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**Veterinary Pathology** 

### Veterinary Pathology

## Six-year follow-up of slaughterhouse surveillance (2008-2013): the Catalan Slaughterhouse Support Network (SESC)

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Keywords:	slaughterhouse, surveillance, pathology, food inspection, one health, food safety, continuing education

Abstract:	Meat inspection has the ultimate objective of declaring the meat and offal obtained from carcasses of slaughtered animals fit or unfit for human consumption. This safeguards the health of consumers by ensuring that the foodstuff coming out of these establishments poses no risk to public health. Concomitantly, it contributes to animal disease surveillance. The Catalan Public Health Protection Agency (Generalitat de Catalunya) identified the need to provide its meat inspectors with a support structure to improve diagnostic capacity: the Slaughterhouse Support Network (SESC). The main goal of the SESC program was to offer continuing education to meat inspectors in order to improve the diagnostic capacity on the lesions observed at slaughterhouses. With this aim, a web-based application was designed. The system allowed meat inspectors to submit their inquiries, images of the lesions and, if needed, samples to conduct laboratory analysis. In this commentary, a review of the cases from the first six years of SESC operation (2008-2013) is presented and the data are analyzed within the context of the covered geographical region, Catalonia. The program not only provides continuing education to inspectors but, in addition, contributes to the collection of useful information on animal health and welfare. Therefore, SESC complements animal disease surveillance programs, such as tuberculosis, and is a powerful tool for early detection of (re)emergence of animal diseases and zoonosis.
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#### Commentary

# Six-year follow-up of slaughterhouse surveillance (2008-2013): the Catalan Slaughterhouse Support Network (SESC)

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#### Abstract

Meat inspection has the ultimate objective of declaring the meat and offal obtained from carcasses of slaughtered animals fit or unfit for human consumption. This safeguards the health of consumers by ensuring that the foodstuff coming out of these establishments poses no risk to public health. Concomitantly, it contributes to animal disease surveillance. The Catalan Public Health Protection Agency (Generalitat de Catalunya) identified the need to provide its meat inspectors with a support structure to improve diagnostic capacity: the Slaughterhouse Support Network (SESC). The main goal of the SESC program was to offer continuing education to meat inspectors in order to improve the diagnostic capacity on the lesions observed at slaughterhouses. With this aim, a web-based application was designed. The system allowed meat inspectors to submit their inquiries, images of the lesions and, if needed, samples to conduct laboratory analysis. In this commentary, a review of the cases from the first six years of SESC operation (2008-2013) is presented and the data are analyzed within the context of the covered geographical region, Catalonia. The program not only provides continuing education to inspectors but, in addition, contributes to the collection of useful information on animal health and welfare. Therefore, SESC complements animal disease surveillance programs, such as tuberculosis, and is a powerful tool for early detection of (re)emergence of animal diseases and zoonosis.

**Keywords:** slaughterhouse, surveillance, pathology, food inspection, one health, food safety, continuing education

#### Introduction

Meat inspection, a task traditionally performed in slaughterhouses by veterinarians (sometimes assisted by meat inspection technicians), has the main objective of declaring the meat and offal obtained from carcasses of slaughtered animals fit or unfit for human consumption. Therefore, the safeguard of consumers' health by ensuring that the foodstuff coming out of these establishments poses no risk to public health is the ultimate goal. In addition, the diagnosis of lesions found during meat inspection might provide useful information regarding animal health and welfare issues that may have a relatively low relevance for public health but might be of great importance to farmers and veterinarians.

By mid 2000s, the Catalan Public Health Agency, belonging to the Health Department of the Catalan Government (*Generalitat de Catalunya*) identified the need to provide its meat inspectors with a support structure. In 2007, the Agency commissioned to *Centre de Recerca en Sanitat Animal* (CReSA) the organization of a system to support the meat inspectors: the Slaughterhouse Support Network (*servei de Suport a ESCorxadors*, SESC) (http://www.cresa.cat/blogs/sesc/).

In this commentary we intend to introduce this innovative system to the readers along with a brief analysis of the data gathered during its first 6 years of operation. The objective is to emphasize the relevant role veterinary pathology has in meat inspection and, consequently, in improving public health and animal disease surveillance. Other relevant aspects are the synergy that is created between the administration's meat inspection services and both academic and research pathologists. Also we think it is proof of the benefits of applying new information technologies to our field. Finally, the data presented on diagnoses is not intended to

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provide novel findings but rather to illustrate the challenges meat inspectors are faced with and how these are managed through the SESC program.

The main goal of the SESC program was to provide meat inspectors (official veterinarians of the Catalan Public Health Agency) with continuing education in their ability to diagnose lesions they might come across in slaughterhouses of Catalonia. With this aim, a web-based application was designed through which meat inspectors could submit their inquiries along with images of the lesions found and, if needed, samples to conduct laboratory analysis. The objective was to reach a final diagnosis of each case and send a final report to the inspector. It is important to note that condemnation of the carcass, a part of it, or any affected viscera has to be based on current legislation and the inspector's criteria, not on the report received form SESC. However, in some instances, the result of the report can influence or refine the inspector's final decision. That would be the case, for example, of Cysticercus bovis compatible lesions confirmation or if a lesion compatible with tuberculosis (TB) was found. In many occasions, the query leads to a final diagnosis, thus supporting the inspectors decision and improving its quality and reliability. Since condemnation is not to be based on SESC's report the time delay between the moment an inquiry is received and when the answer is delivered is not critical. However, as mentioned above, in some occasions the resolution of the case is urgent. In these cases the inspector can label the inquiry as urgent in the web-application. Urgent-labeled inquiries will be answered in 24 hours, unless sample analysis is included, as this might delay the answer depending on the tests performed.

These inquiries, when received at SESC, are forwarded to a number of veterinary pathologists and other animal health and welfare professionals of CReSA and the *Universitat Autònoma de Barcelona* (UAB) Veterinary Faculty. Occasionally, inquiries

are also forwarded to international collaborators. With the answers obtained from the different experts, SESC's technicians elaborate a response that is submitted to the consulting inspector with copy to the public health authorities. The experts include mainly pathologists, but also parasitologists, microbiologists, virologists, immunologists, experts in animal welfare, meat science and food hygiene professionals and animal anatomists.

The web-based application form includes fields that the inspector has to complete with information regarding the slaughterhouse of origin, information about the animal (or animals) affected and a description of the lesions and organs involved. This information and the images of the lesions uploaded to the application form are used by the experts to elaborate a report with the most likely diagnosis and/or a list of possible differentials. Additionally, the meat inspector has the possibility to include information on tissue samples sent to SESC for laboratory analysis. SESC technicians process and distribute the samples to the most appropriate laboratories based on the experts' assessment of each case. Ideally, samples are to be submitted within the first 24 hours and kept refrigerated. Alternatively, when the submission is not done immediately, it is recommended to split the sample in two parts: one half kept frozen and the other fixed in formalin.

#### First six years of SESC in numbers

#### Context in which SESC operates

SESC gives coverage to all slaughterhouses of Catalonia. A total of 254 slaughter lines were active in this territory at the moment of writing this report; including 45 bovine lines, 76 ovine lines, 17 equines lines, 48 porcine lines, 44 poultry lines (9 of

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which cull anseriformes as well) and 24 lagomorph lines. Each slaughter line is covered by, at least, one official meat inspector.

Catalonia is the Spanish autonomous community with the largest numbers of animals slaughtered annually. Regarding the number of farms, poultry is the sector most represented in the region (n=6814 farms) followed by the bovine (n=6579) and porcine (n=5983) sectors (data from 2012, obtained from the Agriculture department website: http://www20.gencat.cat/portal/site/DAR). A significant amount of the livestock culled in Catalonia is imported from other Spanish autonomous communities and from other countries.

The percentage of carcasses that were fully or partially condemned each year in Catalonia is presented in Supplemental Table 1. A system, based on the current legislation, has been implemented to gather condemnation data. However this database includes broad lesion categories and data on specific relevant diseases such as zoonoses. For instance, one of the most frequently reported reasons for total or partial condemnation of carcasses, and indeed of viscera, was "inflammatory lesions", indicating that the diagnosis of these cases either was not reported or had never been established. The scope of this commentary is not to analyze the condemnation data nor is the objective of the SESC program to analyze every condemned carcass. However a thorough systematic data gathering and a greater use of diagnostic tools might be valuable to establish meat inspection as an effective syndromic surveillance tool for animal diseases including zoonoses.

Implementation of SESC between 2008 and 2013

During the period from 2008 to 2013 a total of 975 cases were managed. The first year, only 60 cases were submitted since many inspectors were not still aware of the system. Then, in 2009, a peak of cases was registered, up to 279, but the following years the number of consultations was stabilized around 150 cases per year (Supplemental Table 2). Approximately 12% of each year's inquiries were purely telematic (telematics: methods of sending information between computers in different places), but the majority included submission of samples for laboratory analysis (Supplemental Table 2).

Supplemental table 3 shows a breakdown of each year's inquiries distributed by species. Bovine, porcine and poultry together covered more than 85% of the inquiries. As seen in supplemental Tables 4 (number of animals slaughtered per year) and 5 (annual weight of the meat produced in Catalan slaughterhouses), porcine and poultry are the two biggest production sectors in the region. However, the species with the highest number of inquiries has been the bovine, even though it is only the third species in the meat production ranking (Supplemental Table 5). The explanation is straightforward since many of the submissions, as discussed later, are cases of suspected zoonoses including bovine Cysticercosis (BC), caused by *Cysticercus bovis*, and TB, caused by *Mycobacterium tuberculosis* complex (MTBC) organisms.

The proportion of cases that elicit inquiries to the support service (Supplemental Table 6) was very small compared to the number of condemned carcasses, and would be smaller if data of viscera condemnation would have been considered. This is to be expected, since the use of SESC is not compulsory for meat inspectors, and it depends on their criteria if a case is to be submitted for diagnosis or not. Thus, only cases in which the inspector has doubts regarding the final diagnosis are to be

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submitted. The rate of case submission was higher for large animals (i.e. cattle and horse). This was predictable since the value of a single carcass is comparatively higher and the need of a solid reason for condemnation might have encouraged inspectors to submit more cases. Moreover, the occurrence of zoonosis such as bovine cysticercosis or TB in cattle is also a factor increasing the case submission rate in this specie.

The diagnostic data presented hereon must be interpreted in the context of SESC operation discussed above and, thus, it is not necessarily representative of the actual animal health epidemiological picture of Catalonia but is of interest to illustrate the value of the program with respect to surveillance and public health. Of particular relevance is the bias posed by the fact that only those cases that somehow generated diagnostic uncertainty to the inspector were submitted.

#### Cases from bovine slaughterhouses

Regarding bovine (*Bos taurus*) inquiries, 161 out of 537 (29.9%) were bovine TB suspected cases. From them, 97 out of 161 (60%) were confirmed to be TB by means of pathological analysis, *Mycobacterium tuberculosis* complex (MTBC) detection by direct PCR and/or isolation, and PCR identification of MTBC. Over the years, a substantial reduction of TB cases has been noticed in Catalonia (Figure 1). This is in agreement with the decreasing herd prevalence of TB in the region, which decreased from 0.85% in 2008 to 0.04% in 2013.<sup>18</sup> Thus, the proportion of non-confirmed TB suspected cases should have increased comparatively, as indeed happened in 2010 and 2011. But, in absolute terms, the number of suspected but not confirmed TB cases also diminished. The non-TB submission rate per 1000 culled cattle was calculated and is shown in Table 1. This decreasing rate could be

explained by either (A) a reduced awareness of meat inspectors or (B) an increased experience towards recognizing non-TB lesions, which were consequently not submitted for confirmation. At this time point, when the prevalence curve has reached an asymptotic phase in the TB eradication program, passive surveillance of TB at the slaughterhouse is crucial to detect and control new outbreaks.<sup>6,17</sup> Thus, any granulomatous-like lesion found at slaughterhouse should be tested to rule out putative new TB outbreaks (Figure 2 and 3). A summary of the differential diagnoses of suspected but not confirmed TB cases can be found in Table 2.

Another main reason to submit bovine samples to SESC was to rule out another zoonosis, bovine cysticercosis (BC), caused by the larval stage of *Taenia saginata*: 184 out of 537 (34%) (Figure 4 and 5). These submissions included also those inquiries where the inspectors wanted a differential diagnosis between BC and eosinophilic myositis caused by *Sarcocystis* spp.. Although *Sarcocystis* spp. cysts were not always observed associated to this type of lesions, this diagnosis was made based on the inflammatory cell infiltrate, enriched in eosinophils.

Most suspected BC cases were submitted as muscle tissue samples, mainly in the myocardium, masseter muscles and tongue, but some liver samples were also submitted under this presumptive diagnosis. Of these cases, 110 out of 184 (60%) were confirmed to be lesions indicative of BC. As it happened with TB suspects, a decreasing trend in the numbers of BC suspected cases submitted to SESC (Figure 6) has been noticed over the years. BC prevalence detected through meat inspection in Catalonia is as low as 0.02% (0.01–0.03, 95% confidence interval, CI) and this is an underestimation according to serological studies which calculated a prevalence of 1.1% (0.8–1.8, 95% CI).<sup>3</sup> The ability of meat inspection to detect BC is considered to be limited<sup>2</sup>, particularly in the current epidemiological context where lightly infested

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cases are expected.<sup>8</sup> Thus, sectioning of target muscles, such as myocardium and masseters, to detect BC is at debate since it might increase meat contamination. <sup>1,9</sup> But until more sensitive (antemortem serological) methods are validated and implemented, meat inspection remains the only means for BC prevalence control and consumer protection. In fact, additional myocardial and other tissue cuts have been suggested to increase its effectiveness.<sup>12</sup>

Supplemental Table 7 summarizes all inquiries from bovine carcasses, and a few examples are shown in figures 2-5 and 7-10. For each inquiry, one single diagnosis has been recorded in the table. When a final etiologic diagnosis was not established, a short description of the lesion was given. Also, a few inquiries included more than one animal, but often with the same lesion: these were recorded once. Additionally, 4 inquiries were submitted regarding lesions found in buffalo carcasses (*Bubalus bubalis*). Three of them corresponded to TB suspected lesions, which were not confirmed (they were: unspecific granulomatous lesions (n=2) and foreign lipidic material resorption (n=1)). A fourth case was a parasitic lesion compatible with hydatid cyst

#### Cases from porcine slaughterhouses

Due to its different processing, pig carcasses are commercialized with its skin on. Thus, skin alterations gain relevance in the pathologies detected at the slaughterhouse since these might be cause of carcass condemnation; 47/181 (25%) of the porcine inquiries involved alteration of the skin (Figures 11 to 16). However, the scalding process of the carcasses causes important artifacts that alter the histological appearance of the epidermis and superficial dermis, compromising the proper histopathological interpretation. In many occasions, only unspecific lesions

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can be identified, such as vascular congestion and perivascular dermatitis with variable presence of eosinophils. These lesions, grossly identified as erythema, are often accompanied by blood reabsorption in lymph nodes, which might be generalized (Figure 12). This situation poses a dilemma to the meat inspector since lymphadenopathy might be interpreted as a sign of generalized disease (which would require condemnation of the whole carcass). The causes of skin erythema in pig carcasses are multiple, including incorrect husbandry and/or stress, inappropriate bleeding, and infectious generalized disease among others. Even though the first one might not pose a risk for human consumers, it needs to be studied on animal welfare grounds. The latter demands a careful inspection of the carcass to rule out other signs of sepsis (such as multiple petechiae) and might benefit from laboratory confirmation. Table 4 summarizes all skin conditions reported in this period.

The life cycle of *Taenia solium* is difficult to be completed in current porcine management systems; however, autochthonous and imported human cases of *Cysticercus cellullosae* and taeniosis are still being diagnosed in Europe.<sup>30</sup> During the reported period only two parasitic muscle granulomas in pigs were submitted compatible with *C. cellullosae*, but PCR ruled out the diagnosis. The implementation of the support network allows early detection and confirmation of possible reemergence of this zoonosis in swine. *Cysticercus tenuicollis,* however, was not an unusual finding (n=14). No cases of *Trichinella* spp. were recorded in domestic pigs during this period (data obtained from ASPC). Since *Trichinella* spp. diagnosis is performed at the slaughterhouse, no samples regarding this infestation where submitted.

Several cases of widespread granulomatous lesions were detected in pig carcasses from five different farms in late 2010 and early 2011. A final diagnosis of

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mycobacteriosis due to *M. avium* subsp. *avium* was attained.<sup>23</sup> Interestingly, pigs are highly susceptible to *M. avium* complex infections, displaying lesions undistinguishable from those caused by MTBC.<sup>6</sup> Therefore, a rapid diagnosis becomes crucial in terms of occupational hazards. In that case, the support network allowed for a rapid identification and management of the outbreak as well as for an assessment of the public health and occupational risks associated to it.

A considerable number of neoplastic lesions were submitted during the study period. The most frequent were lymphomas (9/26) (Figure 17) closely followed by melanomas (5/26) (Figure 14). These figures are in accordance with the published literature, but nephroblastoma was only diagnosed in one occasion although it is frequently reported in the literature.<sup>11</sup> The systematic analysis of the tumors allowed the identification of two neoplasms that had not previously described in this species: a liposarcoma<sup>7</sup> and two cases of osteochondromatosis<sup>4</sup>; additionally a rare presentation of multiple cutaneous mast cell tumours was described.<sup>19</sup>

Supplemental table 8 summarizes all inquiries from porcine carcasses. A few examples of lesions found in porcine carcasses are shown in figures 11 to 18.

#### Cases from small ruminant slaughterhouses

Supplemental tables 9 and 10 summarize caprine and ovine enquires, respectively. From the small ruminant cases, zoonoses affecting the skin such as orf (Figure 19), scabies (Figure 20) or ringworm (Figures 21 and 22), affecting particularly goat kids, were diagnosed. It is also noteworthy that 5 cases of granulomatous lesions compatible with TB were detected in adult goat carcasses. Indeed, caprine TB, either caused by *M. caprae* or *M. bovis*, is an emerging disease in a number of European countries.<sup>5,24,26,27</sup> In fact, infected goats may be a source of infection for cattle.<sup>21</sup>The 5 TB goat cases were detected in adult goats, a population which represents only a 0.18% of the number of goats slaughtered (2011 data published by MAGRAMA), since adult goats are rarely sent to slaughterhouse compared to goat kids, which are extensively consumed (Supplemental table 4). Thus, given the small numbers of slaughtered animals, and considering the rather slow evolution of TB lesions, the proportion of TB cases detected in this population should not be underestimated. A few examples of small ruminant cases are illustrated in figures 19 to 24.

#### Cases from poultry slaughterhouses

The majority of inquiries originated in poultry slaughterhouses were of lesions compatible with Marek's disease (MD) (Figure 25). During the study period, 85 cases of MD were histopathologically confirmed mostly in organic or slow-growing chickens (n=78) and less often in layers (n=5) and breeders (n=2). Among the differential diagnoses of MD, the most frequent was squamous cell carcinoma (n=11). Supplemental table 11 summarizes all confirmed diagnostics in cases submitted from poultry slaughterhouses. The emergence of the organic food market has broadened the range of pathologies observed at slaughter. For instance, diseases such as visceral gout (Figure 26), fungal (Figures 27) or viral dermatitis (Figure 28) were found, which are rarely seen in intensively reared chicken. Additional examples of poultry cases submitted are shown in figures 29 and 30.

#### Cases from rabbit and horse slaughterhouses

Supplemental tables 12 and 13 summarize cases submitted from slaughterhouses culling rabbits and horses, respectively. Several rabbit carcasses were submitted with a conspicuous blue discoloration of the rear limb musculature (Figure 31). As the time progressed, the blue "ink-like" coloration extended, affecting larger areas of the

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carcass. Microbiological analysis allowed identification of *Pseudomonas fluorescens*, a contaminating bacterium, as the cause of this change in color (Figure 32).<sup>13</sup> About 50% of rabbit inquiries involved white liver lesions either of bacterial origin or caused by parasites such as coccidia, most likely *Eimeria stiedae*, (Figure 33) and *Cysticercus pisiformis* (Figure 34).

Inquiries from horse slaughterhouses were rather sporadic (Figures 35 and 36).

#### Final remarks

The purpose of this slaughterhouse support network is to provide meat inspectors with continuing education tools to enhance/complement their diagnostic skills. With this objective in mind, a selection of cases is regularly published in a trilingual (Catalan, English, Spanish) free-access blog (www.cresa.cat/blogs/sesc). All the participating inspectors can benefit from the different cases posted and discuss them. Divulgation of updates is accomplished through social networks and a mailing list. Seminars are also organized to update inspectors on how to use the system and to discuss the cases.

A user satisfaction survey yielded a mean result over 9 on a scale from 0 to 10. However several organizational aspects of the network could be improved to promote inspector's engagement. These include a homogeneous sample transportation service for all slaughterhouses and a user-friendly smart phone-based application to obtain images of the lesions and submit information. Another limitation of the system is the low diagnostic efficiency of those inquiries based only on images, sometimes due to the low quality of the submitted images, stressing the need to provide inspectors with appropriate image capture technologies.

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The synergies obtained from the existence of this support network are multiple. Public health inspectors take advantage of the expertise, knowledge and networks of scientists and academic staff. These, in turn, benefit from a source of updated information on the animal diseases appearing at slaughterhouses. Altogether, this system represents a valuable insight to direct research efforts towards the most relevant needs. And this, in turn, benefits the animal production sector. Also, it is a pioneering system with few precedents in other countries where sampling programs at slaughterhouse exist but focused on specific surveillance programs, such as transmissible spongiform encephalopathies or TB. Of course animal health laboratory networks exist, such as California Animal Health & Food Safety Laboratory System (CAHFS) in the USA or Animal and Plant Health Agency (APHA) in the UK (formerly known as the Animal Health and Veterinary Laboratories Agency -AHVLA-) among many others, which include pathological and laboratorial investigation of disease outbreaks, but lack the key focus at the slaughterhouse level.

The "one world, one health" concept, which has been a trending topic over the last decade, emphasizes the impact of animal diseases on public health. Slaughterhouses are a key control point in the food chain: veterinary pathology and post mortem meat inspection has a unique potential of detecting subclinical diseases. Data obtained from slaughterhouses can be used for syndromic surveillance purposes if geographical information is available. <sup>10,28,29</sup> An efficient meat inspection not only helps detecting and controlling some food-borne diseases and zoonoses affecting the consumers, but also serves as sentinel for animal health and animal welfare issues. Collaboration between academia, administration and industry is key to make the most out of the data generated by slaughterhouse surveillance. <sup>15</sup>

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Indeed, SESC has proven to be a helpful tool to coordinate different administrative departments of the Catalan government (Public Health and Agriculture) in the sampling and laboratory diagnosis of animals that tested positive to the tuberculin skin test and to integrate this with the passive surveillance, i.e. reporting of slaughterhouse TB suspected cases. The improvement of surveillance and eradication programs of animal diseases requires holistic strategies. In this regard, slaughterhouse surveillance can effectively complement the active surveillance measures and epidemiological investigations (i.e., related to animal movements, shared pastures, wildlife reservoirs etc). As such, bovine TB control programs in industrialized countries are mainly based on test and slaughter of positive reactors, complemented by slaughterhouse surveillance. <sup>25</sup> During the period 2009-2011, 38% of the new bovine TB outbreaks in Catalonia were detected through the slaughterhouse surveillance conducted by SESC (unpublished data). Bovine TB detection through meat inspection has been shown to be an important tool in the detection of infected herds and, therefore, it should be emphasized.<sup>16,17,22</sup> Moreover, at the final stage of the bovine TB eradication program, there could be a transition from bovine TB-testing at farm level to slaughterhouse surveillance, and meat inspection may become the only surveillance component.<sup>1</sup> In the future, inclusion of inspectors supervising game meat for human consumption could also provide coverage to some of the diseases affecting wildlife. As an example, TB is a significant issue in wild boars in Spain, where these are extensively consumed.<sup>14,20</sup>

On the other hand, risk-based assessments of meat inspection procedures by food safety agencies recommend implementing visual only inspection.<sup>1</sup> For instance, in bovine species, *Salmonella* spp. and pathogenic verocytotoxin-producing *Escherichia coli* (VTEC) have been identified among the higher priority biological hazards. The

manipulations performed to carcasses during actual meat inspection procedures might enhance spreading and cross-contamination of these food-borne bacteria.<sup>1</sup> However, this new approach might reduce the capacity of detecting certain diseases, such as BC or TB.

In summary, SESC not only provides continuing education to inspectors, but also useful information regarding animal health and welfare. Therefore, it complements animal disease surveillance programs, such as bovine TB, and is a powerful tool to detect the (re)emergence of new or atypical animal diseases and zoonosis.

#### Acknowledgments

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#### Figure Legends:

**Figure 1.** Number of TB suspects submitted per year. The percentage of TB confirmed cases is indicated in the blue segment of the bar. During 2013 no new indigenous outbreaks were detected through slaughterhouse surveillance in Catalonia. However the number of TB diagnosed cases in imported veal calves increased compared to previous years.

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**Figures 2-5** Bovine cases (I). An elevated number of bovine cases were submitted to confirm or rule out zoonoses such as bovine cysticercosis or bovine tuberculosis. **Figure 2.** Nocardia sp., granulomatous lymphadenitis, tracheobronchial lymph node, cattle. On cut section, multifocal to coalescing, whitish areas. **Figure 3.** Presumptive Actinomycosis or Actinobacillosis, pyogranulomatous lymphadenitis, tracheobonchial lymph node, calf. Characteristic *Splendore Hoeppli* material surrounded by degenerate and viable neutrophils and necrotic debris. HE **Figure 4.** *Cysticercus bovis*, granulomatous myocarditis, heart, calf. Single, focal well circumscribed granuloma. **Figure 5.** *Cysticercus bovis*, myocardium, calf. Parasite scolex within a vesicle surrounded by granulomatous inflammatory infiltrate, HE.

**Figure 6.** Number of BC suspected cases submitted per year. The percentage of BC confirmed cases is indicated in the blue segment of the bar.

Figures 7-10 Bovine cases.(II) In many occasions sample submission to SESC allows the inspectors to obtain a final etiological diagnosis of their findings. Figure 7. Ringworm., cervical skin, calf. Oval raised crusty lesions, Figure 8. Dermatophytosis, hair, calf. Presence typical spherical arthrochonidia of dermatophytes on the hair surface taken from figure 7. Direct observation under the microscope. Figure 9. Besnoitia sp. pannicullitis, subcutaneous tissue, 3 year-old Gasconne cow. .Ecchymotic haemorrhages and presence of macroscopically visible besnoitia cysts conferring a "gitty" appearance to the caracass. Figure 10. Besnoitia sp. Pannicullitis and myiositis, subcutaneous tissue, 3 year-old Gasconne cow. Pannicullitis and myiositis associated to the presence of large *Besnoitia* spp. cysts within the cytoplasm of multinucleated macrophages, sample form.figure.9. HE.

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**Figures 11 to 14.** Porcine cases (I). Skin conditions, sometimes with involvement of regional lymph nodes, are the findings most commonly eliciting inquiries from pig slaughterhouses. **Figure 11.** Porcine Dermatitits and Nephropathy Syndrome (PDNS), erytematous-necrotizing lesions, skin, 2 years old sow. **Figure 12.** Porcine Dermatitits and Nephropathy Syndrome (PDNS), blood resorption (accumulation of blood in lymph nodes draining sites of hemorrhage), superficial inguinal lymph nodes of the carcass shown in Fig. 11, sow. Slightly enlarged and reddened lymph nodes. **Figure 13.** *Pityriasis rosea,* erythematous lesions, skin, 6 months old pig. Typical swine juvenile psoriasiform pustular dermatitis lesions..**Figure 14.** Melanoma, skin, 5 months old pig. Bulging black single nodular lesion.

**Figures 15 to 18.** Porcine cases (II). In some occasions a final etiological diagnosis could not be reached but those cases are extensively discussed and a list of possible differentials is sent to the submitting inspector. Regarding neoplasms lymphoma was the most frequently diagnosed in pig carcasses. **Figure 15.** Unknown etiology. dermatitis, skin, 6 months old pig. The lesion showed a peculiar radiating pattern, no samples were submitted for laboratorial diagnosis but ringworm was discussed among other possible etiologies. **Figure 16.** Unknown etiology generalized erythema, pig carcass. A severe congestion of the dermis was observed. The cause could not be determined but excessive scalding time and/or incomplete bleeding were discussed as possible causes. **Figure 17.** Multicentric lymphoma, liver, 6 months old pig. Multiple coalescing whitish hepatic nodules.. **Figure 18.** Unknown etiology, fibrinous peritonitis,cross bred, 6 months old pig. This lesion is typically associated to systemic bacterial infections such as *Haemophilus parasuis*, *Streptococcus suis* or *Mycoplasma hyorrhinis* 

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Figure 19-26. Small ruminant cases. Zoonotic skin conditions were frequently submitted for confirmation from small ruminant abattoirs. Figure 19. Orf proliferative lesions, lips and gums, 1 month old lamb. Figure 20. Sarcoptic mange, facial skin, 3 months old lamb.Crusty skin lesions covering the whole face. Figure 21. Ringworm, proliferative crusty skin lesions, facial skin, 1 month old kid. Mulitfocal well circumscribed proliferative-crusty lesions Figure 22. *Trichophyton verrucossum, hair, kid.* Samples cultured from lesions in figure 21.Mycosel agar culture. Figure 23. Mycoplasma ovis, jaundice, lamb carcasses. Figure 24 Ovine cysticercosis, Parasitic miliary granulomatous hepatitis, liver, 2 months old lamb.Miliar white nodular lesions in the liver parenchima

Figures 25-30 Poultry cases. Confirmation of Marek's disease was the most frequent reason for submission from poultry slaughterhouses. A range of pathologies associated to organic breeding of poultry were also noticeable. Figure 25. Marek's Disease, neoplasic lymphoid nodules, liver, broiler chicken. Multifocal coalescing white nodules. Figure 26. Renal gout,, kidney, organic bred chicken. Uric acid crystals deposited on the kidney Insert: Urate crystal eliciting a granulomatous nephritis. HE Figure 27. Fungi (unidentified) granulomatous dermatitis, skin, organic bred chicken. Two soft plaque-like skin lesions (arrowheads). Figure 28. Probable papillomavirus, dermatitis, comb, organic bred chicken. Multifocal crusty lesion in the comb.. Figure 29. Unknown etiology, haemorrhagic lesions, duodenum wall, chicken. Lesion typically found in poultry of no pathological significance. Figure 30. Green muscle disease, deep pectoral muscle, broiler chicken. Greenish discolouration of the deep pectoral muscle also known as deep pectoral muscle myopathy.

Figures 31-34 Rabbit cases. Rabbit livers with white lesions were frequently submitted for etiological diagnosis. Figure 31. *Pseudomonas fluorescens*, muscle, rabbit. Two rabbit carcasses presented with a blue discoloration of the muscular tissue. Figure 32 *Pseudomonas fluorescens*, the contaminating bacteria that caused the color alteration. Cetrimide agar culture.. Figure 33. *Cysticercus pisiformis*, granulomatous hepatitis, liver, rabbit. White multifocal lesions in the liver parenchyma due to larval migration tracts.. Figure 34. Coccidiosis, colangioheaptiits, liver , rabbit. Protozoan structures, compatible with a coccidiosis by *Eimeria stiedae*. HE. Figures 35-36 Equine cases Figure 35. *Gasterophilus intestinalis, gastritis, stomach, horse. Multiple* arthropod larvae attached to the stomach wall. Figure 36. Lipodostrophy, epicardial fat, horse. Brownish discoloration of the adipose tissue due to pigment deposition.

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#### Commentary

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## Six-year follow-up of slaughterhouse surveillance (2008-2013): the Catalan Slaughterhouse Support Network (SESC)

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#### Abstract

Meat inspection has the ultimate objective of declaring the meat and offal obtained from carcasses of slaughtered animals fit or unfit for human consumption. This safeguards the health of consumers by ensuring that the foodstuff coming out of these establishments poses no risk to public health. Concomitantly, it contributes to animal disease surveillance. The Catalan Public Health Protection Agency (Generalitat de Catalunya) identified the need to provide its meat inspectors with a support structure to improve diagnostic capacity: the Slaughterhouse Support Network (SESC). The main goal of the SESC program was to offer continuing education to meat inspectors in order to improve the diagnostic capacity on the lesions observed at slaughterhouses. With this aim, a web-based application was designed. The system allowed meat inspectors to submit their enquiries inquiries, images of the lesions and, if needed, samples to conduct laboratory analysis. In this commentary, a review of the casuistic cases from the first six years of SESC operation (2008-2013) is presented and the data are analyzed within the context of the covered geographical region, Catalonia. The program not only provides continuing education to inspectors but, in addition, contributes to the collection of useful information on animal health and welfare. Therefore, SESC complements animal disease surveillance programs, such as tuberculosis, and is a powerful tool for early detection of (re)emergence of animal diseases and zoonosis.

**Keywords:** slaughterhouse, surveillance, pathology, food inspection, one health, food safety, continuing education

#### Introduction

Meat inspection, a task traditionally performed in slaughterhouses by veterinarians (sometimes assisted by meat inspection technicians), has the main objective of declaring the meat and offal obtained from carcasses of slaughtered animals fit or unfit for human consumption. Therefore, the safeguard of consumers' health by ensuring that the foodstuff coming out of these establishments poses no risk to public health is the ultimate goal. In addition, the diagnosis of lesions found during meat inspection might provide useful information regarding animal health and welfare issues that may have a relatively low relevance for public health but might be of great importance to farmers and veterinarians.

By mid 2000s, the Catalan Public Health Agency, belonging to the Health Department of the Catalan Government (*Generalitat de Catalunya*) identified the need to provide its meat inspectors with a support structure. In 2007, the Agency commissioned to *Centre de Recerca en Sanitat Animal* (CReSA) the organization of a system to support the meat inspectors: the Slaughterhouse Support Network (*Servei-servei\_de sSupport a escerxadorsESCorxadors*, SESC)

(http://www.cresa.cat/blogs/sesc/).

In this commentary we intend to introduce this innovative system to the readers along with a brief analysis of the data gathered during its first 6 years of operation. The objective is to emphasize the relevant role veterinary pathology has in meat inspection and, consequently, in improving public health and animal disease surveillance. Other relevant aspects are the synergy that is created between the administration's meat inspection services and both academic and research pathologists. Also we think it is proof of the benefits of applying new information

technologies to our field. Finally, the data presented on diagnoses is not intended to provide novel findings but rather to illustrate the challenges meat inspectors are faced with and how these are managed through the SESC program.

The main goal of the SESC program was to provide meat inspectors (official veterinarians of the Catalan Public Health Agency) with continuing education in their ability to diagnose lesions they might come across in slaughterhouses of Catalonia. With this aim, a web-based application was designed through which meat inspectors could submit their enquiries along with images of the lesions found and, if needed, samples to conduct laboratory analysis. The objective was to reach a final diagnosis of each case and send a final report to the inspector. It is important to note that condemnation of the carcass, a part of it, or any affected viscera has to be based on current legislation and the inspector's criteria, not on the report received form SESC. However, in some instances, the result of the report can influence or refine the inspector's final decision. That would be the case, for example, of Cysticercus bovis compatible lesions confirmation or if a lesion compatible with tuberculosis (TB) was found. In many occasions, the query leads to a final diagnosis, thus supporting the inspectors decision and improving its quality and reliability. Since condemnation is not to be based on SESC's report the time delay between the moment an inquiry is received and when the answer is delivered is not critical. However, as mentioned above, in some occasions the resolution of the case is urgent. In these cases the inspector can label the inquiry as urgent in the web-application. Urgent-labeled inquiries will be answered in 24 hours, unless sample analysis is included, as this might delay the answer depending on the tests performed.

These enquiries inquiries, when received at SESC, are forwarded to a number of veterinary pathologists and other animal health and welfare professionals of CReSA

and the *Universitat Autònoma de Barcelona* (UAB) Veterinary Faculty. Occasionally, enquiries inquiries are also forwarded to international collaborators. With the answers obtained from the different experts, SESC's technicians elaborate a response that is submitted to the consulting inspector with copy to the public health authorities. The experts include mainly pathologists, but also parasitologists, microbiologists, virologists, immunologists, experts in animal welfare, meat science and food hygiene professionals and animal anatomists.

The web-based application form includes fields that the inspector has to complete with information regarding the slaughterhouse of origin, information about the animal (or animals) affected and a description of the lesions and organs involved. This information and the images of the lesions uploaded to the application form are used by the experts to elaborate a report with the most likely diagnosis and/or a list of possible differentials. Additionally, the meat inspector has the possibility to include information on tissue samples sent to SESC for laboratory analysis. SESC technicians process and distribute the samples to the most appropriate laboratories based on the experts' assessment of each case. Ideally, samples are to be submitted within the first 24 hours and kept refrigerated. Alternatively, when the submission is not done immediately, it is recommended to split the sample in two parts: one half kept frozen and the other fixed in formalin.

#### First six years of SESC in numbers

#### Context in which SESC operates

SESC gives coverage to all slaughterhouses of Catalonia. A total of 254 slaughter lines were active in this territory at the moment of writing this report; including 45

#### **Veterinary Pathology**

bovine lines, 76 ovine lines, 17 equines lines, 48 porcine lines, 44 poultry lines (9 of which cull anseriformes as well) and 24 lagomorph lines. Each slaughter line is covered by, at least, one official meat inspector.

Catalonia is the Spanish autonomous community with the largest numbers of animals slaughtered annually. Regarding the number of farms, poultry is the sector most represented in the region (n=6814 farms) followed by the bovine (n=6579) and porcine (n=5983) sectors (data from 2012, obtained from the Agriculture department website: http://www20.gencat.cat/portal/site/DAR). A significant amount of the livestock culled in Catalonia is imported from other Spanish autonomous communities and from other countries.

The percentage of carcasses that were fully or partially condemned each year in Catalonia is presented in **Supplemental Table 1**. A system, based on the current legislation, has been implemented to gather condemnation data. However this database includes broad lesion categories and data on specific relevant diseases such as zoonoses. For instance, one of the most frequently reported reasons for total or partial condemnation of carcasses, and indeed of viscera, was "inflammatory lesions", indicating that the diagnosis of these cases either was not reported or had never been established. The scope of this commentary is not to analyze the condemnation data nor is the objective of the SESC program to analyze every condemned carcass. However a thorough systematic data gathering and a greater use of diagnostic tools might be valuable to establish meat inspection as an effective syndromic surveillance tool for animal diseases including zoonoses.

Implementation of SESC between 2008 and 2013

 During the period from 2008 to 2013 a total of 975 cases were managed. The first year, only 60 cases were submitted since many inspectors were not still aware of the system. Then, in 2009, a peak of cases was registered, up to 279, but the following years the number of consultations was stabilized around 150 cases per year (Supplemental Table 12). Approximately 12% of each year's enquiries inquiries were purely telematic (telematics: methods of sending information between computers in different places), but the majority included submission of samples for laboratory analysis (Supplemental Table 12).

Supplemental Ttable 23 shows a breakdown of each year's enquiriesinguiries distributed by species. Bovine, porcine and poultry together covered more than 85% of the enquiriesinguiries. As seen in supplemental Tables 42 (number of animals slaughtered per year) and 53 (annual weight of the meat produced in Catalan slaughterhouses), porcine and poultry are the two biggest production sectors in the region. However, the species with the highest number of enquiriesinguiries has been the bovine, even though it is only the third species in the meat production ranking (Supplemental Table 35). The explanation is straightforward since many of the submissions, as discussed later, are cases of suspected zoonoses including bovine Cysticercosis (BC), caused by *Cysticercus bovis*, and TB, caused by *Mycobacterium tuberculosis* complex (MTBC) organisms.

The proportion of cases that elicit <u>enquiriesinguiries</u> to the support service (<u>Supplemental Table 36</u>) was very small compared to the number of condemned carcasses, and would be smaller if data of viscera condemnation would have been considered. This is to be expected, since the use of SESC is not compulsory for meat inspectors, and it depends on their criteria if a case is to be submitted for diagnosis or not. Thus, only cases in which the inspector has doubts regarding the final

diagnosis are to be submitted. The rate of case submission was higher for large animals (i.e. cattle and horse). This was predictable since the value of a single carcass is comparatively higher and the need of a solid reason for condemnation might have encouraged inspectors to submit more cases. Moreover, the occurrence of zoonosis such as bovine cysticercosis or TB in cattle is also a factor increasing the case submission rate in this specie. This was predictable since a much smaller number of these animals are sacrificed, particularly in the case of horses, of which small numbers are culled in Catalonia (hence the ratio of cases submitted per condemned carcass is higher). Also, since the value of a single carcass is comparatively higher the need of a solid reason for condemnation might have encouraged inspectors to submit more cases. The occurrence of zoonosis such as bovine cysticercosis in cattle and TB in cattle and small ruminants is also a factor increasing the case submission rate in these species.

The diagnostic data presented hereon must be interpreted in the context of SESC operation discusses discussed above and, thus, it is not necessarily representative of the actual animal health epidemiological picture of Catalonia but is of interest to illustrate the value of the program with respect to surveillance and public health. Of particular relevance is the bias posed by the fact that only those cases that somehow generated diagnostic uncertainty to the inspector were submitted.

#### Cases from bovine slaughterhouses

Regarding bovine (*Bos taurus*) enquiries inquiries, 161 out of 537 (29.9%) were bovine TB suspected cases. From them, 97 out of 161 (60%) were confirmed to be TB by means of pathological analysis, *Mycobacterium tuberculosis* complex (MTBC) detection by direct PCR and/or isolation, and PCR identification of MTBC. Over the years, a substantial reduction of TB cases has been noticed in Catalonia (Figure 1).

This is in agreement with the decreasing herd prevalence of TB in the region, which decreased from 0.85% in 2008 to 0.04% in 2013<sup>18</sup>, Thus, the proportion of nonconfirmed TB suspected cases should have increased comparatively, as indeed happened in 2010 and 2011. But, in absolute terms, the number of suspected but not confirmed TB cases also diminished. The non-TB submission rate per 1000 culled cattle was calculated and is shown in Table 41. This decreasing rate could be explained by either (A) a reduced awareness of meat inspectors or (B) an increased experience towards recognizing non-TB lesions, which were consequently not submitted for confirmation. At this time point, when the prevalence curve has reached an asymptotic phase in the TB eradication program, passive surveillance of TB at the slaughterhouse is crucial to detect and control new outbreaks<sup>6,17</sup>, Thus, any granulomatous-like lesion found at slaughterhouse should be tested to rule out putative new TB outbreaks (Figure 2 and 3). A summary of the differential diagnoses of suspected but not confirmed TB cases can be found in Table 52.

Another main reason to submit bovine samples to SESC was to rule out another zoonosis, bovine cysticercosis (BC), caused by the larval stage of *Taenia saginata*: 184 out of 537 (34%) (Figure 4 and 5). These submissions included also those enquiries inquiries where the inspectors wanted a differential diagnosis between BC and eosinophilic myositis caused by *Sarcocystis* spp.. Although *Sarcocystis* spp. cysts were not always observed associated to this type of lesions, this diagnosis was made based on the inflammatory cell infiltrate, enriched in eosinophils.

Most suspected BC cases were submitted as muscle tissue samples, mainly in the myocardium, masseter muscles and tongue, but some liver samples were also submitted under this presumptive diagnosis. Of these cases, 110 out of 184 (60%) were confirmed to be lesions indicative of BC. As it happened with TB suspects, a

decreasing trend in the numbers of BC suspected cases submitted to SESC (Figure 6) has been noticed over the years. BC prevalence detected through meat inspection in Catalonia is as low as 0.02% (0.01–0.03, 95% confidence interval, CI) and this is an underestimation according to serological studies which calculated a prevalence of 1.1% (0.8–1.8, 95% CI)<sup>3</sup>. The ability of meat inspection to detect BC is considered to be limited-<sup>2</sup>, particularly in the current epidemiological context where lightly infested cases are expected<sup>8</sup>. Thus, sectioning of target muscles, such as myocardium and masseters, to detect BC is at debate since it might increase meat contamination.<sup>1,9</sup>. But until more sensitive (antemortem serological) methods are validated and implemented, meat inspection remains the only means for BC prevalence control and consumer protection. In fact, additional myocardial and other tissue cuts have been suggested to increase its effectiveness.<sup>12</sup>.

Supplemental Table 4-7\_summarizes all enquiries inquiries from bovine carcasses, and a few examples are shown in figures 2-5 and 7-10. For each enquiry inquiry, one single diagnosis has been recorded in the table. When a final etiologic diagnosis was not established, a short description of the lesion was given. Also, a few enquiries included more than one animal, but often with the same lesion: these were recorded once. Additionally, 4 enquiries inquiries were submitted regarding lesions found in buffalo carcasses (*Bubalus bubalis*). Three of them corresponded to TB suspected lesions, which were not confirmed (they were: unspecific granulomatous lesions (n=2) and foreign lipidic material resorption (n=1)). A fourth case was a parasitic lesion compatible with hydatid cyst

#### Cases from porcine slaughterhouses

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Due to its different processing, pig carcasses are commercialized with its skin on. Thus, skin alterations gain relevance in the pathologies detected at the slaughterhouse since these might be cause of carcass condemnation; 47/181 (25%) of the porcine enquiries involved alteration of the skin (Figures 11 to 16). However, the scalding process of the carcasses causes important artifacts that alter the histological appearance of the epidermis and superficial dermis, compromising the proper histopathological interpretation. In many occasions, only unspecific lesions can be identified, such as vascular congestion and perivascular dermatitis with variable presence of eosinophils. These lesions, grossly identified as erythema, are often accompanied by blood reabsorption in lymph nodes, which might be generalized (Figure 12). This situation poses a dilemma to the meat inspector since lymphadenopathy might be interpreted as a sign of generalized disease (which would require condemnation of the whole carcass). The causes of skin erythema in pig carcasses are multiple, including incorrect husbandry and/or stress, inappropriate bleeding, and infectious generalized disease among others. Even though the first one might not pose a risk for human consumers, it needs to be studied on animal welfare grounds. The latter demands a careful inspection of the carcass to rule out other signs of sepsis (such as multiple petechiae) and might benefit from laboratory confirmation. Table 7-4 summarizes all skin conditions reported in this period.

The life cycle of *Taenia solium* is difficult to be completed in current porcine management systems; however, autochthonous and imported human cases of *Cysticercus cellullosae* and taeniosis are still being diagnosed in Europe\_-<sup>30</sup>, During the reported period only two parasitic muscle granulomas in pigs were submitted compatible with *C. cellullosae*, but PCR ruled out the diagnosis. The implementation of the support network allows early detection and confirmation of possible

### **Veterinary Pathology**

reemergence of this zoonosis in swine. *Cysticercus tenuicollis*, however, was not an unusual finding (n=14). Conversely, nNo cases of *Trichinella* spp. were recorded in domestic pigs during this period (data obtained from ASPC). Since *Trichinella* spp. diagnosis is performed at the slaughterhouse, no samples regarding this infestation where submitted.

Several cases of widespread granulomatous lesions were detected in pig carcasses from five different farms in late 2010 and early 2011. A final diagnosis of mycobacteriosis due to *M. avium* subsp. *avium* was attained  $2^{-23}$ . Interestingly, pigs are highly susceptible to *M. avium* complex infections, displaying lesions undistinguishable from those caused by MTBC  $\frac{6}{2}$ . Therefore, a rapid diagnosis becomes crucial in terms of occupational hazards. In that case, the support network allowed for a rapid identification and management of the outbreak as well as for an assessment of the public health and occupational risks associated to it.

A considerable <u>amount-number</u> of neoplastic lesions were submitted during the study period. The most frequent were lymphomas (9/26) (Figure 17) closely followed by melanomas (5/26) (Figure 14). These figures are in accordance with the published literature, but nephroblastoma was only diagnosed in one occasion although it is frequently reported in the literature.<sup>11</sup>- The systematic analysis of the tumors allowed the identification of two neoplasms that had not previously described in this species: a liposarcoma<sup>7</sup> and <del>a</del>-two cases of osteochondromatosis<sup>4</sup>; additionally a rare presentation of multiple cutaneous mast cell tumours was described.<sup>19</sup>-

Supplemental table <u>5</u>-8 summarizes all <u>enquiries inquiries</u> from porcine carcasses. A few examples of lesions found in porcine carcasses are shown in figures 11 to 18.

Cases from small ruminant slaughterhouses

 Supplemental tables 6.9 and 7-10 summarize caprine and ovine enquires, respectively. From the small ruminant cases, zeonocis zoonoses affecting the skin such as orf (Figure 19), scabies (Figure 20) or ringworm (Figures 21 and 22), affecting particularly goat kids, were diagnosed. It is also noteworthy that 5 cases of granulomatous lesions compatible with TB were detected in adult goat carcasses. Indeed, caprine TB, either caused by *M. caprae* or *M. bovis*, is an emerging disease in a number of European countries <sup>5,24,26,27</sup>. In fact, infected goats may be a source of infection for cattle, <sup>21</sup>. The 5 TB goat cases were detected in adult goats, a population which represents only a 0.18% of the number of goats slaughtered (2011 data published by MAGRAMA), since adult goats are rarely sent to slaughterhouse compared to goat kids, which are extensively consumed (Supplemental table 24). Thus, given the small numbers of slaughtered animals, and considering the rather slow evolution of TB lesions, the proportion of TB cases detected in this population should not be underestimated. A few examples of small ruminant cases are

illustrated in <mark>figures 19 to <del>2</del>6<u>24</u>.</mark>

## Cases from poultry slaughterhouses

The majority of enquiriesinguiries originated in poultry slaughterhouses were of lesions compatible with Marek's disease (MD) (Figure 2725). During the study period, 85 cases of MD were histopathologically confirmed mostly in organic or slow-growing chickens (n=78) and less often in layers (n=5) and breeders (n=2). Among the differential diagnosis-diagnoses of MD, the most frequent was squamous cell carcinoma (n=11). Supplemental table 8-11 summarizes all confirmed diagnostics in cases submitted from poultry slaughterhouses. The emergence of the organic food market has broadened the range of pathologies observed at slaughter. For instance, diseases such as visceral gout (Figure 2826), fungal (Figures 29-27 and 30) or viral

dermatitis (Figure 3128) were found, which are rarely seen in intensively reared chicken. Additional examples of poultry cases submitted are shown in figures 32-29 and to 340.

#### Cases from rabbit and horse slaughterhouses

Supplemental tables 9-12 and 40-13 summarize cases submitted from slaughterhouses culling rabbits and horses, respectively. Several rabbit carcasses were submitted with a conspicuous blue discoloration of the rear limb musculature (Figure 3531). As the time progressed, the blue "ink-like" coloration extended, affecting larger areas of the carcass. Microbiological analysis allowed identification of *Pseudomonas fluorescens*, a contaminating bacterium, as the cause of this change in color (Figure 3632).<sup>13</sup>- About 50% of rabbit enquiries involved white liver white lesions either of bacterial origin or caused by parasites such as coccidia, most likely *Eimeria stiedae*, (Figures 37-and-383) and *Cysticercus pisiformis* (Figure 3934).

(Figures 40-4235 and 36).

## **Final remarks**

The purpose of this slaughterhouse support network is to provide meat inspectors with continuing education tools to enhance/complement their diagnostic skills. With this objective in mind, a selection of cases is regularly published in a trilingual (Catalan, English, Spanish) free-access blog (www.cresa.cat/blogs/sesc). All the participating inspectors can benefit from the different cases posted and discuss them. Divulgation of updates is accomplished through social networks and a mailing list. Seminars are also organized to update inspectors on how to use the system and to discuss the cases.

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A user satisfaction survey yielded a mean result over 9 on a scale from 0 to 10. However several organizational aspects of the network could be improved to promote inspector's engagement. These include a homogeneous sample transportation service for all slaughterhouses and a user-friendly smart phone-based application to obtain images of the lesions and submit information. Another limitation of the system is the low diagnostic efficiency of those <u>enquiriesinguiries</u> based only on images, sometimes due to the low quality of the submitted images, stressing the need to provide inspectors with appropriate image capture technologies.

The synergies obtained from the existence of this support network are multiple. Public health inspectors take advantage of the expertise, knowledge and networks of scientists and academic staff. These, in turn, benefit from a source of updated information on the animal diseases appearing at slaughterhouses. Altogether, this system represents a valuable insight to direct research efforts towards the most relevant needs. And this, in turn, benefits the animal production sector. Also, it is a pioneering system with few precedents in other countries where sampling programs at slaughterhouse exist but focused on specific surveillance programs, such as transmissible spongiform encephalopathies or TB. Of course animal health laboratory networks exist, such as California Animal Health & Food Safety Laboratory System (CAHFS) in the USA or Animal and Plant Health Agency (APHA) in the UK (formerly known as the Animal Health and Veterinary Laboratories Agency -AHVLA-) among many others, which include pathological and laboratorial investigation of disease outbreaks, but lack the key focus at the slaughterhouse level.

The "one world, one health" concept, which has been a trending topic over the last decade, emphasizes the impact of animal diseases on public health.

Slaughterhouses are a key control point in the food chain: veterinary pathology and

post mortem meat inspection has a unique potential of detecting unnoticed subclinical diseases. Data obtained from slaughterhouses can be used for syndromic surveillance purposes if geographical information is available.<sup>10,28,29</sup>- An efficient meat inspection not only helps detecting and controlling some food-borne diseases and zoonoses affecting the consumers, but also serves as sentinel for animal health and animal welfare issues. Collaboration between academia, administration and industry is key to make the most out of the data generated by slaughterhouse surveillance.<sup>15</sup>-

Indeed, SESC has proven to be a helpful tool to coordinate different administrative departments of the Catalan government (Public Health and Agriculture) in the sampling and laboratory diagnosis of animals that tested positive to the tuberculin skin test and to integrate this with the passive surveillance, i.e. reporting of slaughterhouse TB suspected cases. The improvement of surveillance and eradication programs of animal diseases requires holistic strategies. In this regard, slaughterhouse surveillance can effectively complement the active surveillance measures and epidemiological investigations (i.e., related to animal movements, shared pastures, wildlife reservoirs etc). As such, bovine TB control programs in industrialized countries are mainly based on test and slaughter of positive reactors, complemented by slaughterhouse surveillance.<sup>25</sup>- During the period 2009-2011, a 38% of the new bovine TB outbreaks in Catalonia were detected through the slaughterhouse surveillance conducted by SESC (unpublished data). Bovine TB detection through meat inspection has been shown to be an important tool in the detection of infected herds herd breakdown detection through meat inspection has been evidenced to be an important complement for the detection of infected herds <sup>16,17,22</sup>-and, therefore, it should be emphasized.<sup>16,17,22</sup> Moreover, at the final stage of

the bovine TB eradication program, there could be a transition from bovine TB-testing at farm level to slaughterhouse surveillance, and meat inspection may become the only surveillance component\_-<sup>1</sup>- In the future, inclusion of inspectors supervising game meat for human consumption could also provide coverage to some of the diseases affecting wildlife. As an example, TB is a significant issue in wild boars in Spain, where these are extensively consumed\_-<sup>14,20</sup>-

On the other hand, risk-based assessments of meat inspection procedures by food safety agencies recommend implementing visual only inspection.<sup>1</sup>- For instance, in bovine species, *Salmonella* spp. and pathogenic verocytotoxin-producing *Escherichia coli* (VTEC) have been identified among the higher priority biological hazards. The manipulations performed to carcasses during actual meat inspection procedures might enhance spreading and cross-contamination of these food-borne bacteria.<sup>-1</sup>- However, this new approach might reduce the capacity of detecting certain diseases, such as BC or TB.

In summary, SESC not only provides continuing education to inspectors, but also useful information regarding animal health and welfare. Therefore, it complements animal disease surveillance programs, such as bovine TB, and is a powerful tool to detect the (re)emergence of new or atypical animal diseases and zoonosis.

#### Acknowledgments

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## Figure Legends:

**Figure 1.** Number of TB suspects submitted per year. The percentage of TB confirmed cases is indicated in the blue segment of the bar. During 2013 no new indigenous outbreaks were detected through slaughterhouse surveillance in Catalonia. However the number of TB diagnosed cases in imported veal calves increased compared to previous years.

Figures 2-5 and 7-10. Bovine casuistrycases (I). An elevated number of bovine cases were submitted to confirm or rule out zoonoses such as bovine cysticercosis of bovine tuberculosis, Figure 2. Nocardia sp., granulomatous lymphadenitis, tracheobronchial lymph node, cattle. Granulomatous lesions submitted as a TB compatible lesion, *Nocardia* was cultured, tracheobronchial lymph node. On cut section, multifocal to coalescing, whitish areas. Figure 3. Presumptive Actinomycosis or Actinobacillosis, pPyogranulomatous lymphadenitis, <u>in a tracheobonchial lymph</u> node, calf. <u>Characteristic SS</u>plendore Hoeppli material surrounded by degenerate and viable neutrophils and necrotic debris. , typical of bacterial ethiology (HE)HE Figure 4. <u>Cysticercus bovis</u>, granulomatous myocarditis, heart, calf. Single, focal well circumscribed granulomaGranuloma in the myocardium of a 1 year old Friesian calf. Figure 5. <u>Cysticercus bovis</u>, myocardium, calf. Parasitic granuloma, myocardium, suggestive of *Cysticercus bovis* (HE)Parasite scolex within a vesicle surrounded by granulomatous inflammatory infiltrate, HE. Figure 7. Oval crusty skin losions,

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corvical skin of an 11 months old calf submitted as a suspected case of scabies. **Figure 8.** Direct observation under the microscope of the hair from the losion in Fig.7 revealed it was a case of ringworm, confirmed by the presence typical spherical arthrochonidia of dermatophytos on the hair surface. **Figure 9.** Gritty appearance of the subcutaneous tissue in a 3 year old Gasconne cow carcass. **Figure 10.** Panniculitis and myositis associated to the presence of *Besnoitia* spp. cysts of the case shown in Fig.0 (HE).

**Figure 6.** Number of BC suspected cases submitted per year. The percentage of BC confirmed cases is indicated in the blue segment of the bar.

Figures 7-10 Bovine cases.(II) In many occasions sample submission to SESC allows the inspectors to obtain a final etiological diagnosis of their findings. Figure 7. Ringworm., cervical skin, calf. Oval raised crusty-skin lesions, cervical skin of an 11 months old calf submitted as a suspected case of scabies. Figure 8. Dermatophytosis, hair, calf. Presence typical spherical arthrochonidia of dermatophytes on the hair surface taken from figure 7. Direct observation under the microscope. of the hair from the lesion in Fig.7 revealed it was a case of ringworm, confirmed by the presence typical spherical arthrochonidia of dermatophytes on the hair surface. Figure 9. Besnoitia sp. pannicullitis, Gritty appearance of the subcutaneous tissue, in a 3. 3 year-old Gasconne cow, -carcass, Ecchymotic haemorrhages and presence of macroscopically visible besnoitia cysts conferring a "gitty" appearance to the caracass. Figure 10. Besnoitia sp. Pannicullitis and myiositis, subcutaneous tissue, 3 year-old Gasconne cow, Pannicullitis and myositis associated to the presence of large *Besnoitia* spp. cysts within the cytoplasm of multinucleated macrophages, sample form. of the case shown in Ffigure 9. (HE). Formatted: Highlight Formatted: Highlight

Figures 11 to 1814. Porcine casuistrycases (I). Skin conditions, sometimes with involvement of regional lymph nodes, are the findings most commonly eliciting inquiries from pig slaughterhouses. Figure 11. Porcine Dermatitits and Nephropathy Syndrome (PDNS), Eerytematous-necrotizing lesions, skin, 2 years old sow. histopathological examination revealed necrotizing lesions compatible with Porcine Dermatitits and Nephropathy Syndrome (PDNS) Figure 12. Porcine Dermatitits and Nephropathy Syndrome (PDNS), Bblood restorption (accumulation of blood in lymph nodes draining sites of hemorrhage), superficial inguinal lymph nodes of the carcass shown in Fig. 11, sow. sSlightly enlarged and reddened lymph nodes. Figure 13. Pityriasis rosea, Erythematous erythematous lesions, skin, in a-6 months old porciigne carcass. compatible with a Typical swine juvenile psoriasiform pustular dermatitis lesions..-also known as Pityriasis rosea Figure 14. Melanoma, skin, 5 months old pig-with metastasis in the regional lymph node. Bulging black single nodular lesion. Figure 15. Unsolved case of a dormatitis in a 6 months old pig carcass with a poculiar radiating pattern, no samples were submitted for laboratorial diagnosis but ringworm was discussed among other possible etiologies. Figure 16. Generalized erythema in a pig carcass due to severe congestion of the dermis. cause could not be determined but excessive scalding time and/or incomplete bleeding were discussed as possible causes. Figure 17. Multicentric lymphoma, liver, 6 months old pig. This was the most frequently diagnosed neoplasia in swine. Figure 18. Fibrinous peritonitis in a cross bred, 6 months old, pig carcass. This lesion is typically associated to systemic bacterial infections such as Haemophilus parasuis, Streptococcus suis or Mycoplasma hyorrhinis

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Figures 15 to 18. Porcine cases (II). In some occasions a final etiological diagnosis
could not be reached but those cases are extensively discussed and a list of possible
differentials is sent to the submitting inspector. Regarding neoplasms lymphoma was
<u>the most frequently diagnosed in pig carcasses. Figure 15. Un</u> known etiology. <u>solved</u>
<del>case of a </del> dermatitis, <mark>skin in a</mark> , 6 months old pig. <del>carcass</del> The lesion showed a <del>with a p</del>
peculiar radiating pattern, no samples were submitted for laboratorial diagnosis but
ringworm was discussed among other possible etiologies. Figure 16. Unknown
etiology Ggeneralized erythema, in a pig carcass. A due to severe congestion of the
dermis was observed. The cause could not be determined but excessive scalding
time and/or incomplete bleeding were discussed as possible causes. Figure 17.
Multicentric lymphoma, liver, 6 months old pig. This was the most frequently
diagnosed neoplasia in swineMultiple coalescing whitish hepatic nodules Figure 18.
Unknown etiology,
This lesion is typically associated to systemic bacterial infections such as
Haemophilus parasuis, Streptococcus suis or Mycoplasma hyorrhinis,
Figure 19-26. Small ruminant casuistrycases, Zoonotic skin conditions were
frequently submitted for confirmation from small ruminant abattoirs. Figure 19. Orf
proliferative lesions, lips and gums, of a 1 month old lamb. Figure 20. Sarcoptic
mange, facial skin, 3 months old lamb.Crusty skin lesions covering the whole face.
Figure 21. Ringworm, typical proliferative, crusty skin lesions, facial skin, 1 month
old kid. Mulitfocal well circumscribed proliferative-crusty lesions Figure 22. Growth
<del>of</del> Trichophyton verrucossum <u>, hair, kid.</u> from s <u>S</u> amples <u>cultured</u> of <u>rom</u> lesions in
figure 21 <u>.—(</u> Mycosel agar culture <u>.</u> ) Figure 23. <u>Mycoplasma ovis, jaundice, lamb</u>
carcassesSeveral lamb carcasses with icterus and splenomegaly Figure 24 Ovine
cysticercosis, Parasitic miliary granulomatous hepatitis, ,-liver, <u>of a-</u> 2 months old

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Iamb.Miliar white nodular lesions in the liver parenchima, compatible with ovine cysticercosis.. *Mycoplasma ovis* was confirmed by observation of cocoid-shaped bacteria within erythrocytes in a blood smear (arrowheads) form carcases shown in figure 23. **Figure 25.** Parasitis miliary granulomatous hopatitis, liver of a 2 months old lamb, compatible with ovine cysticercosis. **Figure 26.** Myocardial granulomas, heart, 3 months old lamb. Another case of ovine cysticercosis which had also lesions in the diaphragm.

Figures 2725-34-30 Poultry casuistry cases. Confirmation of Marek's disease was the Formatted: Highlight most frequent reason for submission from poultry slaughterhouses. A range of pathologies associated to organic breeding of poultry were also noticeable. Figure **2725.** Marek's Disease, neoplasic lymphoid nodules, liver, broiler chicken. Multifocal coalescing white nodules. Figure 2826. Renal gout Visceral gout, kidney, organic Formatted: Font: Not Bold bred chicken. -uUric acid crystals deposited on the kidneyviscera of an organic chicken Insert: Urate crystal eliciting a granulomatous nephritis. (HE) Figure 2927. Fungi (unidentified)al granulomatous dermatitis, skin, organic bred chicken, carcass evidenced as tT wo soft plaque-like skin lesions (arrowheads). Figure 30. Fungal hyphae within the lesions shown in figure 29 (arrowhead) (Groccott stain) Figure 3128. Probable papillomavirus, --Multifocal dermatitis, c-of the comb, organic bred Formatted: Font: Not Bold chicken., with intranuclear inclusion bodies suggestive of a viral etiology (inclusion bodies were positive to papillomavirus immunohistochemistry but viral particles could not be confirmed ultrastructurally) Multifocal crusty lesion in the comb. Figure 3229. Unknown etiology, Hhaemorrhagic lesions-, duodenum wall, often observed in Formatted: Font: Not Bold poultry, chicken. Lesion typically found in poultry of no pathological significance. Figure 3330. Green muscle disease, deep pectoral muscle, broiler chicken. Greenish discolouration of the deep pectoral muscle also known as or-deep pectoral muscle

myopathy in a broiler. Figure 34. Hypopion secondary to corneal ulceration in an ostrich.

Figures 3531-39-34 Rabbit casuistry and cases Figures 40-42 Equine casuistry. Rabbit livers with white lesions were frequently submitted for etiological diagnosis. Figure 3531. <u>Pseudomonas fluorescens</u>, muscle, rabbit. Two rabbit carcasses presented with a blue discoloration of the muscular tissue. Figure 362 Pseudomonas fluorescens, the contaminating bacteria that caused the color alteration. (Cetrimide agar culture.) Figure 37. White lesions in two rabbit livers. Figure 383. Cysticercus pisiformis, granulomatous hepatitis, liver, rabbit. White multifocal lesions in the liver parenchyma due to larval migration tracts. Another common cause of white granulomatous lesions in rabbit livers is Cysticercus pisiformis migration. Figure 394. Coccidiosis, colangioheaptiits, liver, rabbit. Protozoan structures, compatible with a coccidiosis by Eimeria stiedae., as the cause of the colangiohepatitis in the liver of figure 37 (HE). Figures 35-36 Equine cases Figure 4035. Multiple Gasterophilus intestinalis, gastritis, stomach, horse. Multiple arthropod larvae attached to the stomach wall, Horse. Figure 4136. Lipodostrophy, epicardial fat, horse. B-Brownish discoloration of the adipose tissuepericardial fat in a horse due to pigment deposition. Figure 42. Multiple pigment deposits of varying coloration among adypocites, probably within fagocytic cells. The lesion was suggestive of a wear-and-tear pigment, such as lipofuscin or ceroid, which could be a consequence of adipose tissue degeneration. Thus, the lesion was classified as a lipodystrophy.(HE)

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## Table 1: Non-TB granuloma submission rate

Suspected		ТВ		N° of culled	Non TB granuloma
Year	TB cases	confirmed	Non-TB	cattle	submission rate (1000)
 2009	54	37	17	473824	0.04
2010	42	29	13	480685	0.03
2011	24	11	13	477388	0.03
2012	18	8	10	477549	0.02
2013	15	9	6	479812	0.01

**TB** Tuberculosis

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 Table 2: Bovine TB differential diagnoses (N=64).

Rhodococcus equi (1)	y lesions of known origin (n=4)			
Pasteurella multocida (	(1)			
Nocardia spp. (1) (Figu				
	+Pasteurella multocida (1)			
was not established (n=2				
Bacterial granulomatou	us lymphadenitis (16) (Figure 3)			
Suppurative bronchop				
	h node foreing body granuloma(1)			
Hyperplasic/reactive ly				
Interstitial pneumonia (				
Chronic proliferative pe				
Chronic proliferative po				
Traumatic pericarditis				
Unspecific granulomatous pneumonia (1)				
Fungal (n=13)				
Fungal granulomatous	lymphadenitis (13)			
Parasitic (n=5)				
	us lesions (liver, lymph nodes) (3)			
Hidatidosis(liver and lu				
Bovine cysticercosis (r	nyocardium) (1)			
Neoplasia (n=8)				
Mesothelioma (4)				
Carcinoma (3)				
Histiocytic sarcoma (1)				
Other (n=9)				
Foreign lipid material r nodes) (2)	esorption (prescapular and cervical lymph			
Ectopic splenic tissue (	(1)			
No diagnosis reached				

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 Table 3: Bovine cysticercosis differential diagnoses (n=74)

Parasitic (n=36)
Eosinophylic myositis (34) due to Sarcocystis spp.
Cysticercus tenuicollis <sup>1</sup> (2)
Inflammatory Inflammatory lesions of which an etiological
diagnosis was not established. (n=7)
Bacterial lingual granuloma (2)
Unspecific myositis (3)
Suppurative myocarditis (embolic metastatic) (1)
Necrotic multifocal myocarditis (1)
Neoplasia (n=8)
Myocardial adenomatoid tumour (3)
Fibrovascular benign proliferation (3)
Spindle cell neoplasia (1)
Nerve sheath tumour (1)
<b>Other</b> ( <i>n</i> =23)
Epitelial cyst (7) either endocardial or biliary <sup>2</sup> .
No apparent lesions found (3)
Ectopic lymphoid tissue in myocardium (1)
Focal area of fibrosis and masseter degeneration (1)
Unspecific myocardial degeneration (1)
Unspecific multifocal necrosis (1)
No diagnosis reached (n=9)

<sup>1</sup>Parasitic cysts found in the liver serosa and diagnosed morphologically as *C.tenuicollis*. <sup>2</sup>Biliary serous cysts submitted as suspected parasitic vesicles.

**Table 4:** Summary of porcine enquiries involving the skin (n=47)

Inflammatory Includes inflammatory lesions of which an etiological
diagnosis was not established (n=27)
Perivascular eosinophilic dermatitis (10)
PDNS (Porcine dermatitis and nephopathy syndrome, Figure 11)
(8)
Pityriasis rosea (Figure 13) (5)
Actinic dermatitis (2)
Pimples (1)
Skin necrosis and scar tissue (1)
Infectious Includes inflammatory lesions of known origin (n=1)
Erysipelas (1)
Neoplasia (n=7)
Melanoma (5)
Mastocytoma (1)
Lymphoid cell neoplasia (1)
<b>Other</b> ( <i>n</i> =12)
Skin erythema/ congestion (2)
Multifocal petechiae (2)
Melanosis (1)
Generalized erythema (red pig, Figure 16 ) (1)
Traumatic skin injuries (1)
Undiagnosed dermatitis (rare multifocal dermatitis with a peculiar radial
pattern, Figure 15) (1)
No diagnosis reached $(n=4)^1$
<b>v</b>

<sup>1</sup>Mostly consisting of telematic enquiries in which the images did not provide enough information to obtain a diagnosis, in such cases a differential diagnostic list was provided to the submitting inspector

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Supplemental Table 1: Carcass condemnation data in Catalonia in the period 2008-2013.

		2008	2009	2010	2011	2012	2013
Bovine	Total	825(0.15)	567 (0.11)	625 (0.13)	892 (0.19)	544 (0.11)	457 (0.10)
	Partial	13858 (2.58)	10805 (2.28)	10470 (2.18)	12096 (2.53)	11352 (2.38)	11114 (2.32)
Porcine	Total	33206 (0.20)	26283 (0.16)	27867 (0.16)	30890 (0.18)	29111 (0.16)	26283 (0.14)
	Partial	555606 (3.34)	508001 (3.02)	308318 (1.81)	481342 (2.76)	610614 (3.38)	609842 (3.28)
Poultry	Total	2485204 (1.15)	2091014 (1.00)	1910962 (0.88)	1836412 (0.87)	1688858 (0.82)	1721279 (0.85)
	Partial	10190740 (4.73)	11606971 (5.56)	12818807 (5.92)	13172624 (6.21)	14522735 (7.03)	14455851 (7.13)
Ovine	Total	1612 (0.09)	1568 (0.09)	1402 (0.07)	1796 (0.11)	1401 (0.09)	1222 (0.09)
&caprine	Partial	13246 (0.75)	3702 (0.22)	3337 (0.16)	5358 (0.33)	4104 (0.27)	4464 (0.33)
Equine	Total	14 (0.21)	6 (0.09)	18 (0.26)	28 (0.34)	48 (0.66)	67 (1.01)
	Partial	77 (1.13)	125 (1.94)	106 (1.53)	276 (3.38)	112 (1.54)	133 (2.01)
Rabbit	Total	38936 (0.27)	35674 (0.26)	36940 (0.26)	48812 (0.35)	54270 (0.33)	43958 (0.28)
	Partial	178609 (1.24)	188539 (1.38)	276826 (1.98)	424755 (3.02)	341195 (2.08)	270854 (1.71)

Data provided by the Agència de Protecció de la Salut de Catalunya. This table does not include data on viscera condemnation. **Total**: number of carcasses totally condemned. **Partial**: number of carcasses of which only a part has been condemned. **In brackets**: percentage over the total number of animals slaughtered that year as reported in www.magrama.gob.es.

Supplemental Table 2: Number of enquiries submitted to SESC

Year	Laboratory	Telematic	TOTAL
2008	22	38	60
2009	267	12	279
2010	171	19	190
2011	136	12	148
2012	139	12	151
2013	124	23	147
TOTAL	859	116	975

#### Supplemental Table 3: Species distribution of the enquiries submitted to SESC

	2008	2009	2010	2011	2012	2013	TOTAL
Bovine	38	184	110	73	70	62	537
Porcine	12	21	21	32	57	38	181
Poultry	1	42	30	20	10	30	133
Ovine	5	22	14	11	2	10	64
Caprine	2	5	1	5	7	3	23
Equine	2	2	6	6	2	4	22
Rabbit	0	3	8	0	0	0	11
Buffalo	0	0	0	1	3	0	4
TOTAL	60	279	190	148	151	147	975

Supplemental table 4: Annual number of animals slaughtered in Catalonia<sup>1</sup>

2008	2009	2010	2011	2012	2013	TOTAL
492.678	473.824	480.685	477.388	477.549	479.812	2.881.936
16.729.435	16.717.935	16.898.418	17.449.951	18.042.794	18.615.004	104.453.537
210.836.000	208.593.000	206.072.000	212.182.000	206.482.000	202.790.000	1.246.955.000
1.505.647	1.476.210	1.508.456	1.432.138	1.341.826	1.224.407	8.488.684
125.553	174.616	177.512	179.797	174.412	134.190	966.080
6.697	6.374	6.864	8.167	7.280	6.623	42.005
13.329.000	13.663.000	14.147.000	14.082.000	16.413.000	15.885.000	87.519.000
	492.678 16.729.435 210.836.000 1.505.647 125.553 6.697	492.678         473.824           16.729.435         16.717.935           210.836.000         208.593.000           1.505.647         1.476.210           125.553         174.616           6.697         6.374	492.678473.824480.68516.729.43516.717.93516.898.418210.836.000208.593.000206.072.0001.505.6471.476.2101.508.456125.553174.616177.5126.6976.3746.864	492.678473.824480.685477.38816.729.43516.717.93516.898.41817.449.951210.836.000208.593.000206.072.000212.182.0001.505.6471.476.2101.508.4561.432.138125.553174.616177.512179.7976.6976.3746.8648.167	492.678473.824480.685477.388477.54916.729.43516.717.93516.898.41817.449.95118.042.794210.836.000208.593.000206.072.000212.182.000206.482.0001.505.6471.476.2101.508.4561.432.1381.341.826125.553174.616177.512179.797174.4126.6976.3746.8648.1677.280	492.678473.824480.685477.388477.549479.81216.729.43516.717.93516.898.41817.449.95118.042.79418.615.004210.836.000208.593.000206.072.000212.182.000206.482.000202.790.0001.505.6471.476.2101.508.4561.432.1381.341.8261.224.407125.553174.616177.512179.797174.412134.1906.6976.3746.8648.1677.2806.623

<sup>1</sup>Source: Encuesta de sacrificio de Ganado (<u>www.magrama.gob.es</u>)

Supplemental Table 5: Annual weight of meat (tons) of animals slaughtered in Catalonia<sup>1</sup>

200820092010201120122013TOTALBovine123.958116.390120.918118.241119.570118.502717.579Porcine1.348.8401.338.0571.369.4351.418.1981.454.1561.502.4908.431.176Poultry327.649342.437341.641355.847352.876345.7192.066.169Ovine18.71018.41119.32818.29417.43315.742107.918Caprine5417637677817846004.236Equine1.5701.5431.8202.1121.6991.62210.366Rabbit14.54814.92516.42416.19415.97918.10096.170								
Porcine         1.348.840         1.338.057         1.369.435         1.418.198         1.454.156         1.502.490         8.431.176           Poultry         327.649         342.437         341.641         355.847         352.876         345.719         2.066.169           Ovine         18.710         18.411         19.328         18.294         17.433         15.742         107.918           Caprine         541         763         767         781         784         600         4.236           Equine         1.570         1.543         1.820         2.112         1.699         1.622         10.366		2008	2009	2010	2011	2012	2013	TOTAL
Poultry         327.649         342.437         341.641         355.847         352.876         345.719         2.066.169           Ovine         18.710         18.411         19.328         18.294         17.433         15.742         107.918           Caprine         541         763         767         781         784         600         4.236           Equine         1.570         1.543         1.820         2.112         1.699         1.622         10.366	Bovine	123.958	116.390	120.918	118.241	119.570	118.502	717.579
Poultry         327.649         342.437         341.641         355.847         352.876         2.066.169           Ovine         18.710         18.411         19.328         18.294         17.433         15.742         107.918           Caprine         541         763         767         781         784         600         4.236           Equine         1.570         1.543         1.820         2.112         1.699         1.622         10.366	Porcine	1.348.840	1.338.057	1.369.435	1.418.198	1.454.156	1.502.490	8.431.176
Ovine         18.710         18.411         19.328         18.294         17.433         107.918           Caprine         541         763         767         781         784         600         4.236           Equine         1.570         1.543         1.820         2.112         1.699         1.622         10.366	Poultry	327.649	342.437	341.641	355.847	352.876	345.719	2.066.169
Equine 1.570 1.543 1.820 2.112 1.699 1.622 10.366	Ovine	18.710	18.411	19.328	18.294	17.433	15.742	107.918
	Caprine	541	763	767	781	784	600	4.236
Rabbit 14.548 14.925 16.424 16.194 15.979 18.100 96.170	Equine	1.570	1.543	1.820	2.112	1.699	1.622	10.366
		14.548	14.925	16.424	16.194	15.979	18.100	96.170

<sup>1</sup>Source: Encuesta de sacrificio de Ganado (<u>www.magrama.gob.es</u>)

**Supplemental Table 6**: Percentage (%) of cases submitted to SESC over the total number of condemned carcasses<sup>1</sup>

_	2008	2009	2010	2011	2012	2013
Bovine	0.2520	1.6180	1.0005	0.5698	0.6137	0.5358
Porcine	0.0020	0.0039	0.0062	0.0062	0.0089	0.0060
Poultry	0.0000	0.0003	0.0002	0.0001	0.0001	0.0002
Ovine & caprine	0.0471	0.5123	0.3165	0.2237	0.1635	0.2286
Equine	2.1978	1.5267	4.8387	1.9737	1.2500	2.0000
Rabbit	0.0000	0.0013	0.0025	0.0000	0.0000	0.0000

<sup>1</sup> The percentage has been calculated over the sum of the number of fully condemned carcasses and the number of partially condemned carcasses. Condemnation of viscera has not been accounted for in these calculations.

#### **Supplemental Table 7:** Summary of all bovine enquiries (n=537)

Parasitic (n=194)
Cysticercus bovis (myocardium, masseter, etc.) (110) (Figure 4 and 5)
Eosynophilic myositis, lesions attributed to Sarcocystis spp. (60)
Undetermined parasitic granulomatous lesions (6)
Cysticercus tenuicollis (5)
Besnoitiasis (4) (Figure 9 and 10)
Larvary migratory tracts (3)

Page 57 of 71	Veterinary Pathology		
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3	Fasciolasis (2)		
4	Hypodermabovis (2)		
5	Hydatidosis (2)		
6	Inflammatory Includes inflammatory lesions of which an etiological diagnosis		
7	was not established.(n=71)		
}	Granulomatous-suppurative bacterial lymphadenitis (16) (Figure 3)		
)	Perivascular eosinophylic dermatitis (3) Suppurative pneumonia (probably embolic metastatic) (3)		
0	Suppurative pheumonia (probably embolic metastatic) (3) Suppurative catharral brochopneumonia (5)		
1	Fibrinous/proliferative polyserositis (4)		
2	Reactive/hyperplastic lymph node (4)		
3	Foreign body granuloma (3)		
4	Steatitis (3)		
5	Hepatic cirrhosis (3)		
6	Unspecific myositis (3)		
7	Chronic fibrinous/fibrinohemorrhagic pericarditis (3)		
8	Ulcerative glositis (2)		
9	Pleuropneumonia (2)		
20	Lingual bacterial granuloma (2)		
21	Unspecific granulomatous pneumonia (1)		
2	Interstitial pneumonia (1) Fibrinous pleuritis (1)		
3	Subacute peritonitis (1)		
4	Chronic hepatitis (1)		
5	Hepatic abscess (1)		
6	Granulomatous multifocal epicarditis (1)		
7	Myocardial abscess (1)		
8	Pyogranulomatous necrotizing splenitis (1)		
9	Interstitial nephritis (1)		
0	Fibrinosuppurative panniculitis (1)		
1	Arthritis (1)		
2	Necrotic multifocal myocarditis (1)		
3	Suppurative myocarditis (embolic methastatic) (1)		
4	Infectious Includes inflammatory lesions of known origin. (n=109)		
5	Tuberculosis ( <i>M.bovis / M.caprae</i> ) (97)		
6	Pseudotuberculosis ( <i>C.pseudotuberculosis</i> ) (2)		
7	Paratuberculosis ( <i>M.avium</i> ) (1)		
8	Rhodococcus equi (1)		
9	Tuberculosis ( <i>M.bovis / M.caprae</i> ) (97) Pseudotuberculosis ( <i>C.pseudotuberculosis</i> ) (2) Paratuberculosis ( <i>M.avium</i> ) (1) <i>Rhodococcus equi</i> (1) <i>Nocardia</i> spp. (1) (Figure 2) <i>Pasteurella</i> spp. (1) <i>P. multocida</i> + <i>T. pyogenes</i> (1) Syncitial bovine respiratory virus (1) Pleuropneumonia ( <i>T. pyogenes</i> ) (1)		
0	Pasteurella spp. (1)		
1	P. multocida + T. pyogenes (1)		
2	Syncitial bovine respiratory virus (1)		
3			
4	Necrotizing orchitis ( <i>B.abortus</i> ?) (1)		
5	Bacterial granulomatous lymphadenitis (actinomyces-type bacterial		
6	growth, unidentified) (1) Suppurative lymphadenitis (corineform-type growth, unidentified) (1)		
7	Fungal (n=21)		
3			
9	Granulomatous fungal lymphadenitis (17)		
)	Ringworm (2) (Figure 7 and 8)		
	Pulmonary fungal granuloma (2)		
	Neoplasia (n=40)		
3	Sporadicbovinelymphoma (5)		
Ļ	Lungadenocarcinoma (5)		
5	Mesothelioma (5)		
6	Nerve sheath tumor (3)		
7	Myocardial fibrovascular bening proliferation (3)		
3	Adenomatoid myocardial tumour (3)		
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в	roncogenic carcinoma (2)
F	ibropapilomatosis (2)
	lelanoma (2)
	arcinoma (2)
	emangiopericytoma (1)
	eurofibroma (1)
	quamous cell carcinoma (1) lalignant ovarian neoplasia (1)
	denoma (1)
	pindle cell myocardial neoplasia (1)
	istiocytic sarcoma (1)
	ndetermined (1)
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	ocal chronic lymphangiectasy (1)
	ortic mineralization (1)
	hronic hepatotoxicity (1) enign epithelial hyperplasia (1)
	o diagnosis reached (27) <sup>1</sup>
	on diagnostic enquiries (n=4)
	ow to assess bovine age through dental study (2)
	ow to determine uremia portmortem (1)
	nquiry about TB PCR sensitivity (1)

<sup>1</sup>Mostly consisting of telematic enquiries in which the images did not provide enough information to obtain a diagnosis, in such cases a differential diagnostic list was provided to the submitting inspector.

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7	Supplemental Table 8: Summary of all porcine enquiries (N=181)
8	Parasitic (n=23)
9	Cysticercus tenuicollis (14)
	Hepatitis due to Ascaris suum (3)
10	Hydatidosis (2)
11	Muscular parasitic granuloma (PCR negative to <i>C.cellullosae</i> ) (2)
12	Hepaticparasitic granuloma (1)
13	Parasitic pleuropneumonia (1)
14	Inflammatory Includes inflammatory lesions of which an etiological diagnosis
15	was not established. (n= 49)
16	Perivascular eosinophilic dermatitis (10)
17	PDNS (Porcine dermatitis and nephopathy syndrome, Figure 11) (8)
18	Pityriasis rosea (Figure 13) (5)
19	Chronic fibrinous peritonitis (Figure 18) (4)
20	Actinic dermatitis (2)
21	Suppurative osteomyelitis (2)
22	Chronic mononuclear hepatitis (1)
23	Perihepatic necrotic nodule (1)
24	Fibrinopurulent peritonitis (1)
25	Pimples (1)
26	Interstitial nephritis (1)
27	Bacterial pyelonephritis (1)
	Neutrophil rich enlarged lymph node (1)
28	Chronic suppurative myositis (1)
29	Chronic fibrous pericarditis (1)
30	Multifocal abscesses (1)
31	Fibrinohemorrhagic-necrotizingpleuropneumonia (1)
32	Pneumonia with pulmonary sequestra (1)
33	Granulomatous pneumonia (1)
34	Apostematous pneumonia (1)
35	Embolicmetastatic pneumonia (1)
36	Unspecific colitis (1)
37	Skin necrosis and scar tissue (1)
38	Abscess with necrotic and mineralized content (1)
39	Infectious Includes inflammatory lesions of known origin. (n=18)
40	Mycobacteriosis (Mycobacterium avium subsp. avium) (7)
41	Progressive atrophic rhinitis ( <i>Pasteurella multocida</i> , positive to
42	dermonecrotoxin) (5)
43	Suppurative osteomyelitis ( <i>Trueperella pyogenes</i> ) (2)
44	Pleural and lung abscesses ( <i>Trueperella pyogenes</i> ) (1)
45	Mycoplasma hyopneumoniae (1)
46	Erysipelas (1)
40	Bacterial pleuropenumonia (multiple pathogens isolated) (1)
48	Neoplasia (n=26)
	Lymphoid cell neoplasia (Figure 17) (9)
49	Melanoma (Figure 14) (5)
50	Undifferentiatied sarcoma (2)
51	Osteochondromatosis (2) Mastocytoma (1)
52	Osteosarcoma (1)
53	Hysticitic sarcoma (1)
54	Liposarcoma (1)
55	Malignantpheochromocytoma (1)
56	Teratoma (1)
57	Hepatic adenoma (1)
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	oblastoma (1)
	(n=61)
	lial cysts (10)
	n torsion (4)
	muscle disease (3)
	neal cyst (3)
	mations (3)
	rythema/ congestion (2)
	lymphnodes (2)
	osis (2)
	ocal petechiae (2)
	ic nodular hyperplasia (2)
	ic splenic hemorrhages (1)
	nerative hepatopathy (1)
	s tissue (giant ear) (1)
	tic cyst (1)
	ic capsular fibrosis (1)
	n of liver lobe (1)
	nuscle necrosis (1)
	resorption in lymph nodes (1)
	exia (1)
	is callosities (1)
	/stic kidney (1)
	mination with <i>Pseudomonas fluorescens</i> (1)
	alized erythema due to excessive scalding (red pig ) (1)
	natic skin injuries (1) nosed dermatitis dermatitis (rare multifocal dermatitis with a
	cuous radial pattern, Figure 15) (1)
	ignosis reached (13) <sup>1</sup>
	liagnostic enquiries (n=4)
	ding <i>Trichinella spirallis</i> pathogenesis (1)
	ding digestive contamination of the carcass (1)
	the zoonotic potential of <i>Sarcocystis</i> spp.
	ding total vs partial lung condemnation when lesioned
<sup>1</sup> Mostl	consisting of telematic enquiries in which the images did not provide
enougl	n information to obtain a diagnosis, in such cases a differential diagnos
list was	s provided to the submitting inspector.
Suppl	emental Table 9: Summary of all caprine enquiries (n=23)
	matory Includes inflammatory lesions of which an etiological
	sis was not established. (n=2)
	itial pneumonia (1)
Foreio	n body lymphadenitis (prescapular lymph node) (1)

Foreign body lymphadenitis (prescapular lymph node) (1) **Infectious** *Includes inflammatory lesions of known origin. (n=13)* 

Orf (6) (Figure 19)

Tuberculosis (5)

Corynebacterium pseudotuberculosis (2)

Fungal (n=3)

Ringworm (3) (Figure 21 and 22)

In one of the cases Trichophyton verrucossum was isolated.

 Neoplasia (n=1)

 Melanoma (1)

 Other (n=4)

 Lipomatosis (1)

 Calcinosis circumscripta (preescapular lymph node and muscle) (1)

 Melanosis (1)

 Polycystic kidney + congenital biliary dysplasia (1)

Inflammatory Includes inflamm	natory lesions of which an etiological diagno
was not established. (n=14)	
Chronic interstitial nephritis (5	)
Chronic fibrous pleuropneumo	onia (1)
Fibrinous broncopneumonia (	1)
Chronic suppurative pyelonep	hritis (1)
Foreign body granulomatous I	ymphadenitis (1)
Foreign body granuloma (veg	etable) (1)
Labial ulceration (1)	
Blood resorption and lymph ne	
Suppurative multifocal myositi	is (1)
Suppurative orchitis (1)	
Infectious Includes inflammator	ry lesions of known origin. (n=13)
Corynebacterium pseudotube	rculosis (9)
Mycoplasma ovis (2) (Figure 2	23)
Orf (1)	
Staphilococcus aureus subsp	. anaerobius (1)
Parasitic (n=18)	
Cysticercus ovis (4) (Figure 24	4)
Cysticercus tenuicollis (3)	,
Eosinophilic myositis (Sarcoc	vstis spp.) (6)
Hepatic larval migratory tracts	
Verminous pneumonia (Dyctic	
Scabies (sarcoptic mange) (1)	
Parasitic granulomatous hepa	
Hydatidosis (liver) (1)	
Neoplasia (n=1)	
Hepatocarcinoma (1)	
<b>Other</b> ( <i>n</i> =15)	
Biliary cysts (2)	
Biliary hyperplasia (1)	
Cryptorchidia (1)	
Epidermoid cyst (1)	
Epitelial cyst (1)	
Melanosis (1)	
Muscular hemosiderosis (1)	
Splenic hyperemia (1)	
Testicular hypoplasia (1)	
No significant lesions found (1	
No diagnosis reached $(n=3)^{1}$	,
Non diagnostic enquiries (n	=3)
About the risks associated to	
	onsumption of Sarcocystis infested me
(1).	
	sis of splenomegaly (1).

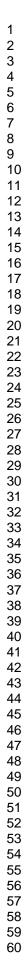
<sup>1</sup>Mostly consisting of telematic enquiries in which the images did not provide enough information to obtain a diagnosis, in such cases a differential diagnostic list was provided to the submitting inspector.

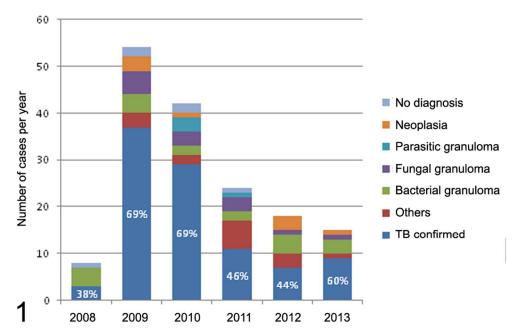
<b>Supplemental table 11:</b> Summary of all poultry enquiries (n=133) <b>Inflammatory</b> <i>Includes inflammatory lesions of which an etiological diagnosis</i> <i>was not established. (n=17)</i>
Septicemia (4)
Polyserositis (3)
Suppurative dermatitis (2)
Pododermatitis (2)
Hypopion (1)
Arthritis (1)
Chronic lymphoplasmacytic hepatitis (1)
Colangiohepatitis (1)
Gout (1) (Figure 26)
Proventriculitis (1)
Infectious Includes inflammatory lesions of known origin. (n=90)
<sup>1</sup> Marek's disease (85) (Figure 25)
Inclusion body hepatitis (Avian adenovirus) (2)
Necrotic enteritis (Clostridium perfringens) (1)
Arthritis (Avian reovirus) and Viral dermatitis (1) (Figure 28)
Avian pox (1)
Fungal (n=1)
Fungal dermatitis (1) (Figure 27)
Neoplasia <sup>1</sup> (n=14)
Squamous cell carcinoma (11)
Spindle cell (nerve sheath) benign tumour (leg muscle) (1)
Teratoma (1)
Adenocarcinoma (1)
<b>Other</b> ( <i>n</i> =11)
Non-pathologic multifocal hemorrhagic spots in the gut (1) (Figure 29)
Deep pectoral muscle myopathy (1) (Figure 30)
No significant lesions found (5)
No diagnosis reached (n=3) <sup>2</sup>
1 The 85 cases of Marek's diseases consisted in a lymphoid neoplasia affecting different organs. These have been classified as infectious even though are also neoplastic in nature. <sup>2</sup> Mostly consisting of telematic enquiries in which the images did not provide enough information to obtain a diagnosis, in such cases a differential diagnostic list was provided to the submitting inspector.
Supplemental table 12: Summary of all rabbit enquiries (n=11)
Inflammatory Includes inflammatory lesions of which an etiological diagnosis
was not established. (n=3)
Granulomatous suppurative hepatitis (3)
<b>Infectious</b> Includes inflammatory lesions of known origin. (n=1)
Suppurative catarral bronchopneumonia ( <i>Pasteurella multocida, Bordetella bronchiseptica</i> ) (1)
Parasitic (n=2)
Cysticercus pisiformis (1) (Figure 33)
Hepatic coccidiosis ( <i>Eimeria stiedae</i> ) (1) (Figure 34)
Other ( <i>n</i> =5)
The blue rabbit case - Contamination with Pseudomonas fluorescens (2)
(Figures 31 and 32)
No diagnosis reached (n=3) <sup>1</sup>
<sup>1</sup> Mostly consisting of telematic enquiries in which the images did not provide enough information to obtain a diagnosis, in such cases a differential diagnostic list was provided to the submitting inspector.

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Supplemental table 13: Summary of all equine enquiries (n=22)

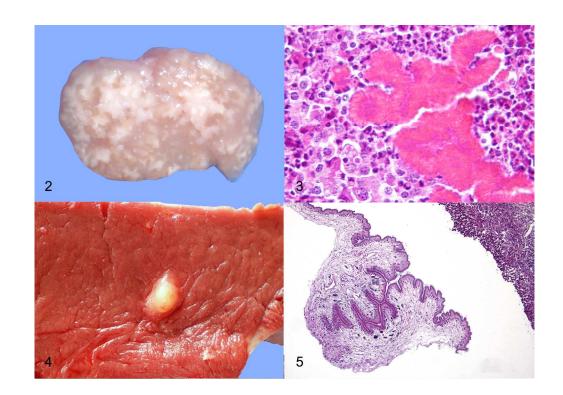
Infla	ammatory Includes inflammatory lesions of which an etiological diagnosis
was	not established. (n=6)
	ine multisystemic eosinophilic disease (1)
	tifocal suppurative pneumonia (1)
	purative dermatitis (1)
	ary granulomatous pneumonia (1)
	pecific lymphadenitis with mineralization (1)
	nulomatous-suppurative pneumonia and hepatitis (1)
-	asitic (n=4)
	sterophilus intestinalis (1) (Figure 35)
	plocephala perfoliata (1)
	toneal parasitic granuloma (1)
	/al hepatic migratory tracts (Strongylus spp.) (1)
	oplasia (n=4)
	oma (2)
	anoma (2)
	er (n=6)
	igenital mesonephric cysts (1)
	tissue necrosis (1)
	tissue degeneration (1) (Figure 36)
	significant lesions found (1)
	diagnosis reached (n=2) <sup>1</sup>
	n diagnostic enquiries (n=2)
	arding the determination of the age of a foetus (1)
1	ut Trichinella spiralis diagnosis (1)
	stly consisting of telematic enquiries in which the images did not provide
	ugh information to obtain a diagnosis, in such cases a differential diagnostic
IISEV	vas provided to the submitting inspector.



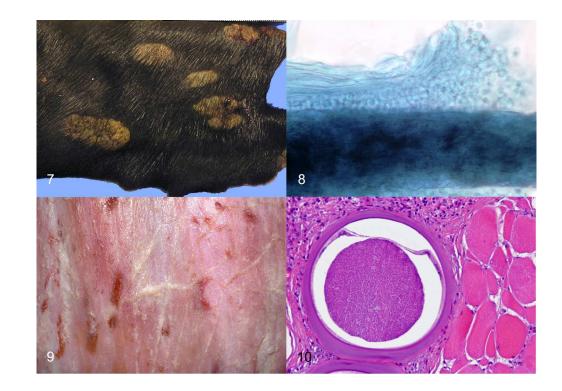


# Number of bovine tuberculosis suspects submitted per year

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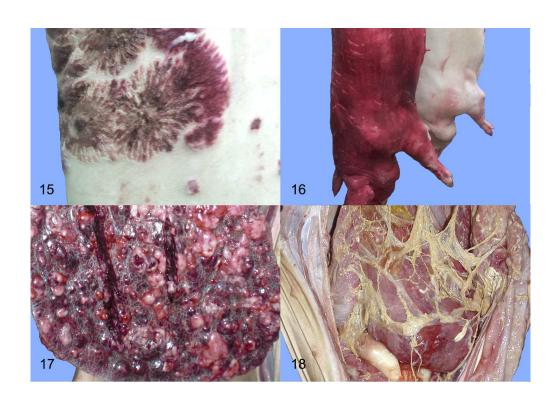
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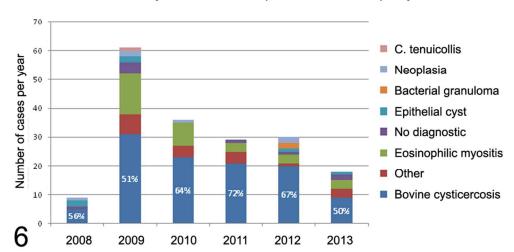


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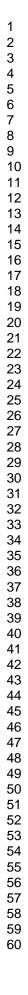
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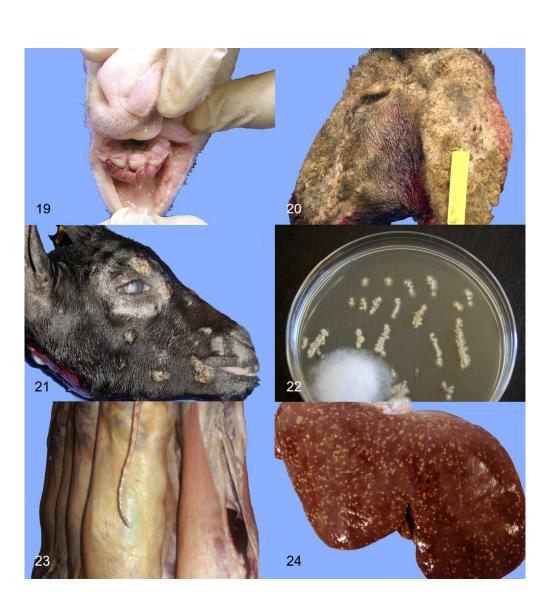


# Number of bovine cysticercosis suspects submitted per year

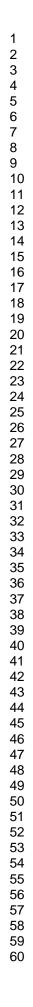
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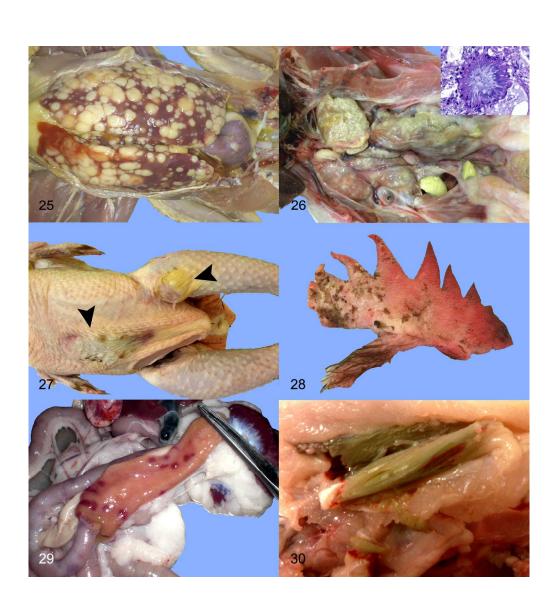




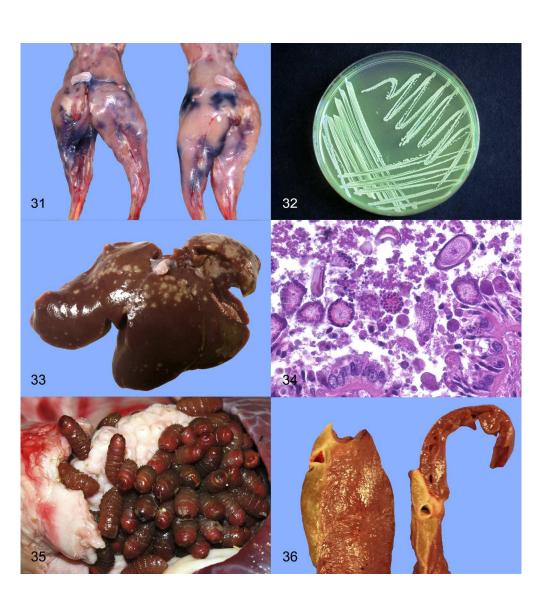


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