

Remote post-mortem veterinary meat inspections in bovine and analysis of post-mortem inspection outcomes: preliminary results

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ABSTRACT

The work aimed to collect and analyse data obtained from the routine post-mortem veterinary inspection of bovine viscera and to evaluate the reliability of remote veterinary meat inspections. A comparative study was conducted on viscera belonging to 30 bovines. For each bovine, findings were recorded simultaneously by two veterinarians, one conducting an on-site post-mortem inspection and another performing the remote inspection through a tablet. A total of 10 (33.33 %) bovines were healthy. In a total of 18 (60.00 %) and 11 animals (33.00 %) pulmonary and hepatic lesions were respectively, observed. The most reported finding was pleuritis recorded in 17 animals (56.67 %), followed by pneumonia (16.67 %). The agreement between the two methods used for the post-mortem inspection of bovines was generally high. In an overall 73.33 % of bovines the findings recorded by the veterinarian who conducted the on-site post-mortem inspection and those recorded by the veterinarian performing the remote inspection were overlapping. In conclusion, a move to a remote post-mortem inspection has a negligible negative impact on public health since the most frequently detected lesions recorded were of scarce significance and in most cases, they were effectively detected by the veterinarian through the tablet.

Section: RESEARCH PAPER

Keywords: Bovines ; viscera; remote post-mortem inspection

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1. INTRODUCTION

Protection of the consumers and good animal health and welfare are achieved at the slaughterhouse by meat inspection and by hygienic slaughtering. Meat inspection is performed to identify slaughtered animals that are unfit for human consumption, with notifiable diseases, and with welfare problems [1]. Meat inspection as it is performed in the European Union (EU) today is based on the procedures laid down in German in 1899 by Robert Ostertag and includes a set of activities before and after stunning (ante- and post-mortem inspection) involving visual inspection, palpation, and incisions [2]. Although significant changes were introduced by the regulations 625/2017, 624/2019, and 627/2019, bovine post-mortem inspection is still more focused on zoonotic agents more

common in the past and is still not risk-based. Indeed, post-mortem inspection still follows a strict protocol regardless of factors, such as the origin of the animals or other information included in the Food Chain Information, which may influence the outcome of it [3].

According to the EU Reg. 624/2019, post-mortem inspection can be performed either by Official Veterinarians (OV) or by Official Auxiliaries (OA) under the responsibility of the OV. According to the EU Reg. 624/2019, the OA can perform post-mortem inspections only when the slaughter is carried out in a low-capacity slaughterhouse that slaughters less than one thousand adult bovine animals per year whilst in larger slaughterhouses post-mortem inspection can be only performed by an OV.

According to the regulation, the presence of the OV is always required during slaughter activities which take place only during part of the working day or during the whole working day but not on each working day of the week in a low-capacity slaughterhouse, and in each working day for all working hours in larger facilities [4].

The demand for increased productivity, including improved quality and reduced time waste, drives the introduction and spread of new technologies [5]. Emerging technologies have constantly been introduced to meet new challenges. The use of remote video transmission was proved to be reliable for swine post-mortem inspection and could be used also for bovine [4]. The use of remote video transmission could allow an increasing implementation of a post-mortem inspection by an OA under the remote supervision of an OV in large establishments.

Moreover, the most frequently detected lesions in bovines are macroscopic abnormalities of scarce significance [6]. Therefore, meat inspection today serves more for quality control rather than public health purposes. However, there is an overall lack of reported data on the most common lesions, which hampers a proper estimation of their true prevalence of these lesions.

Therefore, the present work aimed to collect and analyse data obtained from the routine post-mortem veterinary inspection of bovine viscera and to evaluate the reliability of remote veterinary meat inspections.

2. MATERIALS AND METHODS

2.1. Data collection

A comparative study was conducted between May and June 2022 at an Italian large-scale slaughter plant for bovine, processing around 1000 animals daily. The slaughterhouse was visited six times, weeks apart, to record the post-mortem inspections of viscera (heart, lungs, liver, kidneys, and intestines) belonging to 30 bovines.

All inspections were performed by two study veterinarians with several years of prior work experience in the field of meat inspection. Before the study, the veterinarians performed a weekly training session together to standardize their assessment.

Remote inspections were performed using an action cam (GoPro Hero 3 black). On-site at the slaughterhouse was a veterinarian carrying the action cam mounted on its head. The veterinarian working on-site carried out the post-mortem inspection following the requirement reported in the art. 18 and 19 of the EU Reg. 627/19. The action cam was used to relay video about the animals to the remote veterinarian through a tablet.

For each bovine, findings were registered simultaneously by both veterinarians on a pre-filled sheet reporting the most common lesions or conditions. The veterinarians switched roles two times during each trial.

Moreover, the Food Chain Information (FCI) was collected per each animal and any relevant information was included in the present work. In particular, the following information were recorded: sex, date of birth, country of birth, rearing country, date of arrival to the slaughterhouse, duration of the transport, and date of slaughtering.

2.2 Statistical analysis

The differences between the occurrence of the lesions considering the recorded data (sex, date of birth, country of birth, rearing country, date of arrival to the slaughterhouse, duration of the transport, and date of slaughtering) were assessed by chi-

square test. A probability value of less than 0.05 ($p < 0.05$) was defined as statistically significant.

3. RESULTS AND DISCUSSION

Post-mortem inspection was performed on organs belonging to 15 male and 15 female bovines (Table 1 and Table 2). Considering the category of animals, 15 were young bulls, 11 were heifers, and 4 were cows. Age ranged from 1.1 to 7.75 years (mean values \pm SD = 1.94 ± 1.61). Twenty-one animals (70 %) and nine (30 %) were born respectively in France and Italy but all of them (100%) were reared in Italy.

Bovines were reared in four Italian regions (Emilia-Romagna = 19 animals, Toscana = 5 animals, Veneto = 5, Lombardia = 1). The duration of the transport ranged from 1 to 6 hours (mean values \pm SD = 2.77 ± 1.57).

Based on the information reported in the FCI, the animals did not receive any drugs and there never were any 'yes' answers regarding the questions about the recent illness, the disease status of the holdings, or relevant results from previous ante-mortem or post-mortem inspections of animals from the same holdings. This is consistent with the study of Jacobs et al (2023) in which the FCI forms were not informative at all [3]. In a risk-based approach, the information included in the FCI should serve as prior screening and should serve as a guide for the implementation of post-mortem procedures [7]. However, farmers seem reluctant to report other information than those regarding the use of veterinary medicine and do not understand the importance of this document that instead should serve as a link between the farm and slaughterhouse [3].

In general, a total of 10 (33.33 %) bovines were healthy. In a total of 18 (18/30, 60.00 %) and 11 animals (18/30, 33.00 %) pulmonary and hepatic lesions were respectively, observed.

In particular, the most reported finding was pleuritis recorded in 17 animals (56.67 %), followed by pneumonia (5 animals, 16.67 %) and hemorrhagic aspect in pulmonary lymph nodes (4 animals, 13.33 %).

Macroscopic lung lesions, such as variation in colour (from red to grey), and the presence of mass or exudate were accounted as pneumonia. However, no laboratory tests were performed and therefore an etiological diagnosis was not obtained.

The reported lesions (principally recorded at the lung level), can be considered of little relevance for public health, however, their presence could lead to condemnation. Moreover, the presence of pneumonia may be indicative of Bovine respiratory disease which is an important cause of economic losses [8].

The number of lesions at the lung and hepatic level observed in the present study was higher than those reported by Villani et al (2022) [9]. In the study of Villani et al (2022) the occurrence of lesions at hepatic and lung levels was 4.68 % and 2.67 %, respectively [9]. Moreover, the results are in contrast with the study of Stella et al (2022) who reported a mean prevalence of 8 % of the lesions at the hepatic level [6]. However, a higher prevalence of lung (18 %) and hepatic lesions (20-25 %) were reported in other studies [8], [10], [11]. The prevalence of pneumonia may vary depending on the country, management system, breed, and season [8].

Few and no significant differences were observed in the occurrence of lesions regarding the category of the animal. Lung lesions (pneumonia and pleuritis) were observed in 10 young bulls (10/15, 66.67 %), in six heifers (6/11, 54.54 %), and two cows (50.00 %) ($p > 0.05$). The hemorrhagic aspect in pulmonary lymph nodes was observed only in females (3 heifers and 1 cow).

Liver lesions (telangiectasis, fibrosis/cirrhosis, other liver lesions) were observed in seven young bulls (7/15, 46.67 %), in two heifers (2/11, 18.18 %), and in two cows (2/4, 50.00 %) ($p > 0.05$) (Table 1 and Table 2). These results are in contrast to those reported by Stella et al., 2022 [6] who observed a significant prevalence of hepatic lesions among the animal categories. In particular, a significantly higher prevalence was observed in bovines higher than > 30 months. The most frequently reported hepatic lesion in the study of Stella et al (2022) was steatosis which was never observed in the present study [6]. Moreover, in the study of Stella et al (2022) a higher prevalence of steatosis was observed in animals > 30 months and cows [6].

No significant differences were also observed in the occurrence of lesions regarding the country of birth (Table 3). Lung lesions (pneumonia and pleuritis) were observed in 11

bovines born in France (11/21, 52.38 %) and in seven born in Italy (7/9, 77.78 %) ($p > 0.05$). Liver lesions (telangiectasis, fibrosis/cirrhosis, other liver lesions) were recorded in seven bovines born in France (7/21, 33.33 %) and in four born in Italy (4/9, 44.44 %) ($p > 0.05$).

Results of the present work are in contrast with those of Jacobs et al., (2023) [3] which observed a statistically significant effect on the outcome of the *post-mortem* inspection related to the origin of the animals probably related to the travel circumstances and/or total duration of the transport.

Moreover, an influence of a geographical area of slaughtering on the occurrence of hepatic lesions was observed in the study of Stella et al., 2022 [6], as well. Differences related to the origin observed by these latter authors may also be due to the different farming systems.

Table 1. Findings recorded on young bulls ($n = 15$ animals) during the post-mortem inspection [Y = years, S = sex, T = Duration of the transport (h)].

Animal ID	Category	Y	S	D (h)	Lesions							
					Pleuritis	Pneumonia	hemorrhagic aspect in pulmonary lymph nodes	Faecal material	Telangiectasis	Fibrosis/cirrhosis	Other liver lesions	
866	Young bull	1.84	M	3	X							
867	Young bull	1.41	M	3	X	X						X
868	Young bull	1.33	M	3		X				X		
869	Young bull	1.26	M	3	X							X
870	Young bull	1.25	M	3	X							X
910	Young bull	1.45	M	1								
911	Young bull	1.60	M	1	X							X
912	Young bull	1.70	M	1	X					X		
913	Young bull	1.48	M	1	X							
914	Young bull	1.30	M	1	X					X		
917	Young bull	1.50	M	3								
918	Young bull	1.42	M	3	X							
919	Young bull	1.40	M	3								
920	Young bull	1.36	M	3								
921	Young bull	1.51	M	3								
Total					9	2				3		4

Table 2. Findings recorded on heifer ($n = 11$ animals) and cows ($n = 4$ animals) during the post-mortem inspection [Y = years, S = sex, T = Duration of the transport (h)].

Animal ID	Category	Y	S	D (h)	Lesions							
					Pleuritis	Pneumonia	hemorrhagic aspect in pulmonary lymph nodes	Faecal material	Telangiectasis	Fibrosis/cirrhosis	Other liver lesions	
840	Heifer	1.21	F	1								
841	Heifer	1.82	F	1								
842	Heifer	1.30	F	1								
843	Heifer	1.30	F	1	X	X						
844	Heifer	1.38	F	1								
960	Heifer	1.14	F	5	X							X
961	Heifer	1.10	F	5	X	X						
962	Heifer	1.13	F	5	X	X	X					
963	Heifer	1.13	F	5	X		X					X
964	Heifer	1.14	F	5	X		X					
2094	Heifer	1.23	F	6								
2090	Cow	6.68	F	4	X							
2091	Cow	7.75	F	2				X	X			
2092	Cow	4.30	F	2	X		X					
2093	Cow	3.85	F	4						X		
Total					8	3	4	1	1	1		2

Table 3. Anatomopathological findings recorded during the post-mortem inspection in bovines born in France (n. 21 animals) or Italy (n. 9 animals) and reared in Emilia Romagna (n. 19 animals), Veneto (n. 5 animals), Toscana (n. 5 animals) or Lombardia (n. 1 animal).

Animal ID	Category	Y	C	Region of rearing	S	D (h)	Lesions							
							Pleuritis	Pneumonia	hemorrhagic aspect in lymph nodes	Faecal material	Telangiectasis	Fibrosi/cirrhosis	Other liver lesio	
840	Heifer	1.21	FR	Toscana	F	1								
841	Heifer	1.82	FR	Toscana	F	1								
842	Heifer	1.30	FR	Toscana	F	1								
843	Heifer	1.30	FR	Toscana	F	1	X	X						
844	Heifer	1.38	FR	Toscana	F	1								
866	Young bulls	1.84	FR	Veneto	M	3	X							
867	Young bulls	1.41	FR	Veneto	M	3	X	X						X
868	Young bulls	1.33	FR	Veneto	M	3		X				X		
869	Young bulls	1.26	FR	Veneto	M	3	X							X
870	Young bulls	1.25	FR	Veneto	M	3	X							X
910	Young bulls	1.45	FR	Emilia Romagna	M	1								
911	Young bulls	1.60	FR	Emilia Romagna	M	1	X							X
912	Young bulls	1.70	FR	Emilia Romagna	M	1	X					X		
913	Young bulls	1.48	FR	Emilia Romagna	M	1	X							
914	Young bulls	1.30	FR	Emilia Romagna	M	1	X					X		
917	Young bulls	1.50	FR	Emilia Romagna	M	3								
918	Young bulls	1.42	FR	Emilia Romagna	M	3	X							
919	Young bulls	1.40	FR	Emilia Romagna	M	3								
920	Young bulls	1.36	FR	Emilia Romagna	M	3								
921	Young bulls	1.51	FR	Emilia Romagna	M	3								
960	Heifer	1.14	IT	Emilia Romagna	F	5	X							X
961	Heifer	1.10	IT	Emilia Romagna	F	5	X	X						
962	Heifer	1.13	IT	Emilia Romagna	F	5	X	X	X					
963	Heifer	1.13	IT	Emilia Romagna	F	5	X		X					X
964	Heifer	1.14	IT	Emilia Romagna	F	5	X		X					
2090	Cow	6.68	IT	Emilia Romagna	F	4	X							
2091	Cow	7.75	IT	Emilia Romagna	F	2				X	X			
2092	Cow	4.30	IT	Emilia Romagna	F	2	X		X					
2093	Cow	3.85	IT	Emilia Romagna	F	4							X	
2094	Heifer	1.23	FR	Lombardia	F	6								
Total							17	5	4	1	1	4	6	

In the present study, no significant differences were observed considering the length of the transportation (≤ 1 hour *vs* > 1 hour). Lung lesions (pneumonia and pleuritis) were observed in five bovines transported for less than one hour (5/10, 50.00 %) and in 13 transported for more than one hour (13/20, 65.00 %) ($p > 0.05$). Liver lesions (telangiectasis, fibrosis/cirrhosis, other liver lesions) were recorded in three bovines transported for less than one hour (3/10, 30.00 %) and in eight transported for more than one hour (8/20, 40.00 %) ($p > 0.05$).

To our knowledge, this is the first study that evaluates the use of remote *post-mortem* inspection in bovine.

In an overall 73.33 % of bovines ($n = 22$) the findings recorded by the veterinarian who conducted the on-site post-mortem inspection and those recorded by the veterinarian performing the remote inspection were overlapping.

In eight bovines, most of the discrepancies were observed at the lungs level (Table 4). It has been reported that the detection of abnormalities during the *post-mortem* inspection may differ between meat inspectors [3], therefore in the present study the veterinarians performed a week training session together to standardize their assessment.

A good agreement between the two methods was observed in the present work.

Therefore, as it was already proved for swine by [4] remote inspection appears to constitute a viable alternative for *post-mortem* meat inspection in bovines, given a sufficiently standardized method of inspection and sufficient inspection times. In the study of Almqvist et al. (2021) [4] remote *post-mortem* inspection was effective for the detection of easily distinguished findings whilst for more vague findings in some cases a disagreement was registered.

Table 4. Differences observed by the veterinarian who conducted the on-site post-mortem inspection (S) and those recorded by the veterinarian performing the remote inspection (R).

Findings	Animal ID															
	840		843		868		912		918		962		963		2091	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Pleuritis	X	X	X				X	X	X	X	X	X				X
Pneumonia			X	X				X	X							
Faecal material															X	X
Telangiectasia															X	

However, according to Reg. 627/2019, in adult bovines, post-mortem inspection procedures still include the incisions of retropharyngeal lymph nodes, masseters, bronchial and mediastinal lymph nodes, and the hearth to survey the occurrence of tuberculosis and *Taenia saginata* (tapeworm) cysticercosis. However, the prevalence of these diseases is decreasing or even disappearing in some countries [5].

Moreover, according to the EU Reg. 624/2019, in a low-capacity slaughterhouse post-mortem inspection can be delayed by a maximum period of 24 hours from slaughter if neither the official veterinarians nor the official auxiliaries are present. Especially in this context, a remote post-mortem inspection could be performed without delay after slaughter leading moreover to substantial financial and ecological benefits.

Moreover, the duties of OA during the slaughter activities could increase even in the large facilities if they could work under the remote supervision of an OV.

The use of remote inspection could, therefore, allow a lead to economic gains, (reduced staff or a reduced risk of human error) [4].

4. CONCLUSIONS

In this preliminary study, the prevalence of lesions on the bovine viscera and the reliability of remote post-mortem inspection were evaluated. Data on the present work could provide farmers and veterinary practitioners with information that is not captured by surveillance programs.

Abnormalities were principally recorded at the lung level. Pulmonary lesions can be considered of little relevance for public health; however, no laboratory tests were performed and therefore an etiological diagnosis was not obtained.

No significant differences were observed between the occurrence of the lesions considering the recorded data (sex, date of birth, country of birth, rearing country, date of arrival to the slaughterhouse, duration of the transport, and date of slaughtering). However, the absence of a significant difference observed may be due to the fact that only 30 animals were examined in this preliminary study.

To our knowledge, this is the first study that evaluates the use of remote post-mortem inspection in bovines.

Based on the results the agreement between the two methods used for the post-mortem inspection of bovines was generally high. Although the number of inspected animals was limited, results of the present work demonstrate that remote post-mortem inspection may constitute a viable alternative for the traditional on-site post-mortem meat inspection in bovine, given a sufficiently standardized method of inspection and sufficient inspection times.

A move to a remote post-mortem inspection has a negligible negative impact on public health since the most frequently detected lesions recorded were of scarce significance and in most cases, they were effectively detected by the veterinarian through the tablet. However, additional information should be included in the FCI in order to facilitate the implementation of post-mortem procedures (e.g. remote post-mortem or on-site post-mortem inspection (including palpation and incision)).

However, to confirm these data further research on a higher number of animals is needed.

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REFERENCES

- [1] K. D. C. Stärk, S. Alonso, N. Dadios, C. Dupuy, L. Ellerbroek, M. Georgiev, J. Hardstaff, A. Huneau-Salaün, (+ 5 more authors), Strengths and weaknesses of meat inspection as a contribution to animal health and welfare surveillance, *Food Control* 39 (2014), pp. 154–162. DOI: [10.1016/j.foodcont.2013.11.009](https://doi.org/10.1016/j.foodcont.2013.11.009)
- [2] O. Alvseike, M. Prieto, K. Torkveen, C. Ruud, T. Nesbakken, Meat inspection and hygiene in a meat factory cell – An alternative concept, *Food Control* 90 (2018), pp. 32–39. DOI: [10.1016/j.foodcont.2018.02.014](https://doi.org/10.1016/j.foodcont.2018.02.014)
- [3] P. Jacobs, B. Berends, L. Lipman, The value of current ante mortem meat inspection and food chain information of dairy cows in relation to post mortem findings and the protection of public health: A case for a more risk-based meat inspection, *Foods* 12(3) (2023), pp. 616. DOI: [10.3390/foods12030616](https://doi.org/10.3390/foods12030616).
- [4] V. Almqvist, C. Berg, J. Hultgren, Reliability of remote post-mortem veterinary meat inspections in pigs using augmented-reality live-stream video software, *Food Control* 125 (2021), pp. 107940. DOI: [10.1016/j.foodcont.2021.107940](https://doi.org/10.1016/j.foodcont.2021.107940).
- [5] B. Blagojevic, T. Nesbakken, O. Alvseike, I. Vågsholm, D. Antic, S. Johler, K. Houf, D. Meemken, (+ 6 more authors), Drivers, opportunities, and challenges of the European risk-based meat safety assurance system, *Food Control* 124 (2021), pp. 107870. DOI: [10.1016/j.foodcont.2021.107870](https://doi.org/10.1016/j.foodcont.2021.107870).
- [6] S. Stella, E. Tirloni, C. Bernardi, G. Acerbis, T. Manginelli, Collection and analysis of post mortem inspection outcomes (liver lesions) from different cattle slaughtering plants located in Northern and Southern Italy, *Ital. J. Food Saf.* 11(1) (2022), art. no. 10035. DOI: [10.4081/ijfs.2022.10035](https://doi.org/10.4081/ijfs.2022.10035).
- [7] A. A. Hill, V. Horigan, K. A. Clarke, T. C. M. Dewé, K. D. C. Stärk, S. O'Brien, S. Buncic, A qualitative risk assessment for visual-only post-mortem meat inspection of cattle, sheep, goats and farmed/wild deer, *Food Control* 38 (2014), pp. 96–103. DOI: [10.1016/j.foodcont.2013.10.002](https://doi.org/10.1016/j.foodcont.2013.10.002).
- [8] M. Fernández, M.D.C. Ferreras, F.J. Giráldez, J. Benavides, V. Pérez, Production significance of bovine respiratory disease lesions in slaughtered beef cattle, *Animals* 10(10) (2020), pp. 1–16. DOI: [10.3390/ani10101770](https://doi.org/10.3390/ani10101770).
- [9] C. Villani, R. Piccioni, Effects of post-mortem inspection techniques change on the detection capability of low public health impact diseases of slaughtered pigs: a quasi-experimental study, *Ital. J. Food Saf.* 11(4) (2022), pp. 241–246. DOI: [10.4081/ijfs.2022.10761](https://doi.org/10.4081/ijfs.2022.10761).
- [10] G. Januškevičienė, V. Paulauskas, J. Dailidavičienė, V. Juozaitienė, Analysis of pathologic lesions in the livestock and poultry slaughtered in the meat establishments of Lithuania, *Vet. ir Zootech.* 52(74) (2010), pp. 33–42. Online [Accessed 27 February 2024]. <https://vetzoo.lsmuni.lt/data/vols/2010/52/pdf/januskeviciene.pdf>
- [11] R. O. Mckeith, G. D. Gray, D. S. Hale, C. R. Kerth, D. B. Griffin, J. W. Savell, C. R. Raines, K. E. Belk, (+ 13 more authors), National Beef Quality Audit-2011: Harvest-Floor Assessments of Targeted Characteristics That Affect Quality and Value of Cattle, Carcasses, and Byproducts, *J. Anim. Sci.*, 90(13) (2012), pp. 5135–5142. DOI: [10.2527/jas.2012-5477](https://doi.org/10.2527/jas.2012-5477)