascertain the presence of JSRV. The high P_{neg} indicates that if a negative result is obtained on macroscopic and histological examination, RT-PCR testing is not merited, as the result is likely to be negative. This provides an evidence-base for the diagnosis of JSRV infection.

Keywords: Jaagsiekte sheep retrovirus, Neoplasms, Ovine Pulmonary Adenomatosis,
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Introduction

Jaagsiekte Sheep Retrovirus (JSRV) is an oncogenic retrovirus that causes ovine pulmonary adenocarcinoma (OPA), a progressive, fatal, bronchiolo-alveolar carcinoma of sheep (Griffiths et al., 2015). Infection has been reported in various countries across Europe, Asia, Africa and South and North America (Griffiths, 2015). Evidence indicates that JSRV is transmitted primarily via the respiratory route but transmission via colostrum and milk may also play a role (Grego et al., 2008; Griffiths et al., 2015). Clinical disease is most commonly reported in adult animals and signs include dyspnoea, tachypnoea, condition loss, nasal discharge and coughing (Griffiths et al., 2015). After initial introduction of the virus into a flock, mortality rates may be as high as 30-50% but decrease to 1-5% as the disease becomes endemic (Griffiths et al., 2015). It is likely that owners of infected flocks suffer financial losses due to reduced body weights, early culling and increased mortality. Additionally, the welfare of animals suffering from advanced clinical disease is compromised. Given the long incubation period (months-years) and the difficulty in accurately diagnosing animals in the early stages of OPA, the infection is likely highly underreported (Scott et al., 2013). Knowledge of a flock's infection status is important, as farmers can undertake various methods to control the disease, thus improving production and animal welfare (Scott et al., 2013). A recent survey in Ireland established a preliminary estimate of prevalence of JSRV infection and OPA as 1.6% and 0.5% respectively in Irish sheep (Lee et al., 2017). A similar survey in the UK found an OPA prevalence of 0.9% in adult sheep (Cousens et al., 2015). While the prevalence is therefore likely low, certain flocks have a higher prevalence than others, meaning these flocks are likely disproportionately affected by the virus (Lee et al., 2017). Therefore, an economically viable, evidence-based method for identifying infected flocks is necessary for disease control.

Post-mortem examination, including both macroscopic pathology and histopathology frequently plays a role in disease prevalence surveys and control programmes. Post-mortem macroscopic examination (ME) for the detection of the typical macroscopic lung lesions of OPA followed up with histologic examination (HE) for OPA lesions is currently the standard method of diagnosing JSRV infection within an individual animal or flock (Scott et al., 2013). However, ME will not detect animals that are infected with JSRV but have not yet developed OPA as it is impossible to detect the presence of JSRV based on pathologic changes alone, and routine post-mortem examination may not detect small, early stage lesions. Additionally, lesions of OPA may be obscured by concurrent pathology (e.g. bronchopneumonia, lungworm). Therefore, ME and HE are not without limitations.

Polymerase chain reaction (PCR) on suspect neoplastic lung tissue for JSRV are sometimes used as an aid to post-mortem examination. In the diagnostic setting, this is mainly used as a follow-up test to confirm equivocal cases based on the assumption of a high sensitivity and specificity when performed on OPA tumour tissue (Scott et al., 2013). However, there is, to the authors' knowledge, no evidence-based information as to how PCR should be combined with ME and HE to optimise post-mortem detection of JSRV infection in the literature. This information is vital for diagnostic accuracy as well as for optimising management of resources.

The goal of this study was to determine an optimal way of using ME and HE along with reverse-transcriptase PCR (RT-PCR) in order to detect JSRV infection post-mortem. This provides a diagnostic evidence base to assist veterinary pathologists and veterinarians in their application and interpretation of macroscopic and histological post-mortem findings.

Materials and Methods

The study population consisted of 369 adult (over one year old) lowland and highland breeds of sheep slaughtered at an Irish abattoir between September and November 2015, as described elsewhere (Lee et al., 2017). The sheep originated from 127 Irish flocks in 18 counties with the majority being cull ewes. No animals were slaughtered specifically for the purposes of this study.

Subsequent to slaughter and evisceration, lungs were selected for further examination (ME, HE and RT-PCR), by visual examination and palpation at the abattoir over the course of ten separate visits in September-November 2015. A team of 2 - 3 veterinarians were responsible for initial lung examination and selection on each visit; these included first author (AML) accompanied by veterinarians from the Department of Agriculture, Food and the Marine (DAFM) experienced in post-mortem examination of farm animals. All lungs displaying significant macroscopic lesions of any type were selected for further examination. Most lungs displayed numerous subpleural, dark red, pinpoint foci due to aspiration of blood at slaughter and those with only this change were excluded from the study.

After initial selection, lungs were subject to ME and categorised according to the gross appearance of the lesions present as previously described (Lee et al., 2017). These categories included abscesses, cranioventral consolidation, discolouration, fibrosis, focal firm nodule(s), mineralisation, subpleural parasitic granulomas, macroscopic suspect OPA, uncollapsed lungs and other lesions that did not fit into the aforementioned categories (i.e. other). The macroscopic changes characterising each category have been described in detail previously

(Lee et al., 2017) and the above-mentioned categories were based on commonly described pathologic changes found in sheep lungs (Caswell and Williams, 2016). A section of tissue approximately one cm³ was taken from each pulmonary lesion at the border between the lesion and normal tissue, where possible. These samples were processed routinely for histology and stained with haematoxylin and eosin (H&E). Also during ME, a smaller portion of tissue (0.5 cm³) was taken from the pulmonary lesions directly adjacent to the site sampled for histology and subjected to real-time RT-PCR for JSRV. Sample preservation, nucleic acid extraction and the RT-PCR protocol was carried out according to standard procedure as described previously (Lee et al., 2017). Macroscopic examination and HE was carried out by AML and RT-PCR was carried out by EC (co-author). A diagnosis of JSRV infection (based on the presence of macroscopic OPA lesions) on ME was constituted by locally extensive, well-demarcated, consolidated, heavy, oedematous, grey-purple areas (Caswell and Williams, 2016). A diagnosis of JSRV infection (based on the presence of histologic OPA lesions) on HE was constituted by single or multifocal, well-demarcated-infiltrative tumours with papillary/lepidic/acinar growth patterns consisting of cuboidal-columnar cells supported by a fine fibrous stroma and surrounded by macrophage infiltration (Caswell and Williams, 2016). On RT-PCR, test samples with a cycle threshold value of < 38 were considered positive for JSRV. Only sheep positive for JSRV on RT-PCR were considered to be infected with JSRV, regardless of what macroscopic or histopathologic lesions were present.

Measures of agreement were calculated for the following pairs of tests: ME and RT-PCR, HE and RT-PCR, ME and HE combined in series (ME-HE_{series}) and RT-PCR, and ME and HE combined in parallel (ME-HE_{parallel}) and RT-PCR (Table 1). If two tests are combined *in series* to diagnose a condition, a positive result on *both* tests must be obtained for the overall result to be considered positive (Figure 1A). If two tests are combined *in parallel* to diagnose

the same condition, a positive result on *one or both* tests is sufficient for the overall result to be considered positive (Figure 1B). In the context of this study, the two diagnostic procedures combined in series or in parallel are considered to be one diagnostic test. Measures of agreement calculated included positive agreement (P_{pos}) and negative agreement (P_{neg}) using Microsoft Excel 2010. Positive agreement is the number of positive results that both tests agree on expressed as a proportion of the average number of positive results obtained from both tests. Similarly, P_{neg} is the number of negative results that both tests agree on expressed as a proportion of the average number of negative results obtained from both tests (Cicchetti and Feinstein, 1990). Standard errors (SE) of P_{pos} and P_{neg} for construction of approximate 95% confidence intervals (CIs) were derived using the Delta method (Oehlert, 1992) exploiting their correspondence with Chamberlain's P_{pos} and P_{neg} (Cicchetti and Feinstein, 1990). Formulae for calculations were as follows, based on the 2x2 table shown in Table 2:

$$P_{pos} = \frac{2a}{(a + b + c + d) + (a - d)}$$

$$P_{neg} = \frac{2d}{(a + b + c + d) - (a - d)}$$

$$P_{pa} = \frac{a}{a + b + c}$$

$$SE(P_{pos}) = \sqrt{\left\{ \left[\frac{2}{(1 + P_{pa})^2} \right]^2 \times \left[\frac{P_{pa}(1 - P_{pa})}{a + b + c} \right] \right\}}$$

$$P_{na} = \frac{d}{b + c + d}$$

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$$SE(P_{neg}) = \sqrt{\left\{ \left[\frac{2}{(1 + P_{na})^2} \right]^2 \times \left[\frac{P_{na}(1 - P_{na})}{b + c + d} \right] \right\}}$$

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184 For purposes of comparison, Cohen's kappa co-efficient (Kappa) and 95% CIs was calculated
185 using IBM SPSS Statistics 24 (Cohen, 1960).

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187 In this study, inferences were drawn primarily from the values for P_{pos} and P_{neg} (as opposed to
188 Kappa). This is because P_{pos} and P_{neg} identify how the tests behave separately for infected and
189 non-infected animals, which is more in line with the goals of this investigation. Relying on
190 the value of Kappa alone in studies such as this is misleading, as Kappa is an omnibus index
191 which combines both positive and negative agreement (Cicchetti and Feinstein, 1990). Such
192 omnibus indices may have deceptive effects on the interpretation of results, as the
193 contribution of positive and negative agreement to the value of Kappa cannot be evaluated
194 separately, and the observational process will be obscured (Cicchetti and Feinstein, 1990). To
195 fully appraise results of studies on test agreement, examination of the P_{pos} and P_{neg}
196 individually should be carried out, as this may yield a more nuanced interpretation of the
197 results (Cicchetti and Feinstein, 1990).

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Results

Three hundred and sixty-nine lungs out of the 1911 examined (19%) were selected due to the presence of macroscopic lesions of any type on preliminary examination at the abattoir. As outlined in Figure 2, 18 lungs were classed as positive for JSRV infection (based on the presence of macroscopic lesions consistent with OPA) by ME. Twelve lungs had lesions consistent with JSRV infection (based on the presence of histologic lesions consistent with OPA) by HE. Thirty lungs were positive for JSRV by RT-PCR. These results are described in greater detail elsewhere (Lee et al., 2017). In summary, six cases of JSRV infection were diagnosed by RT-PCR in sheep with macroscopic OPA lesions, seven cases of JSRV infection were diagnosed in sheep with histologic OPA lesions, four cases of JSRV infection without histologic lesions were diagnosed, and five tumours with JSRV RT-PCR negative status (i.e. non-OPA tumours) were detected. Only two sheep were confirmed to be infected with JSRV that had OPA lesions detected on both ME and HE. Nineteen sheep were positive for JSRV by RT-PCR that were negative on ME and HE.

Results for calculations of the measures of agreement are shown in Table 3. P_{pos} values ranged from 0.13 to 0.38, whereas P_{neg} values ranged from 0.95-0.96. The highest P_{pos} was obtained between ME-HE_{parallel} and RT-PCR (0.38). In general, Kappa values were low (0.12 to 0.33) with three values falling into the range of ‘slight agreement’ and two into the range of ‘fair agreement’ (Kundel and Polansky, 2003). The highest Kappa value (0.33) was obtained for agreement between ME-HE_{parallel} and RT-PCR, the same pair of tests for which the highest P_{pos} was obtained.

Discussion

The aim of this study was to determine an optimal way of using ME and HE along with RT-PCR in order to detect JSRV infection post-mortem in a group of sheep.

The P_{pos} between ME-HE_{parallel} and RT-PCR (0.38; 95% CI: 0.32 - 0.44) was the highest obtained, indicating that this particular combination of tests yields the highest percentage of true positives for JSRV. However, given that this value represents a relatively low level of correlation with RT-PCR, RT-PCR is recommended to rule in or out the presence of JSRV infection, although was still low. This approach increases the number of true JSRV-positive cases diagnosed compared to using ME-HE series; (36% [11/30] of JSRV-positive animals were diagnosed using the methods in parallel compared to 6% [2/30] using the methods in series) or individually. If lungs were selected for RT-PCR based on ME alone, only 20% (6/30) cases of JSRV infection would have been diagnosed. If lungs were selected based on HE alone, only 23% (7/30) cases of JSRV infection (the seven sheep with OPA) would have been diagnosed. In other words, if lungs are macroscopically positive for OPA and/or histologically positive for OPA, RT-PCR is a useful adjunct tool to confirm if JSRV is present and to detect false positives which would have resulted by using ME and/or HE alone, without RT-PCR.

A consistently high P_{neg} was obtained for all combinations of tests (0.95 - 0.96), showing a high agreement of negative status between tests. This indicates that when there is a negative test result for ME, HE, ME-HE_{series}, or ME-HE_{parallel}, RT-PCR will almost always be negative.

Therefore the recommendations based on these results are: 1) preselect lungs from the sheep population of interest that have macroscopic lesions of any type for further macroscopic, histopathologic and RT-PCR examination, 2) if a sample of sheep lung contains lesions consistent with JSRV infection (based on the presence of OPA lesions on ME and/or HE) RT-PCR should be performed to determine if JSRV involvement is present and 3) if a sample of sheep lung does not contain lesions consistent with JSRV infection (based on the absence of lesions of OPA on both ME and HE), a subsequent RT-PCR is unnecessary, as it is also very likely to be negative. This guidance differs from the generally-accepted method of post-mortem OPA (and hence JSRV infection) diagnosis in which ME followed by HE are usually considered sufficient for both positive and negative OPA diagnosis (Scott et al., 2013), with PCR on lung tissue only being used in equivocal cases in which other lesions (e.g. bronchopneumonia) may obscure those due to OPA (Scott et al., 2013). In addition, the definition of what constitutes an equivocal case is subjective. The approach outlined above is most useful in detecting whether or not JSRV infection is present in a group of animals (e.g. cohorts from the same flock) whose lungs are examined post-mortem as part of a flock health investigation of respiratory disease, ill-thrift or other relevant problem.

This approach may seem somewhat contradictory, as 19 cases of JSRV infection were missed (those that had no lesions of OPA on ME or HE). However if the goal of the investigation is to ascertain if infection is or is not present in a flock, the approach outlined here is sufficient, as it is not necessary to detect every case, and the cost of carrying out RT-PCR on all animals examined is great. For instance, the cost of an RT-PCR test for JSRV as offered by the Department of Agriculture Laboratory Service in Ireland is €10.10 (\$11.40 US dollars/£8.70 pounds sterling at the time of writing). Therefore, the cost of testing all sheep with any type of gross lesions selected for further testing in this study (n = 369) would have been €3726.90,

compared to the €282.80 required to test all those with macroscopic or histologic lesions (n = 28). This constitutes a cost saving of 92%. Given the low prevalence of JSRV infection and OPA in Ireland and the UK we anticipate that the number of lungs to be tested during flock health surveys would not be excessive (Cousens et al., 2015; Lee et al., 2017).

We note parenthetically that Kappa values obtained for agreement between RT-PCR and the other tests (0.1-0.33) were low, indicating poor agreement when corrected for chance (Cohen, 1960). However, as discussed previously, the single omnibus value of Kappa gives us no indication of how to deal with positive versus negative results. Therefore, the Kappa-coefficient serves little practical use in this study as it provides no help in indicating how to use the diagnostic tests described, although its calculation emphasizes the limitations of this commonly used measure of agreement.

A potential limitation of this study is the assumption of perfect sensitivity and specificity of the RT-PCR for JSRV. It is assumed that current RT-PCR techniques detecting the unique U3 region of the JSRV long terminal repeat approach 100% specificity (Bai et al., 1996). The sensitivity of PCR using the same primers assessed *in vitro* suggests this assay can detect a single molecule of template DNA in a background of 500ng of normal sheep genomic DNA (Palmarini et al., 1996). As all previous studies have, to the authors' knowledge, invariably found OPA-affected lung tissue to be positive by PCR or RT-PCR for JSRV (Bai et al., 1996; Caporale et al., 2005; De Las Heras et al., 2005; Garcia-Goti et al., 2000; Gonzalez et al., 2001; Kycko et al., 2008; Palmarini et al., 1996; Palmarini et al., 1999; Salvatori et al., 2004) and as we are using the same primers and conditions, we consider the assumption regarding sensitivity and specificity to be reasonable.

Our approach was developed using a population of clinically normal slaughtered sheep. Therefore, the most useful application of this study may be in investigations aiming to detect the presence of JSRV infection within an individual population or flock. This is different to the context of post-mortem examination on an individual animal where more information is usually available (e.g. clinical and flock history and full examination of the animal carcass). In this case, the use of RT-PCR may be at the pathologist's discretion and may take into account other factors, such as the value of the animal or the flock. In contrast, in a prevalence survey for JSRV infection, it is important in the context of study design that all animals are tested identically, so all (or none) should have PCR carried out.

This study has value in that it provides evidence-based information regarding the diagnosis of JSRV infection. Traditionally, pathology relies on empirical knowledge to make diagnoses (Wick and Marchevsky, 2011). However, a more evidence-based approach also has merit (Fleming, 1996). Evidence-based medicine “depends on the use of logic and appropriate statistical methods to identify data-based concepts and procedures and separate them from observational ones” (Wick and Marchevsky, 2011). In human medicine many studies have been undertaken to assess the reliability and consistency of anatomical pathological diagnoses, both histologic and macroscopic (Fleming, 1996). We suggest a similar approach be adopted in veterinary medicine as described in the present study.

Conclusion

We have shown that the use of ME and HE in parallel is optimal in terms of reaching a diagnosis of JSRV infection at post-mortem. If a positive result on either or both of these

tests is obtained, a confirmatory RT-PCR for JSRV should be carried out. If a negative result on both tests is obtained, the sample is likely to yield a negative RT-PCR result and therefore testing is unlikely to aid the diagnostic process in the vast majority of cases.

Given that the prevalence of JSRV is generally low in the sheep population, we predict that most animals will be negative for both macroscopic and histologic lesions of OPA and RT-PCR testing will not be required. However, RT-PCR, when applied based on the presence of OPA lesions on ME and/or HE, is a useful adjunct to diagnosing JSRV infection in sheep.

References

- Bai, J., Zhu, R.Y., Stedman, K., Cousens, C., Carlson, J., Sharp, J.M., DeMartini, J.C., 1996. Unique long terminal repeat U3 sequences distinguish exogenous jaagsiekte sheep retroviruses associated with ovine pulmonary carcinoma from endogenous loci in the sheep genome. *J. Virol.* 70, 3159-3168.
- Caporale, M., Centorame, P., Giovannini, A., Sacchini, F., Di Ventura, M., De las Heras, M., Palmarini, M., 2005. Infection of lung epithelial cells and induction of pulmonary adenocarcinoma is not the most common outcome of naturally occurring JSRV infection during the commercial lifespan of sheep. *Virology*. 338, 144-153. <https://doi.org/10.1016/j.virol.2005.05.018>.
- Caswell, J.L., Williams, K.J., 2016. Respiratory System, in: Maxie, M.G. (Ed.), Jubb, Kennedy and Palmer's Pathology of Domestic Animals, sixth ed. Elsevier, St. Louis, pp. 465-591.
- Cicchetti, D.V., Feinstein, A.R., 1990. High agreement but low kappa: II. Resolving the paradoxes. *J. Clin. Epidemiol.* 43, 551-558. [https://doi.org/10.1016/0895-4356\(90\)90159-M](https://doi.org/10.1016/0895-4356(90)90159-M).
- Cohen, J., 1960. A coefficient of agreement for nominal scales. *Educ. Psychol. Meas.* 20, 37-46. <https://doi.org/10.1177/001316446002000104>.

- Cousens, C., Gibson, L., Finlayson, J., Pritchard, I., Dagleish, M.P., 2015. Prevalence of ovine pulmonary adenocarcinoma (Jaagsiekte) in a UK slaughterhouse sheep study. *Vet. Rec.* 176, 413-413. <http://dx.doi.org/10.1136/vr.102880>.
- Dalefield, R.R., Alley, M.R., 1988. An ovine pulmonary tumour of alveolar epithelial Type II cells. *New Zeal. Vet. J.* 36, 25-27.
- De las Heras, M., Ortín, A., Salvatori, D., de Villareal, M.P., Cousens, C., Ferrer, L.M., Cebrián, L.M., de Jalón, J.A.G., Gonzalez, L., Sharp, J.M., 2005. A PCR technique for the detection of Jaagsiekte sheep retrovirus in the blood suitable for the screening of ovine pulmonary adenocarcinoma in field conditions. *Res. Vet. Sci.* 79, 259-264. <https://doi.org/10.1016/j.rvsc.2005.02.003>.
- Feinstein A.R., Cicchetti, D.V., 1990. High agreement but low kappa: I. The problems of two paradoxes. *J. Clin. Epidemiol.* 43, 543-549. [https://doi.org/10.1016/0895-4356\(90\)90158-L](https://doi.org/10.1016/0895-4356(90)90158-L).
- Fleming, K.A., 1996. Evidence-based pathology. *J. Pathol.* 179, 127-128. [https://doi.org/10.1002/\(SICI\)1096-9896\(199606\)179:2<127::AID-PATH519>3.0.CO;2-Z](https://doi.org/10.1002/(SICI)1096-9896(199606)179:2<127::AID-PATH519>3.0.CO;2-Z).
- Garcia-Goti, M., Gonzalez, L., Cousens, C., Cortabarria, N., Extramiana, A.B., Minguíjon, E., Ortin, A., De las Heras, M., Sharp, J.M., 2000. Sheep pulmonary adenomatosis: characterization of two pathological forms associated with jaagsiekte retrovirus. *J. Comp. Pathol.* 122, 55-65. <https://doi.org/10.1053/jcpa.1999.0344>.

393

394 Gonzalez, L., Garcia-Goti, M., Cousens, C., Dewar, P., Cortabarría, N., Extramiana, A.B.,
395 Ortín, A., De Las Heras, M., Sharp, J.M., 2001. Jaagsiekte sheep retrovirus can be
396 detected in the peripheral blood during the pre-clinical period of sheep pulmonary
397 adenomatosis. J. Gen. Virol. 82, 1355-1358. [https://doi: 10.1099/0022-1317-82-6-](https://doi.org/10.1099/0022-1317-82-6-1355)
398 1355.

399

400 Grego, E., De Meneghi, D., Álvarez, V., Benito, A.A., Minguijón, E., Ortín, A., Mattoni, M.,
401 Moreno, B., De Villarreal, M.P., Alberti, A., Capucchio, M.T., 2008. Colostrum and
402 milk can transmit jaagsiekte retrovirus to lambs. Vet. Microbiol. 130, 247-257.
403 <https://doi.org/10.1016/j.vetmic.2008.01.011>.

404

405 Griffiths, D.J., Martineau, H.M., Cousens, C., 2010. Pathology and pathogenesis of ovine
406 pulmonary adenocarcinoma. J. Comp. Pathol. 142, 260-283.
407 <https://doi.org/10.1016/j.jcpa.2009.12.013>.

408

409 Hooper, P.T, Ellard, K.A. A pulmonary adenoma in an Australian sheep, 1995. Aust. Vet. J.
410 72, 477-477.

411

412 Kundel, H.L., Polansky, M., 2003. Measurement of observer agreement. Radiology. 228,
413 303-308. <https://doi.org/10.1148/radiol.2282011860>.

414

415 Kycko, A., Jasik, A., Reichert, M., 2008. Detection of jaagsiekte sheep retrovirus in
416 respiratory tract fluid and lung tissue of experimentally infected lambs. B. Vet. I.
417 Pulawy. 52, 9-13.

Lee, A.M., Wolfe, A., Cassidy, J.P., Messam, L.L.M., Moriarty, J.P., O'Neill, R., Fahy, C., Connaghan, E., Cousens, C., Dagleish, M.P., McElroy, M.C., 2017. First confirmation by PCR of Jaagsiekte sheep retrovirus in Ireland and prevalence of ovine pulmonary adenocarcinoma in adult sheep at slaughter. *Irish Vet. J.* 70, 33. <https://doi.org/10.1186/s13620-017-0111-z>.

Oehlert, G.W., 1992. A note on the delta method. *Am. Stat.* 46, 27-29.

Palmarini, M., Holland, M. J., Cousens, C., Dalziel, R. G., Sharp, J. M., 1996. Jaagsiekte retrovirus establishes a disseminated infection of the lymphoid tissues of sheep affected by pulmonary adenomatosis. *J. Gen. Virol.* 77, 2991-2998. [https://doi: 10.1099/0022-1317-77-12-2991](https://doi.org/10.1099/0022-1317-77-12-2991).

Palmarini, M., Sharp, J.M., De Las Heras, M., Fan, H., 1999. Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. *J. Virol.* 73, 6964-6972.

Salvatori, D., Gonzalez, L., Dewar, P., Cousens, C., de las Heras, M., Dalziel, R.G., Sharp, J.M., 2004. Successful induction of ovine pulmonary adenocarcinoma in lambs of different ages and detection of viraemia during the preclinical period. *J. Gen. Virol.* 85, 3319-3324. [https://doi: 10.1099/vir.0.80333-0](https://doi.org/10.1099/vir.0.80333-0).

Scott, P., Griffiths, D., Cousens, C., 2013. Diagnosis and control of ovine pulmonary adenocarcinoma (Jaagsiekte). In Practice. 35, 382-397.
<https://dx.doi.org/10.1136/inp.f4427>.

Wick, M.R., Marchevsky, A.M., 2011. Evidence-based principles in pathology: existing problem areas and the development of “quality” practice patterns. Arch. Pathol. Lab. Med. 135, 1398-1404. <https://doi.org/10.5858/arpa.2011-0181-SA>.

Tables

Table 1. Agreement between RT-PCR and a) histologic examination, b) macroscopic examination, c) macroscopic examination and histologic examination in series, and d) macroscopic and histologic examination in parallel during ovine lung tissue diagnosis of Ovine Pulmonary Adenocarcinoma in Ireland, 2015.

Test		RT-PCR		
		Positive	Negative	Total
a. Histologic examination	Positive	7	5	12
	Negative	23	334	357
	Total	30	339	369
b. Macroscopic examination	Positive	6	12	18
	Negative	24	327	351
	Total	30	339	369
c. Macroscopic examination and histologic examination in series	Positive	2	0	2
	Negative	28	339	367
	Total	30	339	369
d. Macroscopic and histologic examination in parallel	Positive	11	17	28
	Negative	19	322	341
	Total	30	339	369

Table 2: Two-by-two table as used for the calculation of positive and negative agreement.

Test 2				
Test 1	+		-	
	+	a	b	a + b
	-	c	d	c + d
	a + c		b + d	a + b + c + d

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Table 3. Results for calculations of measures of agreement. P_{pos} ,
Positive agreement; P_{neg} , Negative agreement; κ , Kappa; 95% CI, 95%
confidence interval.

	P_{pos}	P_{neg}	κ
	95% CI	95% CI	95% CI
Histologic examination and RT-PCR	0.33 (0.27-0.39)	0.96 (0.95-0.97)	0.30 (0.12-0.49)
Macroscopic examination and RT-PCR	0.25 (0.21-0.29)	0.95 (0.93-0.96)	0.20 (0.04-0.38)
Macroscopic and histologic examinations in series and RT-PCR	0.13 (0.11-0.14)	0.96 (0.95-0.97)	0.12 (0.00-0.27)
Macroscopic and histologic examinations in parallel and RT-PCR	0.38 (0.32-0.44)	0.95 (0.93-0.96)	0.33 (0.14-0.49)

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Figure legends

Figure 1: Tests interpreted in series and in parallel. A) Two diagnostic tests used in series: A positive result on both tests must be obtained for the overall result to be considered positive. A negative result on one or both tests is considered to be a negative result. B) Two diagnostic tests used in parallel: A positive result on one or both tests must be obtained for the overall result to be considered positive. A negative result on both tests is considered to be a negative result.

Figure 2: Venn diagram showing the process used for selection of lungs as well as distribution of the results of the macroscopic and histological examination for lesions consistent with Ovine Pulmonary Adenocarcinoma and reverse-transcriptase polymerase chain reaction (RT-PCR) for Jaagsiekte Sheep Retrovirus in 369 sheep in Ireland (September-December 2015). HE: Histologic examination; HSO: Histologically-suspect OPA; ME: Macroscopic examination; MSO: Macroscopic-suspect OPA.

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A.	Test 1	Test 2		Positive result: Test in series
	+	+		
	+	-		Negative result: Test in series
	-	+		
	-	-		
B.	Test 1	Test 2		Positive result: Test in parallel
	+	+		
	+	-		
	-	+		Negative result: Test in parallel
	-	-

An approach to diagnosis of Jaagsiekte sheep retrovirus infection in sheep based on assessment of agreement between macroscopic examination, histopathologic examination and reverse-transcriptase polymerase chain reaction.

Declarations of interest: None

Data Statement

[Click here to download Data Statement: data_JSRR_may19.docx](#)