MINISTERIO DE CONSUMO
agencia española de seguridad alimentaria y nutrición

Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the representative sample size for *post-mortem* inspection of poultry and farmed lagomorphs

Reference number: AESAN-2020-006

Report approved by the Scientific Committee in its plenary session on 17 June 2020

Working group

Carlos Manuel Franco Abuín (Coordinator), Carlos Alonso Calleja, Pablo Fernández Escámez, Elena González Fandos, David Rodríguez Lázaro and Alberto Garre Pérez (External contributor)

Abstract

The Implementing Regulation (EU) 2019/627 provides generally that all slaughtered birds must undergo *post-mortem* inspection by the authority, which applies by extension to lagomorphs. However, it also provides for the possibility for the competent authority to decide to submit a representative sample of birds or lagomorphs to inspection, provided that a number of additional requirements are met. A study has thus been carried out to provide a method for establishing what would be a representative sample for *post-mortem* inspection by sampling of these types of animals. The prevalence of seizure in birds and/or lagomorphs, as well as the sizes of the flocks or lots under inspection have been identified as some of the parameters of interest for proposing a sampling method. It has thus been shown from the literature that the prevalence or percentage of seizures in both birds and lagomorphs would never reach 2 %. It has also been shown that most of the flocks or lots inspected will normally consist of more than 8000 animals. However, there are a significant number of cases of flocks or batches of lagomorphs consisting of significantly fewer than 8000 animals, which could be considered as finite

Translated from the original published in the journal: Revista del Comité Científico de la AESAN, 32, pp: 59-83

populations. On the one hand, it has been seen that from a statistical point of view the population under study would fit into a binomial distribution. Approximating this distribution from a normal distribution, it has been possible to calculate for a percentage of seizures of 2 % that in the case of lots larger than 8480 animals the sample size to be submitted to *post-mortem* inspection would be 424 animals. For smaller lots or herds the calculation of the minimum sample size would be given by a hypergeometric distribution. It has been possible to calculate the range of sample sizes for small population sizes, as well as how to perform all calculations. It is recommended that the time of sampling at slaughter should be chosen randomly throughout the period of slaughter of the flock or lot.

Key words

Poultry, rabbit, slaughterhouses, *post-mortem* inspection, sampling.

Suggested citation

AESAN Scientific Committee. (Working group) Franco, C.M., Alonso, C., Fernández, P., González, E., Rodríguez, D. and Garre, A. Informe del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) sobre el tamaño de muestra de aves de corral o lagomorfos, que se puede considerar representativa para su inspección *post mortem* en el matadero. *Revista del Comite Científico de la AESAN*, 2020, 32, pp: 59-83.

1. Introduction

In May 2012, the European Food Safety Authority (EFSA) published a Scientific Opinion on the public health hazards to be covered by inspection of meat (poultry) (EFSA, 2012). In this Opinion, the EFSA identifies and categorises the main public health hazards to be considered in the inspection of meat; evaluates the strengths and weaknesses of the methodology of traditional meat inspection; and recommends adapting meat inspection methods to achieve an equivalent level of protection.

The EFSA concludes that the traditional *post-mortem* system of inspection is unable to detect biological hazards that pose the greatest risk to public health, as well as chemical hazards in general. Nevertheless, it deems the current meat inspection system, both *ante* and *post-mortem*, to be an important tool for maintaining a supply of safe food items, and for a good management of animal health and welfare.

It also indicates that meat inspection is often key to identifying outbreaks of existing or new diseases. With regard to animal welfare, EFSA considers the *post-mortem* inspection of poultry to be an appropriate and practical way to assess the welfare of on-farm chickens.

The Commission Implementing Regulation (EU) 2019/627 of 15 March 2019, laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption, establishes in Article 25.1 that all slaughtered poultry shall undergo *post-mortem* inspection (EU, 2019).

However, taking into account the EFSA Opinion, Article 25.2 includes an exception to this general requirement, establishing that the competent authorities may decide to submit only a representative sample of poultry from each flock for *post-mortem* inspection, provided a series of additional requirements are met.

In accordance with Article 26, this exception shall also apply to lagomorphs, it being necessary to submit a representative sample of farmed lagomorphs slaughtered the same day from a single holding of provenance, for *post-mortem* inspection.

In order to perform *post-mortem* inspections in poultry and farmed lagomorphs from only a representative sample, it is necessary to have a national regulation that authorises this type of inspection and the conditions under which it shall be performed.

This regulation must establish the criteria for determining what percentage of animals from each flock of birds or farmed lagomorphs from a single holding of provenance slaughtered on the same day, constitutes a representative sample.

Therefore, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) is requested to perform an assessment in order to determine the representative sample size for the *post-mortem* inspection of each flock of poultry and farmed lagomorphs slaughtered the same day from a single holding of provenance. If it is not possible to set a general sample size, they shall define how it may be calculated, based on different parameters such as batch size, the prevalence of identified diseases in the *post-mortem* inspection, the problems detected in animals of the same provenance, or others that may be deemed necessary for this calculation.

2. General considerations on sampling methods

The obtaining and study of a sample must adequately represent the population from which it is taken, such that certain variables may be reproduced with calculable margins of error and the findings from said sample may be applied to the general population from which it is taken. There are two main types of sampling: one performed by researchers based on specific criteria (for example, sampling based on suspicion) that may not be representative of the population and others based on probability, such as simple random sampling, where all individuals have the same probability of being included in the sample.

For the case under study, the statistical properties of the sample are those of a binomial distribution, characterised by a series of parameters to be established, such as the confidence interval, margin of error, the size of the studied population and the expected proportion. Usually, the confidence interval and margin of error are set by the researcher, these commonly being 95 % and 5 % respectively in scientific studies. Nevertheless, the other two parameters must be estimated or calculated based on existing bibliography. In this case, the first is the total size of the batch undergoing *post-mortem* inspection and the second, the condemnation rate of that population. The following questions must be considered in this regard:

- What are the poultry condemnation percentages in traditional *post-mortem* inspection (without laboratory testing)?
- What is considered to be poultry based on the definition established in Regulation (EC) No. 853/2004 (EU, 2004a)?: Broilers, turkeys, ducks, egg-laying hens, other birds such as pigeons? Are there differences in data with regard to the number of unfit carcasses after the *post-mortem* inspection of these birds?
- Are the condemnation percentages fixed and stable over time?
- What are the condemnation percentages in traditional *post-mortem* inspection for lagomorphs?
- What other data is available on the species that are included among lagomorphs?
- What is the usual size of the inspected batches of birds and lagomorphs?

3. Statistical focus

3.1 Statistical focus for infinite populations

The problem under study consists of estimating the number of condemnations in a flock on the basis of the number of condemnations detected in a sample smaller than the flock. As not all the animals in the population are tested, it is impossible to know for certain the number of condemnations in the population. One way to express the uncertainty associated with estimates is to use a confidence interval. The Wald method may be used to compute the confidence interval for a proportion of the population (Box et al., 2005):

$$CI: \hat{p} \pm z_{1-\alpha/2}\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

Where $\hat{p}$ is the expected value of the sample proportion, *n* is the sample size and $z_{1-\alpha/2}$ is the percentile (1-α/2) of the normal distribution. For a standard case where α= 0.05, $z_{1-\alpha/2}$ is equal to 1.96, the equation would appear thus:

$$\hat{p} \pm 1{,}96\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

This method is based on the approximation to the binomial distribution using a normal distribution. The precision of this approximation depends on the sample size and the *p* value. For greater precision, it is recommended to ensure that the coefficient of symmetry is less than 1/3 (Box et al., 2005). This condition may be written as:

Condition 1: $$\frac{1}{\sqrt{n}}\left|\sqrt{\frac{1-p}{p}} - \sqrt{\frac{p}{1-p}}\right| < \frac{1}{3}$$

Once the precision of the estimator has been validated using the previous condition, the margin of error (MOE) is calculated by:

$$MOE = z_{1-\alpha/2}\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

This value may be interpreted as the uncertainty associated with the estimator of the population proportion. Thus, a minimum sample size may be defined on the basis of the expected precision of the estimator:

Condition 2: $$n \geq \left(\frac{z_{1-\frac{\alpha}{2}}}{MOE}\right)^2 p(1-p)$$

These two conditions may be used to determine a sample size on the basis of the expected prevalence (*p*), the confidence interval (α) and the margin of error (MOE). The first condition ensures the robustness of the estimator while the second ensures its precision. Note that both conditions are applied *a-priori*, that is to say, before sampling. Therefore, expected values of *p* based on historical data of the installation or literature review must be used.

3.2 Statistical focus for finite populations

The conditions calculated in the previous section assume that sampling results follow a binomial distribution. This is only true when the population size (the size of the flock, or the batch of rabbits in this case) is infinite. When the sample size is small in comparison to the population size, the importance of this deviation is small. Nevertheless, when the population size is small (the sample size is greater than 5 % of the population size), this deviation may affect the results. Therefore, it is necessary to make calculations using a hypergeometric distribution.

Calculating the minimum sample size for a hypergeometric distribution is more complicated than for a binomial distribution. This paper recommends using the iterative method suggested by Guenther (1973). This methodology deems the production system to be under control if the number of positives in a sample with size *n* is lesser than or equal to a total value *c*. As in any hypothesis testing, the sampling system may fail for two reasons: that a batch is accepted when production is out of control (i.e., when the condemnation percentage is higher than expected) (false negative) or the batch is rejected when production is under control (false positive). Note that, from a statistical point of view, there will always be a (small) possibility of a false positive or negative.

Mathematically, the possibility of a false negative (β) may be defined as the probability of accepting the batch when in reality it contains k_1 or more condemnations. On the other hand, the possibility of a false positive (1-α) may be defined as the probability of rejecting the batch when it contains k_0 or fewer condemnations. On the basis of this definition and Wise's (1954) proposal, Guenther (1973) specifies that the adequate sample size (*n*) for the probability of a false negative to be lower than β, and the probability of a false positive to be lower than 1-α, must be within the following limits:

$$\frac{1}{2}\left[\chi^2_{2c+2;1-\beta}\left(\frac{1}{p_1{}'}-0.5\right)+c\right] \leq n \leq \frac{1}{2}\left[\chi^2_{2c+2;\alpha}\left(\frac{1}{p_0{}'}-0.5\right)+c\right]$$

where:

- $\chi^2_{2c+2;1-\beta}$ is the 1-β percentile of the chi-square distribution with 2*c* + 2 degrees of freedom.
- $\chi^2_{2c+2;\alpha}$ is the α percentile of the chi-square distribution with 2*c* + 2 degrees of freedom.
- $p_1' = \dfrac{k_1 - c/2}{M}$ and $p_0' = \dfrac{k_0 - c/2}{M}$ with $M = N - \dfrac{n-1}{2}$

This equation does not have an analytical solution and must be solved using numerical methods. Based on Guenther's (1973) recommendations, the sample size is defined by the minimum values of *c* and *n* for which the earlier condition has a solution. Annex 1 includes a program written in the R programming language (version 3.5.3) that lets us solve this equation using fixed-point iteration from initial values of *c* and *n*.

4. Historical and legislative aspects

We may begin a short historical review by mentioning that the modern regulation of poultry inspections arose from the approval of the Technical and Health Regulation on poultry slaughterhouses, cutting plants, industrialisation, storage, preservation, distribution and marketing of their meat (BOE, 1985). This Technical and Health Regulation specified that a *post-mortem* inspection of poultry would be carried out by the Official Veterinary Services consisting of an examination of the carcass and the viscera, which must be identifiable as belonging to the original carcass until the definitive decision. A noteworthy aspect of this regulation is the little time available for inspection; a speed of 1200 light birds or 600 heavy birds per hour leaves only a few seconds to inspect each animal.

This circumstance demonstrates that the standard is of limited use in the context of a modern poultry slaughterhouse, as a large number of inspectors are required to carry out the inspection, not counting the physical effort required by the veterinarians to continuously monitor the line of carcasses. Likewise, the regulation made it clear that the observation must at least be direct and systematic. A useful aspect of this regulation is that it highlighted a series of essential aspects to be noted: the overall condition of the carcass, effectiveness of the bleeding, colour, smell, state of the serous membranes and air sacs, the presence of lesions, abnormalities or other anomalies.

Spain's entry into the European Union (EU) involved a transposition of the Community Directives that regulated trade in fresh poultry meat, basically Council Directive 71/118/EC and later modifications and updates (EU, 1971). All of this was consolidated in Royal Decree 2087/1994 of 20 October, which laid down the sanitary conditions for the production and trading of fresh poultry meat (BOE, 1994b). In this regulation, the section on *post-mortem* inspection continued to focus on the visual examination of the surface of the carcass, the head and legs when meant for human consumption, and the viscera and body cavity. It also ordered more detailed examinations whenever necessary, such as abnormalities in the consistency, etc. Nevertheless, this Royal Decree already specifies minimum sample values for *post-mortem* sanitary inspection of the viscera and the body cavity, with a sample size of 300 birds from the batch submitted for *post-mortem* inspection. It also establishes a sample value of 5 % of the slaughtered birds, in order to inspect the viscera and the body cavity in the case of partially eviscerated birds. Thus we may consider that the regulation implied that for large batches, the maximum number of birds to be inspected would be 300, and a lower value in the case of smaller batches.

The application of the informally titled "hygiene package" at the beginning of the century led to the entry into force of Regulation (EC) No. 854/2004 (EU, 2004b). This regulation makes it clear that the official veterinarian shall be personally responsible for inspecting the viscera and body cavities of a representative sample of poultry. It also makes the role of the auxiliaries clear, as well as the possible use of slaughterhouse personnel as official specialised assistants when fulfilling a series of requirements. Finally, the Commission Implementing Regulation (EU) 2019/627 consolidates the inspection of a representative sample of the flock under *post-mortem* inspection. Nevertheless, in contrast to Royal Decree 2087/1994, it does not mention the number or how to calculate said sample (BOE, 1994b) (EU, 2019).

In the case of lagomorphs, the first community directives included the type of inspection to be made, similar to that carried out on poultry, as the same reference standards were used, nevertheless, Spanish regulations did not mention the number of samples to be taken, nor did they refer to sampling in *post-mortem* inspection until the publication of the hygiene package at the beginning of this century. Thus within the historical context prior to Spain's entry into the European Community, we have the Technical and Health Regulation on rabbit slaughterhouses, approved by Royal Decree 1915/1984 (BOE, 1984). This Royal Decree establishes, at the very least, a direct and systematic observation of the viscera and other organs as being necessary during the *post-mortem* inspection. It also mentions that special attention must be paid to the overall condition, effectiveness of the bleeding, colour, smell, state of the serous membranes and the presence of lesions, abnormalities or other anomalies. As noted, it is practically identical to the Technical and Health Regulation on poultry. It is also worth highlighting that the Technical and Health Regulation on rabbits was published before the one on poultry. Mention must also be made of Royal Decree 1543/1994, after Spain's entry into the EU, which cites aspects for observation similar to those mentioned in the earlier regulations (BOE, 1994a). However, and in contrast to the Royal Decree on poultry slaughterhouses published in the same year (Royal Decree 2087/1994), it does not mention sampling of the animal consignment (BOE, 1994b). Subsequent to the publication of the hygiene package, it was specified in Regulation (EC) No. 854/2004 and the Commission Implementing Regulation (EU) 2019/627 that the same requirements would apply to both poultry and lagomorphs (EU, 2004b, 2019). Therefore, it would also reference the sampling established for poultry. On 11 February 2009, the plenary session of the AESAN Scientific Committee (AESAN, 2009) approved a report on the evisceration of lagomorphs where evisceration was not complete and the carcasses retained the thoracic viscera and the liver, highlighting that at least 5 % of the carcasses should be directly inspected by the Official Veterinary Services. Thus it basically adopts the provisions of the standards for poultry in Royal Decree 2087/94 for partially eviscerated birds.

5. Prevalence of detectable processes in the *post-mortem* inspection

From the previous section, we may deduce a series of aspects to be identified in the *post-mortem* inspection of poultry as well as lagomorphs, and which may be reflected in some of the guidelines of the previously mentioned regulations. It is necessary to point out that the earlier regulations often focus on guidelines that arise as the direct result of an etiological agent, but not on the pathological appearance that is visible upon inspection such as salmonellosis or myxomatosis in the case of rabbits. These guidelines are not useful in and of themselves, however they mention other aspects that we shall list below:

- For poultry:
  - General mycosis.
  - General infectious diseases.
  - Extensive parasitism.
  - Abnormal smell, colour, taste.
  - Multiple tumours.
  - Stains or contamination.
  - Serious lesions and ecchymosis.
  - Extensive mechanical lesions, including those caused by excessive scalding.
  - Insufficient bleeding.
  - Ascites.
- For rabbits:
  - General or partial putrefaction (smell and sticky consistency).
  - Bloody carcasses or bruising or meat haemorrhages.
  - Multiple abscesses.
  - Multiple tumours.

- Massive parasitic infestation.
- Serious trauma.
- Large muscle tears.
- Fractures.
- Meat with abnormal smell, taste or colour.
- Abnormal consistency, oedemas.
- Cachexia.
- Contamination.

Detailed information on these causes of condemnation along with illustrations may be obtained from Grist (2006) and Lara Moreno (2015) in the case of poultry. For rabbits, the photo records of Fàbregas i Comadran (1993) may be consulted.

A bibliographic review of the causes of condemnation in inspections at poultry slaughterhouses should tell us which of the aforementioned aspects are the most important. Nevertheless, it must be highlighted that the differences between countries with regard to production and hygiene practices, and the rapid evolution of these aspects of hygiene, production and slaughter of these types of animals, make it necessary to take into account the most recent information on a nearby environment.

One example of this is in the total percentage of condemnations in less developed countries such as Algiers where condemnation percentages are around 8.4 % (Alloui et al., 2012) or Brazil with a condemnation percentage of 8.3 % (Santana et al., 2008). On the contrary, data from 320 slaughterhouses in the United States within a similar time frame displays a distinctly lower condemnation percentage (1.01 %) (USDA, 2008).

Undoubtedly, the research carried out by Salines et al. (2017) is an example that considers both the data of our surroundings and up to date information, as it includes relatively recent data from 10 slaughterhouses in France. We may also include the data published by Part el al. (2016) owing to the volume of inspected animals, with data from the United Kingdom. Salines et al. (2017) also provide data on principal poultry animals such as chickens, turkeys and ducks as well as other less frequent birds such as guinea-fowl. They calculate a global condemnation percentage of 1.04 % for broilers, 1.85 % for turkeys and 1.23 % for lean ducks, 1.42 % for fattened ducks and 1.20 % for guinea-fowl. Salines et al. (2017) also point out that there are variations depending on slaughterhouses, season and sex. Other authors not referenced by Salines et al. (2017) have also detected similar condemnation percentages, for example, Ghaniei et al. (2016) notes that 0.92 % of broilers are condemned in Azerbaijan. If other types of poultry are considered, then the condemnation percentage may vary significantly. Thus, Vecerek et al. (2019) highlight notable differences with regard to the condemnation of laying hens, turkeys and broilers, with an elevated number of condemnations in laying hens. On a global basis and focusing on our country, we would have values of 1.04 % for broilers, 1.85 % for turkeys, between 1.23 % and 1.42 % for ducks and 1.20 % for guinea-fowl.

Condemnation in the case of poultry is primarily due to five reasons: cellulitis, cachexia, congestion/septicaemia, ascites and cyanosis, as listed in Salines et al. (2017). These causes are generally consistent among different countries and researchers and mention specific aspects that are visible upon inspection or macroscopic sensory examination (changes in aspect in the case of cellulitis, cachexia and ascites, with changes in the form, bony appearance, etc. or colour changes in the case of cyanosis or congestion/septicaemia); and are already included in more traditional regulations. Salines et al. (2017) mention values of 41.8 % as the rate for cachexia in the total number of condemnations, 29.3 % for general congestion, and 14.2 % for non-suppurating skin lesions in broilers; while for ducks the values are 58.6 % for abnormalities in conformation, 14.61 % for cachexia and 14.56 % for ascites. As may be observed from this data, three or four causes account for almost 90 % of all declared condemnations.

At the same time, it must be pointed out that significant changes have been detected in the number of condemnations based on the season, thus in winter, a large number of chickens are affected, which also leads to a significant change in condemnation percentages (Part et al., 2016).

With regard to animal welfare indicators in chickens, the pathological aspects that inspectors may focus on are dermatitis of the feet, blisters on the chest, bruises and scratches (Gouveia et al., 2009).

In the case of rabbits, we must consider that it is a less frequently consumed species and therefore, there is less bibliography on its consumption by humans. Nevertheless, after China, the EU is the largest producer of this type of meat.

Within the EU, production is centred on southern European countries, with France, Italy and Spain being the most important countries (*European Commission Directorate-General For Health And Food Safety DG* (SANTE, 2017)). According to the EU Report, Spain is the largest producer. Nevertheless, the size of rabbit farms in our country is below what may be considered ideal (Baviera-Puig et al., 2017) in spite of the progressive intensification and concentration that has taken place in the Iberian peninsula during the first decade of this century (Rosell et al., 2009).

For a time-based review of the data on condemnations of rabbit carcasses, we may begin with the data provided by Tantiñá et al. 2000) in Spain, highlighting a condemnation percentage between 0.5 and 1 % for 185 483 inspected carcasses. Next, we shall examine the data of Kozak et al. (2002). These Czech researchers found up to 3.04 % of rabbit carcasses that were unfit for human consumption in the period between 1989 and 1994. This percentage decreased to 1.2 % of condemned rabbit carcasses between 1995 and 2002. Subsequently, we may cite Rampin et al. (2008) who report a 1.9 % of condemned rabbit carcasses, with a significant change in the pathological aspects of this species when compared to broilers, as most condemnations were due to subcutaneous abscesses that make up 37.70 % of all condemned carcasses and 0.4 % of the slaughtered animals. More recent and nearby data are those revealed by Ferreira et al. (2014), who studied the *post-mortem* results of nearly 300 000 slaughtered rabbits in Portugal, culminating in a condemnation percentage of 1.09 %, also caused primarily by subcutaneous abscesses. An interesting data with regard to the study carried out by these researchers is that it was performed over 8 months, included all four seasons, and highlighted a relatively stable condemnation percentage, between 0.94 % in April and up to 1.39 % in June. Finally, we must mention Conficoni et al. 2020) who provide the most complete, up to date and representative data from the *post-mortem* inspections of more than 100 million rabbits.

These Italian authors published lower values in the case of rabbits with a global condemnation percentage of 0.72 %. Nevertheless, in the case of breeding females, the condemnation percentage was much higher, reaching a maximum of 15 % while it was less than 0.5 % in young animals, thus slaughtered breeding females constitute approximately 1 % with regard to young rabbits. Conficoni et al. (2020) also point to subcutaneous abscesses, cachexia and enteritis as the main reasons for condemnation. It seems logical to limit our focus to young rabbits for consumption and to discard certain excessively high condemnation percentages that may also be found in the bibliography, such as the case of breeding females and other minor types. Condemnation percentages would then range between 0.5 % and 1.4 % if we focus on these works. From the information in this section, we may conclude that the condemnation percentage is never higher than 2 % for any species mentioned here, excluding minority cases (for example, slaughtering diseased batches or animals where age or other causes may lead to higher condemnation percentages). Therefore, we may take 2 % as a base value.

6. Size of poultry flocks in Spain and size of rabbit batches

As explained in the introduction, it is necessary to sample a significant number of carcasses from the same flock. In this regard, we shall define "flock" *as all poultry of the same health status kept on the same premises or in the same enclosure and constituting a single epidemiological unit; in the case of housed poultry, this includes all birds sharing the same airspace* Regulation (EC) No. 2160/2003 (EU, 2003). Therefore, it is essential to be aware of the size of the flocks in advance. According to the national plans for controlling *Salmonella* and other zoonotic agents laid out in Regulation (EC) No. 2160/2003, the sampling unit is the flock. According to 2019 data from the Sub-Directorate General for Livestock Farming on broiler farms controlled according to the aforementioned regulation, there were 4467 farms with a certain number of birds distributed over a variable number of flocks in each farm. The average number of broilers per flock may be established as 18 337 with a minimum of 6 and a maximum of 105 333. The frequency distribution of flock sizes is displayed in Table 1.

Table 1. Frequency distribution of flock size in broilers, Year 2019

Class	Frequency
100	144
1000	99
10 000	650
20 000	1771
50 000	1788
100 000	14
>100 000	1

Note: the class indicates the maximum number of animals in it. For example, there are 99 flocks of 100 to 1000 animals.

It may be observed that most of the flocks have between 10 000 and 100 000 broilers, but the number of flocks with values below 10 000 broilers is not inconsiderable (approximately 900). Nevertheless, in the case of other birds, the flock size is smaller. Thus, in the case of the data for 2019 on turkeys for fattening, the number of farms is smaller and the average flock size is 7108 turkeys, with a minimum of 150 and a maximum of 37 079. In the following table, we can see the frequency distribution of flock size in turkeys for fattening:

Table 2. Frequency distribution of flock size in turkeys for fattening, Year 2019

Class	Frequency
100	0
1000	8
10 000	556
20 000	99
50 000	9
>50 000	0

Note: See Table 1.

The frequency distribution for laying birds may be seen in Table 3.

Table 3. Frequency distribution of flock size in laying birds, Year 2019

Class	Frequency
100	83
1000	268
10 000	377
20 000	143
50 000	130
>50 000	71

Note: See Table 1.

There is less data compiled on rabbits, given that there is no national *Salmonella* control plan for them as there exists for birds. Nevertheless, based on data from the Ministry of Agriculture, Fisheries and Food (MAPAMA, 2019), there were more than 3000 rabbit farms in Spain, although only 2000 farms were not for on-farm consumption but for breeding and commercialisation. The primary rabbit breeding Regions are Catalunya, Castilla y León and Galicia. Data from large slaughterhouses indicate a slaughter of around 20 000 animals per day and farm sizes range between 300 and 10 000 animals with the largest batches dispatched to slaughterhouses being around 7000 to 8000 animals. The slaughter volume per day is consistent with the data for a large Italian slaughterhouse mentioned by Conficoni et al. (2020). Therefore, and similar to the case of large poultry slaughterhouses, various batches submitted for inspection would be slaughtered within the course of a day.

7. Calculating confidence intervals and sample sizes

Based on Section 3, we may take an example of a confidence interval calculation:

For example, if 5 condemnations are detected in a sample of 450 animals, the sample proportion would be $\hat{p} = 5/450 = 0{,}011$. Thus the confidence interval values would be calculated as:

Left side: $$0{,}011 - 1{,}96 \cdot \sqrt{\frac{0{,}011 \cdot (1 - 0{,}011)}{450}} = 0{,}0014 = 0{,}14\%$$

Right side: $$0{,}011 + 1{,}96 \cdot \sqrt{\frac{0{,}011 \cdot (1 - 0{,}011)}{450}} = 0{,}021 = 2{,}1\%$$

Considering the values mentioned in the bibliography, in this case, the system would be considered to be under control.

If, on the other hand, out of 500 animals 24 were condemned, $\hat{p} = 24/500 = 0{,}048$ the confidence interval would be defined by:

Left side: $$0{,}048 - 1{,}96 \cdot \sqrt{\frac{0{,}048 \cdot (1 - 0{,}048)}{500}} = 0{,}029 = 2{,}9\%$$

Right side: $$0{,}048 + 1{,}96 \cdot \sqrt{\frac{0{,}048 \cdot (1 - 0{,}048)}{500}} = 0{,}067 = 6{,}7\%$$

Therefore, given the values mentioned in the bibliography, in this case the system would be deemed out of control as both sides of the confidence interval broadly exceed the 2 % established in Section 5 of this report.

Additionally, with regard to the sample size we must point out that in the case of an infinite population it must fulfil Condition 1 and Condition 2 of Section 3 of the report.

To determine the sample size, it must be remembered that the goal of a sampling system is to detect when the installation is out of control (in our case, when the condemnation percentage exceeds that which is expected). In accordance with the data available in the bibliography, the system may be deemed "out of control" (i.e., the condemnation percentage is above the expected percentage) when 2 % of the sampled animals display defects (p= 0.02) as has been considered. Substituting in Condition 1:

$$n > 9 \cdot \left(\sqrt{\frac{1-p}{p}} - \sqrt{\frac{p}{1-p}} \right)^2 = 9 \cdot \left(\sqrt{\frac{1-0{,}02}{0{,}02}} - \sqrt{\frac{0{,}02}{1-0{,}02}} \right)^2 = 423{,}2$$

Therefore, to fulfil Condition 1, the sample size must be at least 424 animals.

Substituting in Condition 2, considering α= 0.05 and MOE= 0.02:

$$n \geq \left(\frac{1{,}96}{0{,}02}\right)^2 0{,}02 \cdot (1-0{,}02) = 188{,}2$$

To fulfil Condition 2, the sample size must be at least 189 animals. Therefore, for an infinite population, the recommended sample size is at least 424 animals per batch or flock (regardless of the latter's size).

Given that the value of 424 carcasses is the minimum sample size for an infinite population, a greater number of carcasses may be sampled. We have also established a 2 % threshold value of the percentage of condemnations, nevertheless, this threshold value (*p*) may eventually be different, higher or lower, depending on the slaughterhouse history and the specific type of animal in question. In order to facilitate sampling without the need for calculations with the described system and the limit of total condemnations to be considered, Table 4 indicates the threshold values that determine whether the system is out of control for different sample sizes and condemnation percentages considered to be expected (1 %, 1.5 %, 2 %, 3 %) for "infinite" flock populations.

Table 4. Threshold values of *c* from which it is determined that the system is out of control for different sample sizes and threshold values under the infinite population hypothesis

Sample size	*c* (limit of 3 %)	*c* (limit of 2 %)	*c* (limit of 1.5 %)	*c* (limit of 1 %)
280(1)	**15**	-	-	-
424(2)	**21**	**15**	-	-
450	22	15		-
574(3)	**26**	**19**	**16**	-
700	31	22	18	-
874(4)	**37**	**27**	**21**	**16**
1000	41	30	23	18
1200	48	34	27	20

Note: the values of sample size in bold determine the minimum sample size for a threshold of condemnations of 3 %(1), 2 %(2), 1.5 %(3), or 1 %(4), respectively.

For example, if the sample size is 574 and there are a total of 26 condemnations, the system would be under control for a maximum threshold of expected condemnations of 3 %, but it would be out of control for maximum thresholds of expected condemnations of 2 % or 1.5 %, as in these cases the system would be out of control if values are above 19 condemnations (2 %) or 16 condemnations (1.5 %), respectively.

If the flock has less than 8481 animals (424/0.05= 8480), we do not recommend using the previously mentioned formulas as they do not fulfil the infinite population hypothesis. In this case, the iterative method suggested by Guenther (1973) is recommended. Tables 5 and 6 display the estimated values of *c* and *n* for different flock sizes (However, although the R-code program included as an appendix returns a range of sample size, fixed values have been selected from that range to simplify). A traditional value of α= 0.05 has been chosen as the control parameter. A lower value has been fixed for β (β= 0.01) as, in this case, Type II error (to conclude that the flock is under control when it isn't) is more relevant than Type I error (to conclude that the flock is not under control when it is). The limits for population proportion have been set considering k_0= 0.5 % and k_1= 2 % as minimum and maximum thresholds corresponding to a threshold of expected condemnations of 2 %. For other flock sizes not included in the table (or for other values of the control parameters) it is recommended to use the R code attached to this document.

Table 5. Sampling plans (simplified) for small flocks (<8481 animals) and values that indicate that the system is out of control for a threshold of expected condemnations of 2 %

Flock size	Sample size range	*c*
5200 - 8480	800	7
2200 - 5199	710	6
1200 - 2199	630	5
600 - 1199	495	4

Values calculated for α= 0.05, β= 0.01, k_0= 0.5 % of *N* and k_1 = 2 % of *N*.

Table 6. Sampling plans (simplified) for small flocks (<8481 animals) and values that indicate that the system is out of control for a threshold of expected condemnations of 4 %

Flock size	Sample size range	*c*
2801 - 8480	400	7
1051 - 2800	350	6
541 - 1051	295	5
300 - 540	245	4

Values calculated for α= 0.05, β= 0.01, k_0= 1 % of *N* and k_1 = 4 % of *N*.

For example, for a flock of 6000 animals, it is recommended to take a sample of 400 animals. In this case, the system would be deemed out of control (that is, the condemnation percentage is higher than the expected 4 %) if 7 or more condemnations are detected in the sample.

Given that the proposed sampling system is probabilistic, the sample selection must be random. Given the high speed of work in slaughter lines at these slaughterhouses with speeds that may exceed 10 000 animals per hour, it is scarcely feasible to think of a random individual selection of each animal in the sample, rather they are usually picked off the line one after another. However, a random selection should at least be made when inspecting, for example, by drawing from the number of hours or fractions of the time that the slaughter of said batch or flock is expected to take.

The proposed sampling method also displays values that are relatively consistent with those that may be found in the repealed legislation (300 birds as opposed to 424 in this document, for infinite populations).

Symbol	General meaning	Interpretation in this study
p	Proportion of defects in the population	Proportion of condemnations in the flock
N	Population size	Number of animals in the flock or batch sent for slaughter
$\hat{p}$	Proportion of defects in the sample	Number of condemnations divided between the number of animals that are tested (inspected by sampling)
n	Sample size	Number of animals that are (tested) inspected by sampling
MOE	Margin of Error	Given that not all carcasses in a flock are inspected, it is impossible to know for certain the proportion of defects in the population. Instead, it is estimated with regard to the proportion of defects in the sample and the sample size. The MOE indicates the uncertainty linked to the estimator
α	Probability of Type I error	Probability of concluding that the system is out of control (percentage of condemnations beyond that which is expected) when it actually is not
β	Probability of Type II error	Probability of concluding that the system is under control (percentage of condemnations is as expected) when it actually is not
c	Admissible number of defects	If the number of condemnations in the sample is greater than c, the system is deemed to be out of control (percentage of condemnations beyond that which is expected)

Conclusions of the Scientific Committee

The scientific bibliography has allowed us to establish a value of 2 % as the threshold percentage to be used as condemnation prevalence value for poultry and lagomorphs (domestic rabbits) to establish the representative sample size for the *post-mortem* Inspection of these animals. At the same time, it has established that this value is relatively consistent for different types of birds.

The average sizes of bird flocks or lagomorphs slaughtered and inspected in our country have been determined, and in most cases they may be considered as "infinite population". Nevertheless, the number of cases of smaller batches or flocks is also considerable.

It has been calculated that assuming a 2 % rate of condemnations for an infinite population, the minimum sample size must be at least 424 animals. It also describes how to calculate the confidence interval for the number of condemnations in the population based on the percentage found in the sample, which can be used to determine when the number of condemned carcasses would indicate that the process is out of control, that is to say, when it exceeds 2 % at both sides of the interval (there is a percentage of condemnations beyond that which is expected). With regard to the sample size it may be mentioned that a finite population would consist of flocks or batches of less than 8481 animals (for which a sample of 424 animals would be equivalent to or more than 5 %). For the latter case, it has been established that the method of establishing sample size is by means of numerical calculation using fixed-point iteration, as the equation that defines the sample size cannot be resolved analytically. The calculations have established the intervals at which the sample size must be found for flock or batch values lower than 8481 animals as well as the number of condemnations at which the system is “out of control” (Tables 5 and 6).

Random sample selection is recommended, for example, when making the inspection, so it may be as random as possible. The Annex includes the program written in the R programming language (version 3.5.3) to make calculations when the condemnation percentages or other control variables or flock or batch sizes to be sampled are different.

References

AESAN (2009). Agencia Española de Seguridad Alimentaria y Nutrición. Informe del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) sobre la evisceración de los lagomorfos. *Revista del Comité Científico de la AESAN*, 9, pp: 31-38.

Alloui, N., Guettaf, L., Djeghouri, F., Alloui, M. and Lombarkia, O. (2012). Quality of broilers carcases and condemnation rate during the veterinary control in the Batna slaughterhouse. *Journal of Veterinary Advances* 2, pp: 70-73.

Baviera-Puig, A., Buitrago-Vera, J., Escriba-Perez, C. and Montero-Vicente, L. (2017). Rabbit meat sector value chain. *World Rabbit Science*, 25, pp: 95-108.

BOE (1984). Real Decreto 1915/1984, de 26 de septiembre, por el que se aprueba la Reglamentación Técnico-Sanitaria de mataderos de Conejos, salas de despiece, industrialización, almacenamiento, conservación, distribución y comercialización de sus carnes. BOE 260 de 30 de octubre de 1984, pp: 31433-31441.

BOE (1985). Real Decreto 179/1985, de 6 de febrero, por el que se aprueba la Reglamentación Técnico-Sanitaria de Mataderos de aves, salas de despiece, industrialización, almacenamiento, conservación, distribución y Comercialización de sus carnes. BOE 40 de 15 de febrero de 1985, pp: 3830-3839.

BOE (1994a). Real Decreto 1543/1994, de 8 de julio, por el que se establecen los requisitos sanitarios y de policía sanitaria aplicables a la producción y a la comercialización de carne de conejo doméstico y de caza de granja. BOE 216 de 9 de septiembre de 1994, pp: 28097-28108.

BOE (1994b). Real Decreto 2087/1994, de 20 de octubre, por el que se establece las condiciones sanitarias de producción y comercialización de carnes frescas de aves de corral. BOE 301 de 17 de diciembre de 1994, pp: 37965-37986.

Box, G.E.P., Hunter, J.S. and Hunter, W.G. (2005). In book: *Statistics for Experimenters: Design, Innovation, and Discovery*. Wiley-Blackwell, Hoboken, Nueva Jersey.

Conficoni, D., Cullere, M., Lago, N., Alberghini, L., Rossin, T., Dallezotte, A. and Giaccone, V. (2020). Prevalence of *post mortem* lesions recorded in a large Italian Rabbit Slaughterhouse over a fifteen-year period (2003-2017). *World Rabbit Science*, 28, pp: 39-47.

EFSA (2012). European Food Safety Authority. Scientific Opinion on the public health hazards to be covered by inspection of meat (poultry). *EFSA Journal*, 10 (6), 2741, pp: 178.

EU (1971). Council Directive 71/118/CEE of 15 February 1971 on health problems affecting trade in fresh poultrymeat. OJ L 55 of 8 March 1971, pp: 23-39.

EU (2003). Regulation (EC) No. 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents. OJ L 325 of 12 December 2003, pp: 1-15.

EU (2004a). Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. OJ L 139 of 30 April 2004, pp: 55-205.

EU (2004b). Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. OJ L 226 of 25 June 2004, pp: 83-127.

EU (2019). Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No. 2074/2005 as regards official controls. OJ L 131 of 17 May 2019, pp: 51-100.

Fábregas i Comadran, X. (1993). Inspección veterinaria *post mortem* de conejos: causas de no aptitud. Depósito digital de la Universidad Autónoma de Barcelona. Available at: https://ddd.uab.cat/record/130602 [accessed: 15-06-20].

Ferreira, A., Monteiro, J.M. and Vieira-Pinto, M. (2014). The importance of subcutaneous abscess infection by *Pasteurella* spp. and *Stapylococcus* aureus as a cause of meat condemnation in slaughtered commercial rabbits. *World Rabbit Science*, 22, pp: 311-317.

Ghaniei, A., Mojaverrostami, S. and Sepehrnia, P. (2016). Survey of Poultry carcass condemnations in abattoirs of west Azerbaijan province (North West of Iran). *Journal of the Hellenic Veterinary Medical Society,* 67 (3), pp: 183-188.

Gouveia, K.G., Vaz-Pires, P. and Martins Da Costa, P. (2009). Welfare assessment of broilers through examination of haematomas, foot-pad dermatitis, scratches and breast blisters at processing. *Animal Welfare*, 18, pp: 43-48.

Grist, A. (2006). In book: *Poultry Inspection 2nd Edition. Anatomy, physiology and disease conditions.* Context Products Ltd, Packington, UK.

Guenther, W.C. (1973). A Sample Size Formula for the Hypergeometric. *Journal of Quality Technology*, 5, pp: 167-170.

Kozak, A., Vecerek, V., Steinhauserova, I., Chloupek, P. and Pistekova, V. (2002). Results of slaughterhouse carcass classification (capable for human consumption, capable for processing and condemned) in selected species of food animals. *Veterinarni Medicina*, 47, pp: 26-31.

Lara Moreno, A. (2015). In book: *Broiler Meat Inspection.* Servet, Zaragoza, España.

MAPAMA (2019). Ministerio de Agricultura, Pesca y Alimentación. El Sector Cunícola en Cifras: Principales Indicadores Económicos. Available at http://publicacionesoficiales.boe.es/ [accessed: 15-06-20].

Part, C.E., Edwards, P., Hajat, S. and Collins, L.M. (2016). Prevalence rates of health and welfare conditions in broiler chickens change with weather in a temperate climate. *Royal Society Open Science*, 3: 160197.

Rampin, F., Piccirillo, A., Schiavon, E., Poppi, L. and Grilli, G. (2008). Detection of pathological lesions in slaughtered rabbits. *Italian Journal of Animal Science*, 7: 1, pp: 105-111.

Rosell, J.M., de la Fuente, L.F., Badiola, J.I., Fernández de Luco, D., Casal, J. and Saco, M. (2009). Study of urgent visits to commercial rabbit farms in Spain and Portugal during 1997-2007. *World Rabbit Science*, 17, pp: 127-136.

Salines, M., Allain, V., Roul, H. and Le Bouquin, S. (2017). Rates of and reasons for condemnation of poultry carcases: harmonized methodology at the slaughterhouse. *Veterinary Record*. Doi 10.1136/vr.104000.

Santana, A.P., Murata, L.S., De Freitas, C.G., Delphino, M.K. and Pimentel, C.M. (2008). Causes of condemnation of carcases from poultry in slaughterhouses located in State of Goias, Brazil. *Cienda Rural*, 38, pp: 2587-2592.

SANTE (2017). European Commission. Directorate-General for Health and Food Safety DG. Overview report Commercial Rabbit Farming in the European Union. Available at: https://doi.org/10.2772/62174 [accessed: 15-06-20].

Tantiñá, M., Rosell, J.M. and Facchin, E. (2000). Salud Pública. In book: *Enfermedades del Conejo*. Rosell, J.M. Ch. IX, Ediciones Mundi-Prensa, Madrid, Spain, pp: 465-513.

USDA (2008). National Agricultural Statistics Service. Poultry Slaughter. Washington DC: United States Department of Agriculture, pp: 17.

Vecerek, V., Vecerkova, L. and Voslarova, E. (2019). Comparison of the frequency of patho-anatomic findings in laying hens with findings in broiler chickens and turkeys detected during post-mortem veterinary inspection. *Poultry Science*, 98, pp: 5385-5391.

Wise, M.E. (1954). A Quickly Convergent Expansion for Cumulative Hypergeometric Probabilities, Direct and Inverse. *Biometrika*, 41, pp: 317-329.

Annex I

```r
## Load libraries

# In case 'tidyverse' was not installed, it shall be with
# install.packages("tidyverse")

library(tidyverse)

## Calculation based on doi.org/10.1080/00224065.1973.11980599

get_intermediate_stuff <- function(n, c, N, k0, k1) {

    M <- N - (n-1)/2
    p0 <- (k0 - c/2)/M
    p1 <- (k1 - c/2)/M

    list(M = M, p0 = p0, p1 = p1)

}

get_lhs <- function(n, c, N, beta, k0, k1) {

    inter <- get_intermediate_stuff(n, c, N, k0, k1)

    df <- 2*c+2

    ( qchisq(1-beta, df)*(1/inter$p1-.5) + c )/2

}

get_rhs <- function(n, c, N, alpha, k0, k1) {

    inter <- get_intermediate_stuff(n, c, N, k0, k1)

    df <- 2*c+2

    ( qchisq(alpha, df)*(1/inter$p0-.5)+ c )/2

}

## Functions for iterations

#' LHS limit of the sampling size
#'
#' Parameters
#'     niter: Number of iterations of the numerical algorithm
#'     n_start: Initial value of the sampling size
#'     c: Threshold value used in the sampling
```

```
#'     N: Population size
#'     beta: Probability of type II error
#'     k0: Number of failures defining error type I
#'     k1: Number of failures defining error type II
#'
iterate_lhs <- function(niter, n_start, c, N, beta, k0, k1) {

    my_ns <- numeric(length = niter)
    my_ns[1] <- n_start

    for (i in 1:niter) {
        new_n <- get_lhs(my_ns[i], c, N, beta, k0, k1)
        my_ns[i+1] <- new_n
    }

    my_ns

}

#' RHS limit of the sampling size
#'
#' Parameters
#'     niter: Number of iterations of the numerical algorithm
#'     n_start: Initial value of the sampling size
#'     c: Threshold value used in the sampling
#'     N: Population size
#'     beta: Probability of type II error
#'     k0: Number of failures defining error type I
#'     k1: Number of failures defining error type II
#'
#'
iterate_rhs <- function(niter, n_start, c, N, alpha, k0, k1) {

    my_ns <- numeric(length = niter)
    my_ns[1] <- n_start

    for (i in 1:niter) {
        new_n <- get_rhs(my_ns[i], c, N, alpha, k0, k1)
        my_ns[i+1] <- new_n
    }

    my_ns

}


## Example

# Population parameters
N <- 2000
alpha <- .05
```

```
beta <- .01
k0 <- .01*N
k1 <- .04*N

# Estimation of c
# We seek the mínimum value of c for which ‘lower’ is lower than
‘upper’


# For c=4 ‘upper’ is lower than ‘upper’: not valid

tibble(
    iter = 0:25,
    lower = iterate_lhs(niter = 25, n_start = 500, c = 4,
                        N, beta, k0, k1),
    upper = iterate_rhs(niter = 25, n_start = 500, c = 4,
                        N, alpha, k0, k1)
)  %> %
    gather(side, n, -iter)  %> %
    ggplot(aes(x = iter, y = n, colour = side)) +
    geom_point() +
    geom_line()

# In this case, we need c=6

tibble(
    iter = 0:25,
    lower = iterate_lhs(niter = 25, n_start = 500, c = 6,
                        N, beta, k0, k1),
    upper = iterate_rhs(niter = 25, n_start = 500, c = 6,
                        N, alpha, k0, k1)
)  %> %
    gather(side, n, -iter)  %> %
    ggplot(aes(x = iter, y = n, colour = side)) +
    geom_point() +
    geom_line()

# We take the last value of iterate_lhs (rounding up) and iterate_rhs
(rounding down) as sample size

iterate_lhs(niter = 25, n_start = 500, c = 6, N, beta, k0, k1)  %> %
tail(1)  %> % ceiling()
iterate_rhs(niter = 25, n_start = 500, c = 6, N, alpha, k0, k1)  %> %
tail(1)  %> % floor()
```