

A STUDY OF ABATTOIR REJECTIONS OF SLAUGHTERED SHEEP DUE TO PARASITIC INFECTION

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Abstract

The aim of the study was to determine which diseases go unnoticed throughout the sheep's life cycle and that, thanks to the abattoir management system, do not enter the human food chain. The study was carried out in an abattoir in central Spain from October 2010 to September 2011. A batch of animals was selected at random each month. Rejections were subject to post mortem inspection and lesions were described in accordance with the terms generally used by official veterinarians. Of the total 577 rejections, 220 (38.13%) were due to parasites. Rejections were relatively high in lambs (16.04%) and very high in adults (54.75%). Particular attention should be paid to rejections due to hydatidosis and cisticercosis accounting for 21.66% and 4.99% respectively of the adults and lambs analysed. Rejections caused by trematodes were due to *Dicrocoelium dendriticum* and the prevalence was very low. Very low percentages of verminous pneumonia and sarcosporidiosis were also detected. No other parasitic processes were detected. Based on these results, it is possible to conduct a more specific classification and quantification of processes that tend to go undetected in health campaigns and programmes and which could constitute a health risk if they entered the food chain.

Keywords / Palabras clave

Slaughter, Parasitic Diseases, Sheep, Public Health.

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1. Introduction

Health inspection in the food industry is carried out on a systematic basis in accordance with standardised European Union criteria and is performed by veterinarians. Abattoirs play a key role among the different types of food-handling facilities insofar as this is where Hazard Analysis and Critical Control Point (HACCP) plans are applied and supervised and health checks are performed on all meat intended for human consumption. A large amount of meat is rejected at these health inspections due to a wide range of pathologies that are not always properly identified.

Most rejections associated with post mortem inspection are related to the liver and lungs and to a lesser extent to other organs and tissues [1-3]. There is large degree of variability in rejected livers and lungs in different lots from the same stockbreeder [3] and a small group of farms might account for a large proportion of the rejected meat removed from an abattoir [4]. In some diseases there is a close link between lungs and livers of affected sheep. These include hydatidosis and other parasitoses (pulmonary verminosis, fasciolosis and dicrocoeliosis) [5].

Despite the economic importance of data on the etiology of rejected sheep meat for both abattoirs and stockbreeders, such data are not kept up to date and are highly variable as Table 1 shows. This makes it difficult to draw comparisons between studies as the related list of motives or causes for rejection and the percentages attributed to each of these do not coincide [6]. Therefore, there is a predominance of one pathology or another depending on the area, with a significant number of rejections caused by parasitic processes. An example of this can be found in Great Britain. Depending on where the study was carried out and the age of the animals, among other factors, there is a predominance of verminous bronchopneumonias, cysticercoses or trematodiasis [7, 4, 8; respectively]. The object of this study is to provide up-to-date data on parasitic processes in sheep that lead to rejection in an abattoir located in central Spain.

2. Materials and Methods

This study commenced in October 2010 and ended in September 2011. It was conducted in an abattoir in the province of Madrid in central Spain. The sheep came from either breeding or fattening farms in the case of lambs, and from breeding farms only in the case of older sheep. Most are sacrificed for meat with a predominance of Merino stock, although this is frequently crossed with heavy French and German breeds as well as with native breeds ("Manchega", "Castellana" or "Talaverana") and, in smaller numbers, with other breeds. The "Manchega" breed usually predominates among sheep kept on milk-producing farms.

A batch of animals was selected at random each month (40-50 lambs and 10-20 sheep over the age of two). The animals rejected in the post mortem inspection were placed in specially prepared buckets which were duly identified. Each rejection was photographed and underwent a preliminary macroscopic examination in which the lesions were described in accordance with the terms generally used by official veterinarians.

Once post mortem inspection of the batch had been carried out, all of the rejected material was removed for immediate processing in another abattoir building and a maximum of twenty samples of lambs and another twenty of older sheep were collected for the study. A portion of each sample was preserved at -20° C prior to processing in the laboratory. Samples intended for histo-

logical sections, measuring approximately 1 cm³, were preserved in 70% alcohol.

Laboratory analysis was conducted at the laboratory of the Animal Parasitology Group at the Animal Health Research Centre (CISA-INIA). Samples were defrosted in order to repeat the macroscopic anatomo-pathological study. Following are the main parasitic processes identified and the alleged diagnosis criteria:

- Hydatid cyst: intraparenchymatous cystic formations characterised by a thin wall with an intracystic granular membrane and hydatid sand.
- Cysticercosis: unilocular subcapsular cystic duct filled with transparent fluid, with a single parasitic scolex and samples of liver with a subcapsular component even in the absence of the cystic duct.
- Dicrocoeliosis: enlargement of the main bile ducts (periangiocholitis) and/or parasitic organisms in the bile ducts or gallbladder.

The histological sections were prepared in an automated embedder at the Pathological Anatomy Laboratory of the CISA-INIA following the usual protocol.

Data were analysed using the SPSS 17.0 statistical programs for Windows (SPSS Inc., Chicago, IL) and Epi-Info 4.0. The Z-test for proportional comparison was used to establish associations between the variables. OR and 95% confidence intervals were also determined.

3. Results

Samples were taken from a total of 1945 lambs and 484 adult sheep, accounting for 3.82% and 1.70%, respectively of the total volume of animals slaughtered at the abattoir during the year under study (50,973 lambs and 28,401 adult sheep which, in turn, account for 0.02% and 0.07% of the total number of animals slaughtered in Spain during the study period, i.e. 11,281,304 lambs and 673,002 adults). The slaughter of lambs throughout the study year was relatively stable, with the exception of the Christmas season when there was a rise in demand (Table 2). Most lambs were from the province of Badajoz, followed by Ciudad Real and Cordoba, and to a much lesser extent, Madrid, Salamanca and Cuenca. There was greater fluctuation in the case of adult animals; more animals were slaughtered in the summer months and their area of origin was more extensive. Most adult sheep included in this study came from the provinces of Cuenca, Albacete and Toledo (Table 3).

The total number of rejections was 577 (312 lambs and 265 adult sheep, see Table 2) and 220 of these (38.13%) were due to a parasitic infection: dicrocoeliosis, cysticercosis, hydatidosis, verminous pneumonia and sarcosporidiosis. Liver and lungs were the most rejected viscera. Other pathologies detected in the rejections were abscesses, adhesions, septic arthritis, cholangitis, fibrosis, Marek's disease, splenomegalia, hepatitis, hepatosis, caseous lymphadenitis, purulent lymphadenitis, micoplasmosis, miocarditis, necrosis, enzootic pneumonia, pneumonitis and pericarditis [9].

A total of 74 livers and 32 lungs, accounting for nearly a quarter of the adult animals analysed (21.66%), were rejected due to hydatidosis, which was not detected in lambs. Histopathological

preparations done on hydatid cyst lesions showed inflammatory reactions around the protective capsule of the parasite, although not in every case. The percentage of rejections due to this disease in adults was higher in spring and lower in autumn (Table 4). There were considerable differences between the provinces, with the highest percentage of rejections in Madrid (66.67%) and the lowest in Ciudad Real (7.21%).

A significant number of cases of another metacestodes, cysticercosis, were also identified, accounting for the rejection of 97 lamb livers (4.99%) but was not detected in adult sheep. All the samples studied exhibited common features typical of chronic cysticercosis lesions: notable hyperplasia of the bile ducts, dilated lymph vessels and inflammatory polymorphonuclear infiltration around the porta hepatis, in some cases with fibrous tissue, as well as areas of necrosis caused by the migration of larvae with predominantly polymorphonuclear neutrophil and eosinophil infiltrations surrounded by areas of fibrosis where infiltration was mostly mononuclear. Cysticercosis data showed statistically significant differences between seasons ($p < 0.05$) with most rejections occurring in summer (7.14%) and the fewest in autumn (1.74%). With respect to cysticercosis in lambs, no differences were observed between the provinces studied, all having low rejection rates (Table 8).

The lesions on thirteen livers from adult animals (2.69%) indicated, a priori, the presence of trematodes, *Dicrocoelium dendriticum* subsequently being identified as the species responsible for rejection. Necrotic macroscopic lesions, haemorrhaging and tissue reaction were observed along the migratory course of the parasite. At histopathological level, destruction of the hepatic cells, leukocyte infiltration, proliferative lesions and fibrosis of the bile ducts were observed, as well as hypertrophic fibrosis of the liver. None of the lamb liver rejections was due to dicrocoeliosis. Statistical analyses showed significant seasonal differences ($p < 0.05$), the highest percentages occurring in winter (10.00%) while in summer and autumn no cases were detected. This process was detected in only a few flocks made up of adults from Cuenca, Albacete and Avila.

In table 7 the number of rejections due different parasitoses are described in relation to the breed.

Other parasitoses that caused rejection of the sheep studied were sarcosporidiosis, with only two adults rejected in February, both belonging to the same farm from Cuenca province. Finally, and the verminous bronchopneumonias, again in very low numbers (two lambs and one adult from Badajoz, Córdoba and Cuenca, respectively) and different months: April, May and September, respectively.

With respect to co-infections found in the sheep rejected at the abattoir, hydatidosis was the most prevalent in adults and cysticercosis in lambs. In both cases, these were associated with a wider range of pathologies and lesions, notably abscesses, either purulent or calcified (Table 9). As regards the rest of the pathologies, the only concurrences found were in animals rejected due to dicrocoeliosis, a quarter of which also presented hepatic necrosis.

4. Discussion

Analysis of these results should must consider that the aim of the study was not to determine the prevalence of certain diseases in the sheep population (that would call for a different type of study) but rather to discover which ones go unnoticed throughout the sheep life cycle and which, thanks to the abattoir management

system, do not reach the human food chain. Immediately evident was the fact that the percentage of rejections was relatively high in lambs (16.04%) and very high in adults (54.75%), mostly consisting of rejected livers and lungs, which coincides with previous findings by other authors [1-2, 10]. Nearly forty per cent of rejections had a parasitic origin, mainly associated with a small number of diseases, coinciding with previous findings [4]. These diseases were hydatidosis, cysticercosis and dicrocoeliosis and rejections varied considerably, depending on age, province of origin and season of the year.

While we are aware that these results are not a reflection of the health status of the entire livestock population, they do allow us to more accurately classify and quantify processes that usually go undetected in health campaigns and programmes and that could constitute a health risk if they entered the food chain.

Therefore, in view of possible repercussions on Public Health, attention must be paid to rejections due to hydatidosis affecting nearly a quarter of the adult animals analysed. Liver and lung are the preferred organs for hydatid cysts but they are also found in other organs including the heart, spleen, kidney, brain, muscle tissue and bones [6]. Our data coincides with the main target for hydatidosis in a ratio of approximately 2.4:1 (74 livers and 31 lungs) but it was not found in the less typical organs. These results differ from those of Euzéby [11] who found a similar frequency in both locations, although we agree with this author that it is difficult to quantify the frequency of lesions in the liver and lung. The variability observed between different areas could be related to geoclimatic conditions and intraspecific variations of *E. granulosus* [12] and to animal age and species [13]. As Moreno [6] pointed out, due to the tender age of lambs or very recent infections, cysts are not yet large enough to seriously compromise the functioning of the viscera where they form. As a consequence, there are no visible clinical signs thus explaining why they were not detected in lambs. Retrospective long-term studies [14] have shown a degree of seasonality. However, given the chronic nature of hydatidosis, seasonal differences should be minimal. The differences we observed (highest incidence in spring and lowest in autumn) indicate that the seasonality of rejections is due to work dynamics at the abattoir since, as mentioned above, there is no regular schedule when it comes to which provinces send adult animals for slaughter and when. In this case, a single consignment of animals from the province of Salamanca caused "false seasonality". The histopathological preparations from hydatid cyst lesions showed the classical cystic structure [15], although inflammatory reactions around the parasite's protective capsule were not observed in all cases.

The distribution of hydatidosis is worldwide and quite variable, highly prevalent in some areas and appearing only sporadically in others. Very few countries are reported to be free of hydatidosis [16]. In Spain, the presence of *E. granulosus* is also highly variable from one province to the next: Extremadura (10.5%), Manserja-Ciudad Real (11.5%), Navarra (19.8%), Madrid (35.0%), La Rioja (47.8%), Guadalajara (80.3%), Aragón (79.8%) [17-18]. On a global scale, these results situate Spain at an intermediate level. The data appear to coincide with those from Ciudad Real and Toledo but the high prevalence in Madrid and Extremadura (Table 5) is due to the fact that the number of adult animals inspected in these provinces was very low and varied greatly and this is likely to have skewed the percentages found. Studies examining more livestock over a longer period of time could probably confirm or rule out this regional variability.

In the case of cysticercosis, the most common lesions are caused by migration, followed by vesicles and, to a lesser extent,

fibrotic foci and intraparenchymal cysts below Glisson's capsule [6]. While not having the same zoonotic significance as hydatidosis, detection of cysticercoses in the abattoir is an important indicator of health conditions on the farm of origin and the resulting rejections can cause heavy economic losses. In this study, nearly five percent of lamb livers were rejected. Considering the number of lambs slaughtered annually, this implies a high economic cost for both the abattoir and livestock farmers. While these figures differ greatly from those registered in countries such as Ethiopia with a rate of 79% [19], and in abattoirs in central Spain with 20% [20-21], we agree with other authors [22] on the importance of cysticercosis as a cause for rejection of lamb livers. In this case we assume that infections are recent given the young age at which lambs are slaughtered. Therefore, the seasonality observed may well reflect the situation among the sheep population with similar levels in winter-spring-summer and a much lower prevalence in autumn. These results partially coincide with those of other authors who found the highest rate of cysticercosis in the colder seasons [20, 23]. It is no easy task explaining why the incidence drops off in autumn but this is possibly related with official deparasitation campaigns against cestodes in domestic pets which tend to take place in spring and summer. Therefore, lower infection rates could logically be expected in sheep slaughtered in autumn.

In this study we observed something for which we can find no objective explanation. Despite the assumption that cases of cysticercosis were recent, all the histopathological samples exhibited the same features typical of chronicity: notable hyperplasia of the bile ducts, dilated lymph vessels and inflammatory polymorphonuclear infiltration around the porta hepatis, in some cases with fibrosis and areas of necrosis caused by the migration of *Cysticercus tenuicollis* with predominantly polymorphonuclear neutrophil and eosinophil infiltrations, surrounded by areas of fibrosis where infiltration was mostly mononuclear.

As regards rejections caused by trematodes, all were due to *Dicrocoelium dendriticum* and the observed prevalence was low (3%), coinciding with Luzón et al., [21]. The typical lesions were observed in livers rejected for this reason [24]. The few animals in which this pathology was detected belonged to very specific farms, areas and time periods, with significant differences ($p < 0.05$) in terms of the season (winter: 10.00%, summer and autumn: 0.00% in both). A similar situation was described for *F. hepatica* by Froyd [25] in England. This was explained by the close link between local climatic-environmental conditions and the prevalence of trematodiasis, making it difficult to generalise about the prevalence of this disease over a wide area.

With respect to sarcosporidiosis, there seems to be great variability between flocks [26-27], i.e. reported incidence ranging from 6% to 54% [18, 26-29], which is much higher than our findings (0.4%) and those of Moreno [6]. Mature sarcocysts do not normally cause an inflammatory reaction in the surrounding tissues, except when they burst or undergo excessive degeneration, giving rise to eosinophilic myositis [24]. Cysts are sometimes microscopic and this should also be taken into consideration as they are not easily detected in the post mortem inspection [6, 24]; only 20.5% of sarcosporidiosis are visible to the naked eye as macrocysts [30], resulting in rejection.

The total absence of *Fasciola hepatica* is particularly noteworthy. This stands in sharp contrast to bibliographic findings which show that fasciolosis is more frequent in Spain and other countries [24, 31-32], although according to Manga et al. [33], *D. dendriticum* infestations are also very common, especially in sheep. The absence of fasciolosis is at least partly explained by the fact

that the slaughtered sheep come from areas with a dry climate. It should also be noted that, because of the characteristics of the study, the only affected animals considered were those with macroscopic lesions that led to rejection of the liver; animals in the prepatent period or those with subclinical infestation were not considered.

We would also draw attention to the absence of coenurosis and oestrosis, two processes that are frequent in many of the animals' provinces of origin. In this study, we were unable to test the animals for these conditions as slaughtering regulations require the animals' heads to be discarded. We did not observe any clinical indications of these processes during the ante-mortem inspection but such observations lack the objectivity of the other processes analysed.

Rejections of parasitic origin confirm that hydatidosis in sheep and cysticercosis in lambs were associated with a wider variety of pathologies and lesions, with a high incidence of abscesses, either purulent or calcified, in both cases (Table nº 6). Respaldiza & Respaldiza [5] also detected a close link between the presence of hepatic abscesses and hydatidosis in sheep. This is to be expected as in both processes a relatively frequent form of evolution is the formation of an abscess caused by pyogenic microorganisms possibly introduced at the larval stage [6]. Not many concurrences were found with the rest of the pathologies. Almost a quarter of the animals rejected for dicrocoeliosis also exhibited hepatic necrosis, probably because the migratory channels of the parasites through the bile canaliculi triggered ischaemic and inflammatory processes leading to hepatic necroses, especially where trematodiasis caused massive infestations in the liver.

5. Conclusion

Sanitary rejections of sheep, essentially liver and lung, correlated closely with the age of the animal. It was significant that only a few diseases accounted for most of the rejections. The most important parasitoses were hydatidosis in sheep more than one year old and cysticercosis in lambs, with rejections reaching their highest level for lambs in spring and for adult sheep in spring and summer.

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Table 1. Previous references on economic losses through abattoir rejections. * Only parasitic causes analysed.

Author/s	Species	Cause	Place
[34] Sinclair (1969)	Bovine, ovine and caprine	Fasciolosis	United Kingdom
[7] Arrigo et. al (1984)			Great Britain
[8] Martin (1996)			Great Britain
[4] Jepson & Hinton (1986)	Lambs	Cysticercosis	Great Britain
[35] Chiew & Chua	Porcine	Any	Singapore
[2] Morales (1996)	Bovine, ovine, caprine and porcine	Any	Chile
[36] Theodoropoulos* et al. (2002)	Bovine, ovine, caprine and porcine	Hydatidosis in lung, Hydatidosis and trematodiasis in liver	Greece
[37] Lima et al. (2005)	Bovine	Any	Cuba
[21] Luzón et al. (2007)	Ovine	<i>C. tenuicollis</i>	Spain (CLM)
[38] Cadmus & Adesokan (2009)	Bovine	Any	Nigeria
[39] Brito et al. (2010)	Bovine	Fasciolosis	Cuba
[14] Ahmadi & Meshkehkar	Bovine, ovine and caprine	Hydatidosis	Iran
[22] Brown et al. (1984)	Sheep livers	<i>Cysticercus tenuicollis</i>	
[23] Trees et al. (1985)	Lamb livers	Cysticercosis	
[17] García (1987)			Spain
[10] Cubero & Morollón (1993)	Adult sheep	Emaciation or cachexia	
	Lambs	Emaciation, arthritis	Spain
[40] Fábregas et al. (2005)	Lamb livers	Cysticercosis 2.5%	Spain
[21] Luzón et al. (2007)			Spain
[41] Moreno (1994)	Lamb livers	Cysticercosis >85%	Spain
[29] Pérez & González		Ovine sarcosporidiosis 6%	Spain
[28] Martínez et al. (1989)		Ovine sarcosporidiosis 15%	Spain
[18] Sánchez et al. (1983)		Ovine sarcosporidiosis 54%	Spain

Table 2. Monthly and seasonal distribution of slaughtered and inspected animals and samples taken for the study.

	Autumn			Winter			Spring			Summer			TOTAL
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	
Lambs													
Inspected	345	200	200	250	150	150	75	125	100	100	150	100	1,945
Rejected	44	27	15	24	26	30	22	23	22	30	19	30	312
%	12.75	13.50	7.50	9.60	17.33	20.00	29.33	18.40	22.00	30.00	12.67	30.00	16.04
Adults													
Inspected	155	25	-	60	25	35	32	34	18	45	27	28	484
Rejected	49	13	-	26	22	19	28	18	22	23	27	18	265
%	31.61	52.00	-	43.33	88.00	54.29	87.50	52.94	122.22	51.11	100.00	64.29	54.75

Table 3. Distribution by province of animals inspected and samples collected.

	Badajoz	Ciudad Real	Cuenca	Albacete	Toledo	Córdoba	Ávila	Salamanca	Madrid	Cáceres
	Lambs									
Inspected	1,170	250	75	-	-	250	-	100	100	-
Rejected	210	25	15	-	-	40	-	14	8	-
%	17.95	10.00	20.00	-	-	16.00	-	14.00	8.00	-
Adults										
Inspected	52	52	111	100	90	-	18	40	12	9
Rejected	31	32	57	44	35	-	22	23	17	4
%	59.62	61.54	51.35	44.00	38.89	-	122.22	57.50	141.67	44.44

Table 4. Seasonal distribution of liver rejections in adult sheep due to hydatidosis in the abattoir and in Spain.

	Sheep over two years old				Older lambs			
	Winter n=120	Spring n=84	Summer n=100	Autumn n=180	Winter n=550	Spring n=300	Summer n=350	Autumn n=745
Hydatidosis	34*(25+9) 28.33%	36*(23+13) 42.86%	18*(14+4) 18.00%	17*(12+5) 9.44%	-	-	-	-
Dicrocoeliosis	12 10.00%	1 1.19%	-	-	-	-	-	14
Cysticercosis	-	-	-	-	39 7.09%	20 6.67%	25 7.14%	13 1.74%
Sarcosporiosis	2 1.57%	-	-	-	-	-	-	-
Verminous pneumonia	1 0.21%	-	-	-	-	-	2 0.57%	-

Table 5. Distribution by province of rejections of parasitic origin in the abattoir studied.

		Córdoba	Cuenca	Ciudad Real			Albacete	Toledo	Salamanca	Ávila	Madrid	Cáceres	Badajoz
Hydatidosis in adult sheep	AA	ns	15	8	12	11	16	14	8	2	19		
	PR		28.85	7.21	12.00	12.22	40.00	44.44	66.67	22.22	36.54		
Cysticercosis in lambs	AA	12	4	9	ns	ns	3	ns	1	ns	68		
	PR	4.80	5.33	3.6			3.00		1.00		5.81		
Dicrocoeliosis in adult sheeps	AA	ns	11	-	1	-	-	1	-	-	-		
	PR		21.15		0.90			5.56					
Sarcosporiosis	AA	ns	2	-	-	-	-	-	-	-	-		
	PR		1.80										

AA: animals affected by the pathology. PR = estimated prevalence of the pathology (%). ns = no animals from this province were inspected.

Table 6. Pathologies of parasitic origin with other concurrent pathologies in the abattoir under study, showing the number of rejected organs with both pathologies and the percentage it represents of the total organs affected by the cause of rejection.

Cause of rejection	Concurrent pathology/pathologies					
Hydatidosis in adult sheep	ns	15	8	12	11	16
		28.85	7.21	12.00	12.22	40.00
Cysticercosis in lambs	12	4	9	ns	ns	3
	4.80	5.33	3.6			3.00
Dicrocoeliosis in adult sheeps	ns	11	-	1	-	-
		21.15		0.90		
Sarcosporiosis	ns	2	-	-	-	-
		1.80				

Table 7. Distribution of different rejections according to the breed in the abattoir under study (n= 226 samples).

Breed	Cysticercosis	Dicrocoeliosis	Hydatidosis in liver	Hydatidosis in liver & lung	Hydatidosis in lung	Verminous pneumonia	Sarcosporidiosis
Alcarreña	4		7		3		
Assaf			1				
Castellana			11		4		
Lacaune			2				
Manchega	4		11		2		
Merina	83		19		5	2	
Mestiza	8	11	24	2	18	1	2
Talaverana			2				
Total	99	11	77	2	32	3	2