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The impact of liver fluke infection on steers in Ireland: A meta-analytic approach

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ABSTRACT

Post-mortem liver inspection results together with production parameters are often used to estimate the impact of liver fluke infection on farm animal populations. However, post mortem liver inspection is an imperfect method of determining the liver fluke infection status of cattle. This work estimates the difference in mean lifetime weight gain at 819 days (ΔLWG_{819}) between steers assigned liver fluke negative (LFN) and liver fluke positive (LFP) status at post-mortem meat inspection, quantifies the potential impact of imperfect sensitivity and specificity on these results and estimates the economic impact of these differences.

The study population is 32,007 steers that never moved from their birth herd in the Republic of Ireland and were slaughtered in one of two Irish meat processors in 2014. Individual animal-level data are used to generate 46 county - processor level estimates of ΔLWG_{819}. Standard errors and confidence intervals for these estimates are derived using bootstrapping. A meta-analytic approach is then used to obtain 3 overall estimates of the effect of liver fluke status on the ΔLWG_{819} in all the county - processor combinations, assuming post - mortem liver inspection Se = Sp = 1, 0.99 and 0.95. A random effects model is used and 95% prediction intervals (95% PI) are calculated.

Assuming Se = Sp = 1 for post - mortem liver inspection, the random effects summary estimate of ΔLWG_{819} ($\Delta LWG_{819(RE)}$) is 36 kg (95% PI: -1, 73). There is a minor change in $\Delta LWG_{819(RE)}$ (38 kg, 95% PI: -1, 77) when Se = Sp = 0.99 is assumed but this increases to 46 kg (95% PI: -2, 94) assuming Se = Sp = 0.95. The corresponding cost in euros of these differences between the LFN and LFP steers, assuming a price per kg of €3.90, are €77.01 (95% PI: -2.57, 156.37), €80.65 (95% PI: -3.43, 164.74) and €98.67 (95% PI: -5.15, 202.27) respectively.

Our results demonstrate an association between liver fluke infection and reduced weight gain. We show that the effect of liver fluke infection on weight gain in cattle is underestimated due to misclassification resulting from imperfection in post mortem meat inspection. These findings will aid researchers, farmers and veterinary practitioners to make informed decisions on the control of liver fluke on farms.

1. Introduction

Fasciola hepatica or liver fluke is a trematode parasite that is common worldwide and can infect a wide variety of ruminants, including cattle (Khan et al., 2013). A variety of studies have shown the negative impact of liver fluke infection on beef cattle, with the type and magnitudes of the effects varying from study to study. A Scottish study in 2013/2014 estimated that cattle with livers classified as having liver fluke damage had, on average, 10 days greater slaughter age than animals with no evidence of fasciolosis (Mazeri et al., 2017). An earlier Scottish abattoir survey found that carcases with liver fluke pathology had carcase weights that were 0.63 kg lighter than those with no pathology and that the presence of liver fluke was associated with lower carcase conformation and lower fat scores (Sanchez-Vazquez and Lewis, 2013). In 2009, a Belgian project linked liver fluke infection status, as determined by a liver fluke antibody test on meat juice, to a decrease in herd mean carcase weight of 3.4 kg (Charlier et al., 2009). When estimating the financial cost of liver fluke in Switzerland, in 2005, Schweizer et al. summarized the available published literature on weight loss in cattle due to liver fluke and used a mean reduction in weight gain of 8·9% for their subsequent calculations (Schweizer et al., 2005).

The Irish beef sector accounts for over 30% of the value of Irish agricultural output at producer prices (Hanrahan, 2016). In 2010 there

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were over 79,000 farms breeding beef cattle, and overall, more than 90,000 breeding and non-breeding beef farms (Central Statistics Office, 2012). An abattoir study in Ireland in 2002–2003 found pathology attributable to liver fluke in the livers of 65% of beef and dairy cows (Murphy et al., 2006). In 2013, using a bulk milk tank liver fluke antibody ELISA test, another study of Irish dairy cows showed that cows in 78% of herds had been exposed to liver fluke (Selemetas et al., 2015). Most recently, a large scale examination of abattoir records in Northern Ireland from 2011 to 2013 found that 24% of animals were recorded with signs of liver fluke infection (Byrne et al., 2016). Notwithstanding the importance of the beef industry in Ireland and the high prevalence of liver fluke infection in Irish cattle, we have not found any abattoir surveys on the impact of liver fluke on Irish beef cattle.

Post-mortem liver inspection is an imperfect method for identifying the liver fluke infection status of cattle (Mazeri et al., 2017; Rapsch et al., 2006). Though many researchers acknowledge this limitation when estimating liver fluke prevalence (Bellet et al., 2016; Byrne et al., 2016; Olsen et al., 2015) and economic impact (Bellet et al., 2016; Mazeri et al., 2017; Sanchez-Vazquez and Lewis, 2013), we have not identified any study that has taken post-mortem meat inspection sensitivity and specificity into account in their calculations.

In this work we aim to:

(a) Estimate the difference in mean lifetime weight gain between steers assigned liver fluke negative (LFN) and liver fluke positive (LFP) status, based on findings from post-mortem liver inspection in the Republic of Ireland,
(b) Quantify the potential impact of imperfect post - mortem liver inspection on these results, and
(c) Estimate the economic impact of these differences in mean lifetime weight gain

2. Methods

2.1. Data origin

A database containing animal records from steers which had moved directly from their birth herd to slaughter at one of two meat processors in Ireland in 2014, was obtained from the Irish Cattle Breeding Federation. Processor 1 included six separate abattoirs and Processor 2, one abattoir. The database contained animal-level information: animal identifier, birth and slaughter dates, sex, carcase weight, sire type (beef or dairy) and liver fluke infection status, supplemented by county location of the herd from which each animal came. The dataset for analysis was restricted to steers of 365 days and older. The data are presented in Table 1.

2.1.1. Exposure determination

All animals in abattoirs in Ireland undergo post-mortem meat inspection as set out in legislation (*REGULATION (EC) No 854/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004*, 2004). Information from liver inspections was recorded by Temporary Veterinary Inspectors using touch screens at inspection points. Livers were recorded as “active”, “cured”, or “unaffected” in Processor 1. “Active” indicated that liver fluke was detected, while “cured”, that liver damage attributable to liver fluke was observed but no liver fluke was seen and “unaffected” indicated that no abnormalities related to liver fluke were observed. In Processor 2, livers were recorded as “chronic liver fluke”, “recent liver fluke” or “fluke damaged liver”. “Chronic” and “recent”, represented livers in which liver fluke was observed but with different levels of liver pathology. “Fluke damaged liver” was reported in situations where no liver fluke was noted but pathological changes attributable to liver fluke were observed. In Processor 2, recording was done by exception and livers with no observations were assumed to have no changes attributable to the presence of liver fluke. A binary variable was created and steers with infection status recorded as “active”, “cured”, “chronic liver fluke”, “recent liver fluke” and “fluke damaged” designated “liver fluke positive” (LFP). Those recorded as “normal” and those with no observations from Processor 2 were assigned “liver fluke negative” (LFN) status. Throughout the manuscript, “liver fluke infection” is used to denote lifetime liver fluke infection.

2.2. Deriving the outcome of interest

Individual animal-level data were then used to generate county - processor level estimates (Table 2). The outcome of interest was the difference in mean lifetime weight gain at 819 days (ΔLWG_{819}) between LFN and LFP steers in each county - processor combination. This therefore is the expected difference in average weight between LFN and LFP steers from a given county – processor combination when both are 819 days old. The lifetime age was set at 819 days, as this was the average lifetime (time to slaughter) of steers in the database. Thus, the expected difference in weight is the difference if the animals were slaughtered at 819 days. We chose ΔLWG_{819}, as opposed to a difference in average daily gain, as we felt that this was easier for the reader to appreciate. The average daily gain of a steer is generally less than 1 kg and thus average daily gain differences would be very small fractional numbers.

The average daily gain (A) of a steer is given by:

$$A = \frac{\text{Live weight at slaughter - Birth weight}}{\text{Age at slaughter}} \quad (1)$$

The live weight (LW) of each steer at slaughter was calculated from its carcase weight and kill out percentage (McKiernan et al., 2007):

Where

$$LW = \frac{1}{\text{Kill out\%}} \text{x Carcase weight} \quad (2)$$

Based on available literature, we assumed a kill out percentage of 55% (Clarke et al., 2009; Conroy et al., 2010) and a birth weight of 40 kg (Dhakal et al., 2013; Nelson et al., 2016) for all steers. Assuming that misclassification of liver fluke status at slaughter was independent

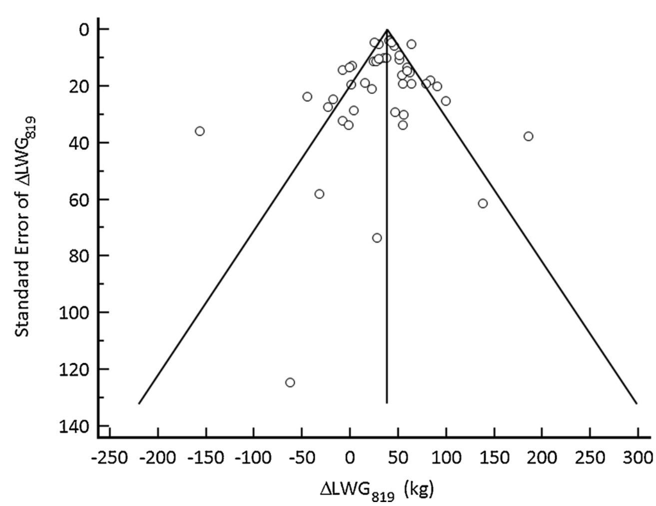

Fig. 1. Funnel plot of point estimates of the difference in mean lifetime weight gain (ΔLWG_{819}) for processor 1 and 2 counties assuming Se = Sp = 1 for post - mortem liver inspection.

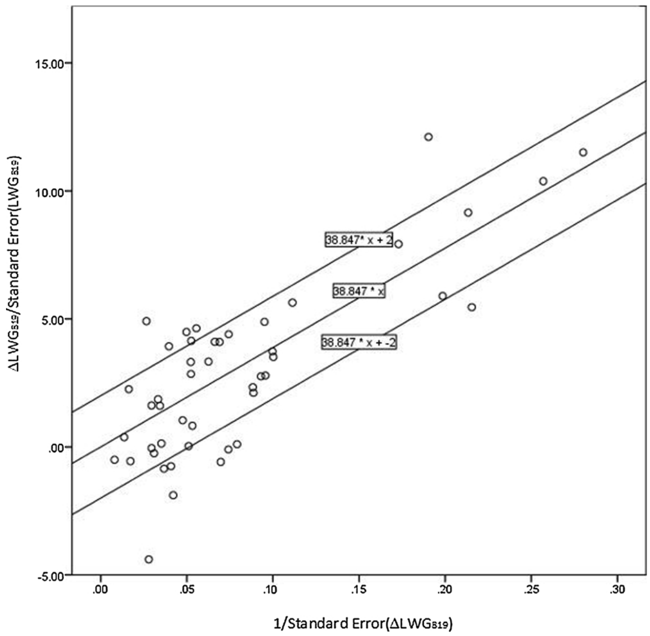

Fig. 2. Galbraith plot of point estimates of the difference in mean lifetime weight gain (ΔLWG_{819}) divided by their standard errors against the inverse of their standard errors for all county – processor combinations assuming Se = Sp = 1 for post – mortem liver inspection.

of the time to slaughter, the mean average daily gain for the LFN and LFP steers for each county - processor combination was calculated using Eqs. 3 and 4 (L. L. McV. Messam, personal communication: derivation available on request):

$$\overline{A_{D-}} = \frac{(\sum_{i}^{n_{T-}} A_{i(T-)}) - (\sum_{j}^{n_{T+}} A_{j(T+)} + \sum_{i}^{n_{T-}} A_{i(T-)})(1\text{-}Se)}{n_{T-} - N(1\text{-}Se)} \quad (3)$$

$$\overline{A_{D+}} = \frac{(\sum_{j}^{n_{T+}} A_{j(T+)}) - (\sum_{j}^{n_{T+}} A_{j(T+)} + \sum_{i}^{n_{T-}} A_{i(T-)})(1\text{-}Sp)}{n_{T+} - N(1\text{-}Sp)} \quad (4)$$

Where,

$\overline{A_{D-}}$ is the mean average daily gain for LFN steers in a herd,
$\overline{A_{D+}}$ is the mean average daily gain for LFP steers in a herd,
$A_{i(T-)}$ is the average daily gain of the i[th] LFN steer in a herd,
$A_{j(T+)}$ is the average daily gain of the j[th] LFP steer in a herd,
Se and Sp are the sensitivity and specificity of post - mortem liver inspection respectively,
n_{T-} is the number of LFN steers in a herd,
n_{T+} is the number of LFP steers in a herd and
N is the total number of steers (i.e. $n_{T-} + n_{T+}$).

From (3) and (4) the difference in $\overline{A_{D-}}$ and $\overline{A_{D+}}$ ($\Delta\overline{A_D}$) was calculated:

$$\text{Where, } \Delta\overline{A_D} = \overline{A_{D-}}\text{-}\overline{A_{D+}} \quad (5)$$

Initially, perfect sensitivity and specificity (Se = Sp = 1) for post - mortem liver inspection was assumed. We then employed bootstrapping to derive standard errors and confidence intervals for $\Delta\overline{A_D}$ using the Microsoft Excel 2013 Add-in Resampling Stats (http://www.resample.com/excel/). The individual steers' average daily gains in each county - processor combination were resampled with replacement and Eqs. 3–5 were reapplied to the resampled values. For each county - processor combination 10,000 samples (Hesterberg, 2015) were calculated to form a sampling distribution for $\Delta\overline{A_D}$. From these, a standard error and 95% confidence interval (95% CI) was calculated for each county - processor estimate of $\Delta\overline{A_D}$. The estimates and 95% CIs were then multiplied by 819 days to get ΔLWG_{819} and its 95% CI for each county –

Table 1
County-level mean ages at slaughter (MA) and mean carcase weights (MCW) for liver fluke test positive and test negative steers, apparent prevalence (AP) and percentages of steers in herds with a beef sire (% Beef) by processor 1 and 2, Ireland, 2014.

County	PROCESSOR 1								PROCESSOR 2							
	Test Negatives			Test Positives			AP	% Beef	Test Negatives			Test Positives			AP	% Beef
	n	MA (days)	MCW (kg)	n	MA (days)	MCW (kg)			N	MA (days)	MCW (kg)	n	MA (days)	MCW (kg)		
Carlow	139	874	382	23	871	394	0.14	82	372	803	341	70	815	348	0.16	65
Cavan	377	821	356	38	955	365	0.09	89	12	779	295	1	796	390	0.08	100
Clare	213	829	359	97	887	352	0.31	87	159	911	359	105	939	353	0.40	92
Cork	6346	798	324	986	859	322	0.13	58	230	792	312	60	806	304	0.21	67
Donegal	25	740	387	0	-[a]	-[a]	0.00	100	0	-[a]	-[a]	0	-[a]	-[a]	-[a]	-[a]
Galway	322	884	380	80	1017	368	0.20	95	112	835	325	35	839	330	0.24	78
Kerry	604	760	314	212	835	323	0.26	63	216	794	340	86	878	338	0.28	70
Kildare	51	792	359	17	816	337	0.25	100	18	896	356	41	841	350	0.69	90
Kilkenny	1718	806	344	684	855	347	0.28	63	1260	820	343	452	860	340	0.26	65
Laois	148	810	334	25	824	332	0.14	73	354	813	345	161	892	348	0.31	82
Leitrim	31	859	346	9	860	350	0.23	100	0	-[a]	-[a]	0	-[a]	-[a]	-[a]	-[a]
Limerick	1753	793	325	646	876	320	0.27	64	139	805	318	55	810	308	0.28	79
Longford	85	880	377	2	669	316	0.02	97	18	934	314	17	997	298	0.49	100
Louth	125	903	353	13	929	334	0.09	83	0	-[a]	-[a]	0	-[a]	-[a]	-[a]	-[a]
Mayo	72	831	383	7	907	414	0.09	91	23	809	329	2	875	311	0.08	100
Meath	131	900	380	3	1104	397	0.02	96	87	857	357	36	837	352	0.29	95
Monaghan	806	805	355	79	937	366	0.09	84	2	857	337	2	723	295	0.50	100
Offaly	338	807	331	78	863	321	0.19	77	39	788	332	165	789	320	0.81	78
Roscommon	67	921	393	18	975	371	0.21	100	14	931	339	4	824	282	0.22	100
Sligo	120	958	386	19	1079	367	0.14	99	12	761	371	3	965	349	0.20	100
Tipperary	3619	791	329	1129	857	331	0.24	66	362	773	326	112	829	329	0.24	62
Waterford	1117	832	331	621	879	325	0.36	66	497	821	331	159	900	328	0.24	64
Westmeath	32	962	354	5	1090	305	0.14	97	24	916	336	43	919	347	0.64	88
Wexford	459	804	356	195	840	352	0.30	78	1659	800	352	382	877	357	0.19	76
Wicklow	109	782	357	26	760	370	0.19	82	530	787	342	57	878	368	0.10	85

Table 2
County - Processor (1 and 2) level differences in mean life time weight gain at 819 days between liver fluke negative and liver fluke positive steers (ΔLWG_{819}) along with 95% confidence intervals (CIs), by post – mortem meat inspection sensitivity (Se) and specificity (Sp) level.

County - processor	Se = Sp = 1			Se = Sp = 0.99			Se = Sp = 0.95		
	ΔLWG_{819} (kg)	95% CI		ΔLWG_{819} (kg)	95% CI		ΔLWG_{819} (kg)	95% CI	
		Lower	Upper		Lower	Upper		Lower	Upper
Carlow - 1	−18	−68	27	−20	−73	29	−27	−100	40
Cavan - 1	63	26	101	71	29	113	133	55	212
Clare - 1	53	22	84	55	23	87	62	26	97
Cork - 1	41	34	48	44	37	52	63	52	73
Galway - 1	79	40	115	82	42	120	101	51	147
Kerry - 1	37	18	57	39	18	59	45	21	68
Kildare - 1	55	−8	124	57	−8	128	66	−10	149
Kilkenny - 1	25	16	35	26	17	36	30	19	41
Laois - 1	22	−19	63	23	−20	67	32	−28	92
Leitrim - 1	4	−52	60	4	−54	62	5	−64	74
Limerick - 1	64	53	74	66	55	76	76	63	88
Longford - 1	−63	−253	128	−110	−443	224	[b]	[b]	[b]
Louth - 1	47	−15	99	52	−17	109	95	−30	200
Mayo - 1	−2	−64	66	−2	−72	74	−3	−141	145
Meath - 1	99	52	149	178	92	266	[b]	[b]	[b]
Monaghan - 1	59	33	86	66	37	96	128	72	185
Offaly - 1	59	30	87	62	32	91	78	40	114
Roscommon - 1	56	−2	117	58	−2	122	70	−3	147
Sligo - 1	90	51	128	97	54	137	136	76	193
Tipperary - 1	40	33	48	42	34	50	49	40	59
Waterford - 1	43	34	52	44	35	53	49	39	59
Westmeath - 1	138	20	259	148	22	277	210	31	393
Wexford - 1	29	8	50	30	9	51	34	10	58
Wicklow - 1	−45	−93	1	−47	−97	1	−58	−121	2
Carlow - 2	−8	−37	20	−9	−39	21	−12	−51	28
Cavan - 2	−157	−219	−78	−179	−249	−89	−428	−594	−212
Clare - 2	30	9	51	30	9	52	33	10	57
Cork - 2	24	2	46	25	2	48	30	2	58
Galway - 2	1	−24	25	1	−25	26	2	−29	31
Kerry - 2	62	32	91	64	33	94	73	38	107
Kildare - 2	−23	−77	29	−24	−79	30	−27	−90	34
Kilkenny - 2	30	20	40	31	20	41	35	24	47
Laois - 2	51	31	72	53	32	74	59	36	83
Limerick - 2	26	4	49	27	4	50	31	5	57
Longford - 2	54	18	92	55	18	94	60	20	102
Mayo - 2	84	49	120	95	55	135	212	124	304
Meath - 2	−1	−27	26	−1	−28	26	−2	−32	30
Monaghan - 2	−32	−132	67	−33	−134	69	−36	−146	75
Offaly - 2	16	−21	52	16	−22	54	20	−28	68
Roscommon - 2	28	−132	146	29	−137	152	35	−164	182
Sligo - 2	185	119	263	193	124	274	237	152	336
Tipperary - 2	35	16	55	36	16	57	43	19	67
Waterford - 2	51	33	68	52	34	70	62	40	82
Westmeath - 2	−8	−71	57	−8	−72	58	−9	−80	65
Wexford - 2	46	35	57	48	36	60	60	45	75
Wicklow - 2	1	−38	39	1	−42	43	1	−75	76

[b]Indicates studies excluded from the meta-analysis because AP < 1 – Sp.

processor combination (Table 2).

2.3. Analysis of county - processor level estimates

The 46 county - processor level estimates of ΔLWG_{819} (Table 2) were obtained and a meta-analytic approach was used to analyse these results. Initially, we calculated both fixed ($\Delta LWG_{819(FE)}$) and random ($\Delta LWG_{819(RE)}$) effects estimates (Borenstein et al., 2009; DerSimonian and Laird, 1986) using MedCalc Version 18.11.3 software (https://www.medcalc.org/).

2.4. Examining heterogeneity

The results were assessed visually for heterogeneity using funnel and Galbraith plots (Bax et al., 2009). Additionally, we calculated I^2 values (Higgins, 2003) and their associated 95% CIs (Borenstein et al., 2009) from the county - processor level point estimates and the $\Delta LWG_{819(FE)}$, reflecting the estimated percentage variation in the pooled estimates due to county - processor variation beyond that caused by chance.

Both the funnel (Fig. 1) and Galbraith plots (Fig. 2) showed evidence of considerable heterogeneity in the county - processor level estimates. In the funnel plot (Fig. 1) 26% (12/46) of the ΔLWG_{819}

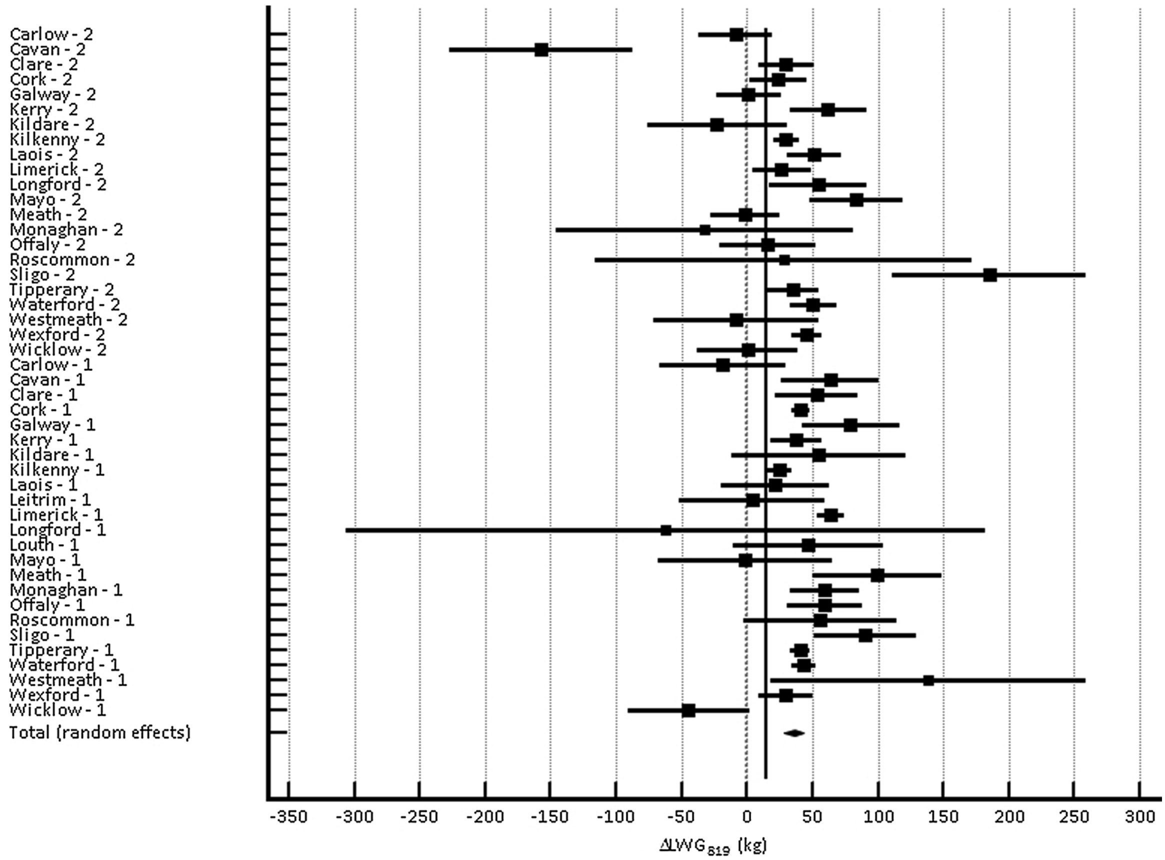

Fig. 3. Forest plot of estimates of the difference in mean lifetime weight gain (ΔLWG_{819}) and associated confidence intervals between the liver fluke negative and liver fluke positive steers for Processor 1 (county - 1) and 2 (county - 2) counties assuming Se = Sp = 1 for post - mortem liver inspection. The random effects summary estimate and confidence interval are represented by the diamond marker. Marker sizes for county – processor point estimates are proportional to their weight in the random effects analysis. The solid vertical line represents the assumed practically important threshold of 15 kg.

estimates were outside the lines representing the 95% CIs around the summary estimate. The Galbraith plot (Fig. 2) showed similar numbers of outliers. I^2 was found to be 76 (95% CI: 69, 82) (Table 3) indicating a high level of heterogeneity (Higgins, 2003).

As a result of the heterogeneity observed in the plots and high I^2 value, scatter plots were created to examine the relationship between the county - processor level estimates of ΔLWG_{819} and (a) the corresponding apparent prevalences (AP) of liver fluke and (b) the percentage of animals with a beef sire in the county to determine if these variables were related to the observed heterogeneity.

2.5. Prediction intervals

To allow for the differences in the effect of liver fluke between the 46 county - processor combinations, we present the results from the random effects model in this paper. In order to quantify the dispersion present in county – processor ΔLWG_{819} estimates, 95% prediction intervals (PI) (IntHout et al., 2016) for $\Delta LWG_{819(RE)}$ were calculated, using the following formulae (Riley et al., 2011):

$$\Delta LWG_{819(RE)} \pm t_{k-2}\sqrt{\tau^2 + SE(\Delta LWG_{819(RE)})} \quad (6)$$

Where $\Delta LWG_{819(RE)}$ is the random effects summary estimate of ΔLWG_{819},

t_{k-2} is the 100(1 − α/2) percentile of the t distribution with k–2 degrees of freedom,

k is the number of county – processor combinations $\alpha = 0.05$,

τ is an estimate of the standard deviation of ΔLWG_{819} across county – processor combinations and

$SE(\Delta LWG_{819(RE)})$ is the standard error of $\Delta LWG_{819(RE)}$

2.6. Economic impact

The economic impact of ΔLWG_{819} was estimated by first calculating the corresponding carcase weight and then multiplying by the price per kilogram carcase weight:

$$\text{Economic Inpact} = \Delta LWG819 \text{ x Kill Out\%x Price Per Kg.} \quad (7)$$

We employed an approach to inferences which focused on the practical importance of the magnitudes of the weight differences (Braitman, 1991) and a threshold of $\Delta LWG_{819} = 15$ kg was selected as practically important. At current (February 2019) market prices, a typical steer carcase could be expected to earn approximately €3.90 per kg carcase weight at slaughter (Department of Agriculture Food and the Marine, 2019), giving the 15 kg live weight difference a monetary value of approximately €32.00 (15*0.55*3.90). According to the 2017 Teagasc Farm survey, the average gross profit per livestock unit that can be expected on beef breeding farms that produce animals for slaughter is approximately €340 (Dillon et al., 2018). A 15 kg difference was considered practically important because it represents approximately 10% of the gross profit of a steer in this analysis and is likely to represent a figure at which a farmer would be motivated to treat or prevent liver fluke infection. To derive the inferences, the point estimates of the ΔLWG_{819} for each county - processor combination, and their 95% CIs,

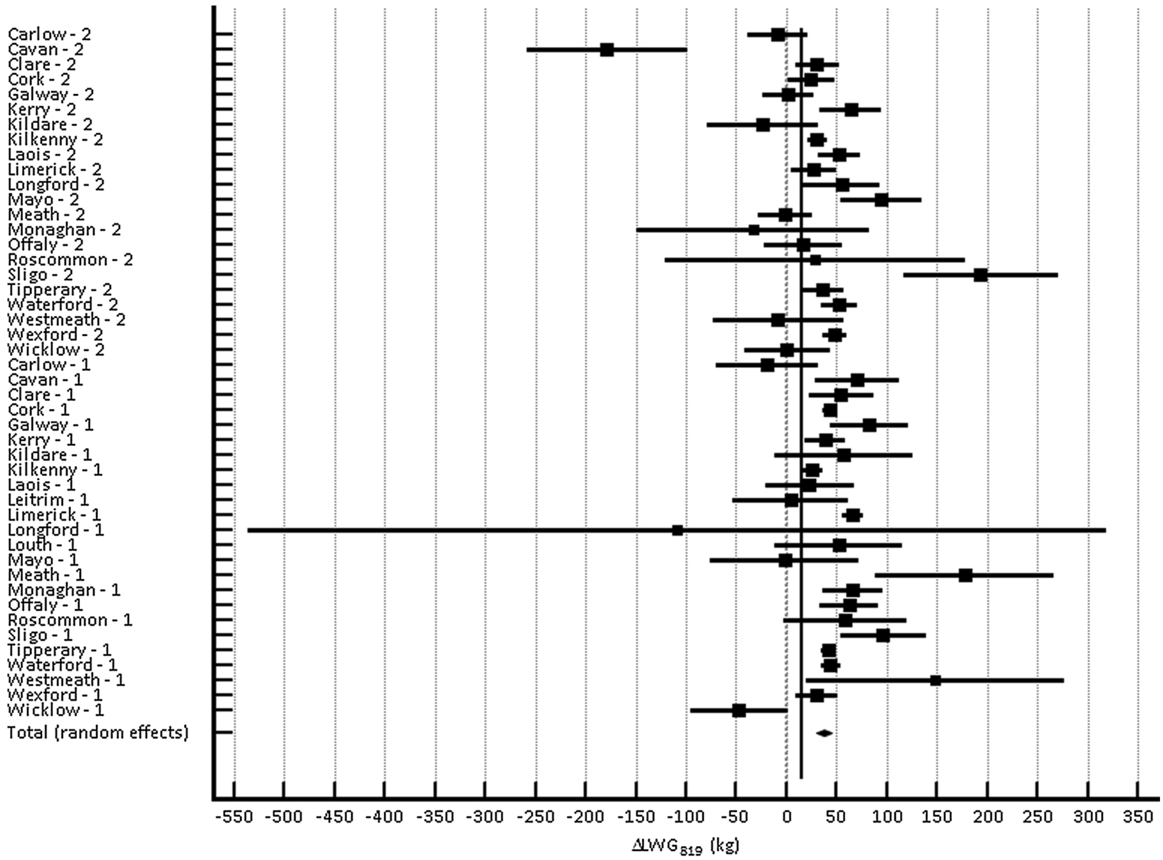

Fig. 4. Forest plot of estimates of the difference in mean lifetime weight gain (ΔLWG_{819}) and associated confidence intervals between the liver fluke negative and liver fluke positive steers for Processor 1 (county - 1) and 2 (county - 2) counties assuming Se = Sp = 0.99 for post - mortem liver inspection. The random effects summary estimate and confidence interval are represented by the diamond marker. Marker sizes for county – processor point estimates are proportional to their weight in the random effects analysis. The solid vertical line represents the assumed practically important threshold of 15 kg.

as well as the $\Delta LWG_{819(RE)}$ and its 95% PI were compared to the 15 kg threshold. Values above 15 kg represent a substantial difference (ie one of practical importance).

2.7. Secondary analyses

To explore the impact of disease misclassification on ΔLWG_{819}, the entire analytic procedure was repeated for Se = Sp = 0.99 and Se = Sp = 0.95. Thus, two further analyses were carried out for the county - processor combinations assuming Se = Sp = 0.99 and 0.95. Only county - processor combinations with AP > 1 - Sp were included in these analyses. Thus, Longford - 1 (Longford, Processor 1) and Meath - 1 for Se = Sp = 0.95 are omitted because the AP of LFP steers was 0.02 in both cases.

3. Results

3.1. Individual level data

3.1.1. Data description

The total number of steers 365 days of age and older was 32,007, of which 23,820 were slaughtered by Processor 1 and 8187 by Processor 2. Of the 26 counties in the Republic of Ireland, results were available from 25 counties from Processor 1 and 22 counties from Processor 2 (Table 1). There were no data from Dublin - 1, Donegal - 2, Dublin - 2, Leitrim - 2 or Louth - 2. There were data from 25 steers for Donegal - 1 but none were LFP steers and hence the difference between LFN and LFP steers could not be estimated and thus these data were excluded from the analysis.

Table 1 lists the numbers of LFN and LFP steers, their mean carcase weights, ages at slaughter and the percentage of steers with a beef breed sire for each county - processor combination. The number of steers in each county - processor combination ranged from 4 (Monaghan - 2) to 7332 (Cork - 1) (Table 1). The mean age at slaughter ranged from 740 (Donegal - 1) to 962 days (Westmeath - 1) and between 669 (Longford - 1) and 1104 days (Meath - 1) for LFN and LFP steers, respectively (Table 1). The mean carcase weights for the LFN steers ranged from 295 (Cavan - 2) to 393 kg (Roscommon - 1) and from 282 (Roscommon - 2) to 414 kg (Mayo - 1) for the LFP steers. The percentage of steers with a beef sire per county ranged from 58 (Cork - 1) to 100% (several counties) (Table 1).

3.2. ΔLWG_{819} and $\Delta LWG_{819(RE)}$

3.2.1. Se = Sp = 1

The ΔLWG_{819} for 80% (37/46) of the individual county - processor combinations showed that the LFN steers were heavier than LFP steers at the end of an 819 day life time (Table 2 and Fig. 3). Approximately 72% (33/46) of county - processor estimates had a ΔLWG_{819} of greater than 15 kg (Fig. 3). All ΔLWG_{819} values within the 95% confidence interval were considered practically important in 50% (23/46) of cases but 2 county - processor combinations (4%) had point estimates and 95% CIs that were entirely below 15 kg (Fig. 3). The median ΔLWG_{819} for the 46 county - processor combinations was 36 kg, with 5th and 95thpercentiles of −56 kg and 125 kg, respectively. The summary $\Delta LWG_{819(RE)}$ estimate for the county - processor combinations for Se = Sp = 1 was 36 kg, suggesting that at the end of a lifetime of 819 days a LFN steer would be, on average, 36 kg heavier than a LFP steer (Fig. 3, Diamond symbol).

3.2.2. Se = Sp = 0.99

The direction of corresponding ΔLWG_{819} estimates for the individual county - processor combinations were the same for Se = Sp = 0.99 as for Se = Sp = 1 with the magnitudes marginally increased (Table 2 and Fig. 4). The median ΔLWG_{819} for all the county - processor combinations was 37 kg with 5th and 95th percentiles of −88 kg and 167 kg, respectively. The number of individual county - processor combinations showing differences of practical importance remained the same as for Se = Sp = 1. Compared to the summary $\Delta LWG_{819(RE)}$ estimate for Se = Sp = 1 (36 kg), the increase assuming Se = Sp = 0.99 (38 kg) was marginal.

3.2.3. Se = Sp = 0.95

The ΔLWG_{819} estimates of the individual county - processor combinations assuming Se = Sp = 0.95 were greater than both Se = Sp = 1 and Se = Sp = 0.99 (Table 2 and Fig. 5). The median ΔLWG_{819} was 44 kg with 5th and 95th percentile values of −53 kg and 211 kg, respectively. The number of county - processor combinations showing differences of practical importance was similar to when Se = Sp = 1 and Se = Sp = 0.99. Individual county – processor ΔLWG_{819} estimates were greater than 15 kg in approximately 73% of cases (32/44) (Fig. 5). The ΔLWG_{819} estimates and their confidence intervals were greater than 15 kg in 50% (22/44) of the county – processor combinations (Fig. 5). The change in the summary $\Delta LWG_{819(RE)}$ estimate was noticeable for Se = Sp = 0.95 at 46 kg compared to 36 kg for Se = Sp = 1 and 38 kg for Se = Sp = 0.99.

3.3. Heterogeneity

Scatter plots did not show a relationship between the county - processor level estimates of ΔLWG_{819} and either the apparent prevalence of liver fluke (Fig. 6) or the percentage of animals with a beef sire in the county (Fig. 7) assuming Se = Sp = 1, 0.99 or 0.95. Scatter plots assuming Se = Sp = 0.99 and 0.95 are not shown because the patterns were the same the plot assuming Se = Sp = 1.

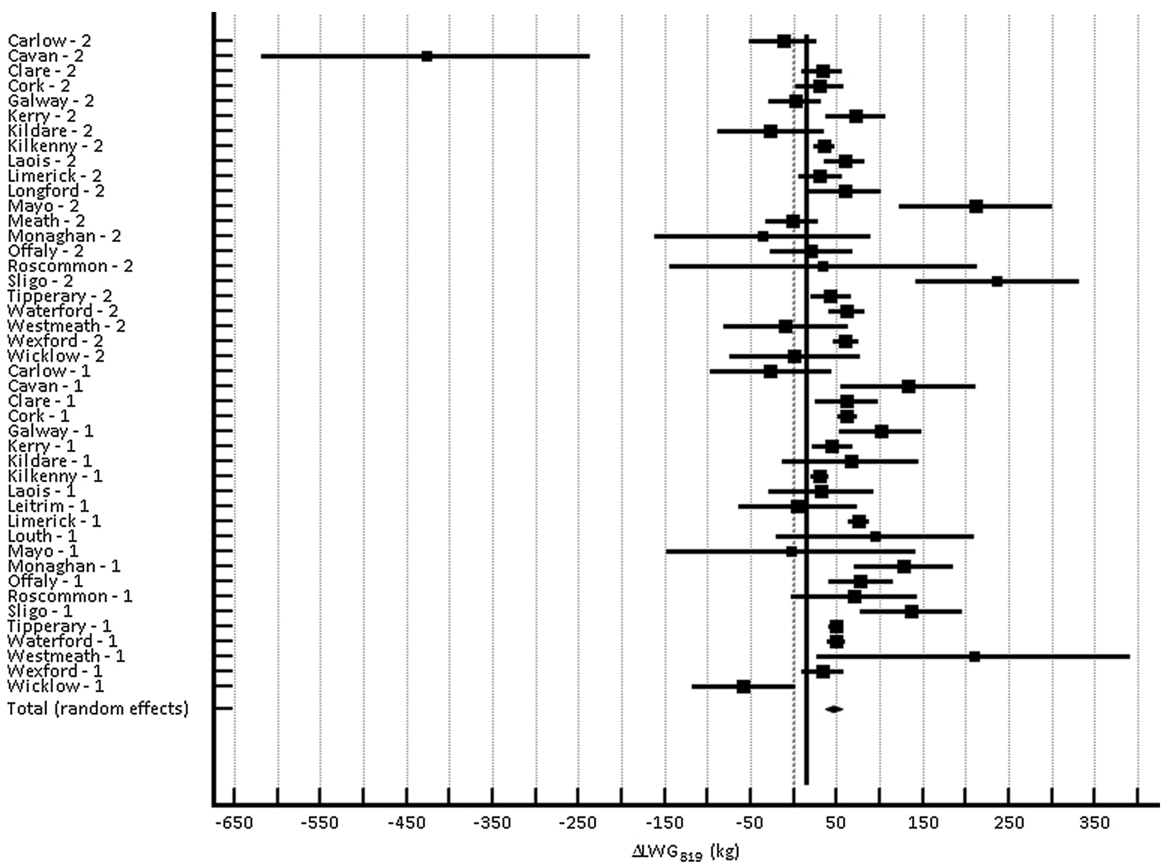

Fig. 5. Forest plot of estimates of the difference in mean lifetime weight gain (ΔLWG_{819}) and associated confidence intervals between the liver fluke negative and liver fluke positive steers for Processor 1 (county - 1) and 2 (county - 2) counties assuming Se = Sp = 0.95 for post - mortem liver inspection. The random effects summary estimate and confidence interval are represented by the diamond marker. Marker sizes for county – processorpoint estimates are proportional to their weight in the random effects analysis. The solid vertical line represents the assumed practically important threshold of 15 kg.

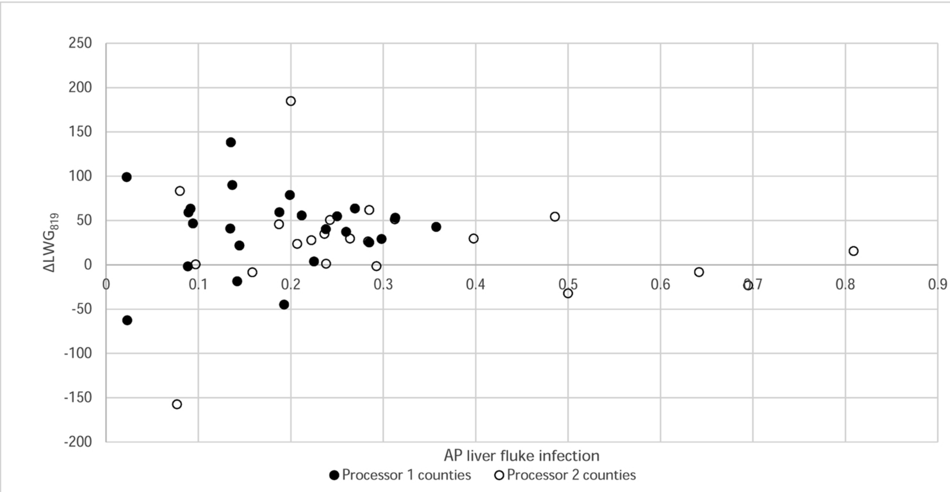

Fig. 6. Scatter plot of the estimates of the difference in mean lifetime weight gain at 819 days (ΔLWG_{819}) between the liver fluke negative and liver fluke positive steers in Processor 1 and 2 counties assuming Se = Sp = 1 against the apparent prevalence of liver fluke infection (AP liver fluke) in each county – processor combination.

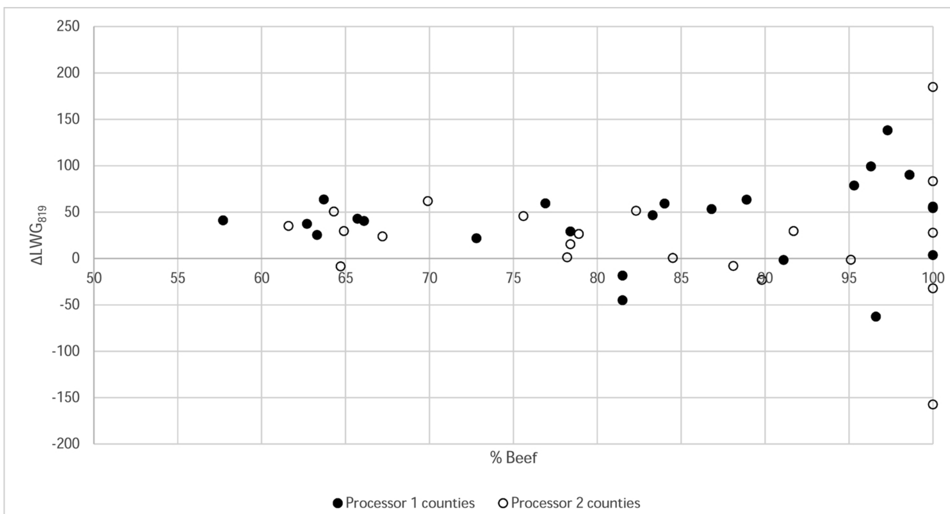

Fig. 7. Scatter plot of the difference in mean lifetime weight gain at 819 days (ΔLWG_{819}) between the liver fluke negative and liver fluke positive steers in Processor 1 and 2 counties assuming Se = Sp = 1 against the percentage of steers with a beef sire (% Beef) in each county – processor combination.

3.4. *Prediction intervals*

The 95% PI assuming Se = Sp = 1 was -1 to 73 kg. While a portion of this PI is below the 15 kg threshold, 78% of the values within the PI are practically important. The 95% PI was marginally wider assuming Se = Sp = 0.99 (-2, 77) but noticeably so assuming Se = Sp = 0.95 (-2, 94). In this case 82% of the values within the PI are greater than the 15 kg threshold of practical importance.

3.5. *Economics*

Table 4 shows the economic value of the $\Delta LWG_{819(RE)}$ and 95% PIs. Assuming Se = Sp = 1, the summary estimate value was €77.01 (95% PI: -2.57, 156.37) and this increased by 5% to €80.65 (95% PI: -3.43, 164.74 and 28% to €98.67 (95% PI: -5.15, 202.27) when Se = Sp = 0.99 and 0.95 were assumed, respectively (Table 4).

4. Discussion

The term meta – analysis is most commonly used when undertaking a qualitative or quantitative review of the published literature on a given topic. Nevertheless, meta - analysis has been defined as the statistical analysis of results from individual studies for the purpose of integrating the findings (Dickersin and Berlin, 1992). In this work, each county - processor combination is treated as a unique study, for which the ΔLWG_{819} is estimated and meta-analytic methods are employed to analyse these results and arrive at a summary estimate ($\Delta LWG_{819(RE)}$). The majority of these studies showed a difference of more than 15 kg between the LFN and LFP steers. When we assume that post - mortem liver inspection has perfect sensitivity and specificity for identifying liver fluke infection status, a difference of 36 kg is found between the LFN and LFP steers ($\Delta LWG_{819(RE)}$ = 36 kg), suggesting a negative association between liver fluke infection and weight gain. The 95% PI goes from -1 kg, to 73 kg, i.e. from being consistent with a small positive association between liver fluke infection and weight gain to a substantial negative association between liver fluke infection and weight gain. Nonetheless, when the interval is compared to the 15 kg threshold difference considered practically important, 78% of the values within the interval show an important (> 15 kg.) difference. Based on all this evidence, it is reasonable to infer that LFN steers have a substantial advantage in terms of weight gain over LFP steers.

Meta-analyses use a fixed or random effects statistical model. The fixed effects model assumes all studies are estimating a common effect and that variation in observed study estimates is due to chance. The random effects model assumes the effect differs between studies and provides an estimate of the average effect (Riley et al., 2011).

Table 3
Random effects summary estimates of the difference in mean life time weight gain between liver fluke negative and liver fluke positive steers at 819 days ($\Delta LWG_{819(RE)}$) and 95% prediction intervals (95% PI) for combined county-processor 1 and 2 data assuming test sensitivities (Se) and specificities (Sp) of 1, 0.99 and 0.95 for meat inspection. Measures of heterogeneity (I^2) and 95% Confidence Intervals (CI) are provided.

	Number of county – processor combinations	$\Delta LWG_{819(RE)}$ (kg)	95 % PI		I^2(%)	95% CI	
			Lower	Upper		Lower	Upper
County – Processor Combinations Se = Sp = 1	46	35.9	−1.2	72.9	76.4	68.7	82.2
County – Processor Combinations Se = Sp = 0.99	46	37.6	−1.6	76.8	76.9	69.5	82.5
County – Processor Combinations Se = Sp = 0.95	44	46.0	−2.4	94.3	78.4	71.4	83.7

Table 4
Values in Euros corresponding to random effects summary estimates (and 95% prediction intervals (95% PI)) of the difference in mean life time weight gain between liver fluke negative and liver fluke positive steers at 819 days ($\Delta LWG_{819(RE)}$) for combined county-processor 1 and 2 data, assuming test sensitivities (Se) and specificities (Sp) of 1, 0.99 and 0.95 for meat inspection.

	Economic impact ΔLWG_{819}		
	RE Sum Est (€)	PI Lower (€)	PI Upper (€)
County – Processor Combinations Se = Sp = 1	76.95	−2.47	156.36
County – Processor Combinations Se = Sp = 0.99	80.65	−3.43	164.74
County – Processor Combinations Se = Sp = 0.95	98.55	−5.14	202.24

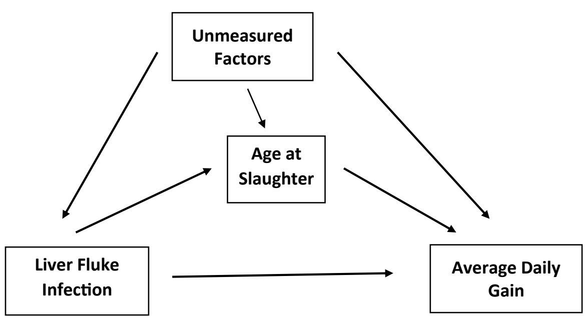

Fig. 8. Directed Acyclic Graph showing the hypothesized relationship between Liver Fluke Infection, Age at Slaughter and Average Daily Gain in steers.

Prediction intervals measure the dispersion of the estimates and illustrate the range of true effects that can be expected in future studies (estimating the same effect under the same conditions (IntHout et al., 2016). Random effects meta-analysis and 95% PIs are used here because of the heterogeneity identified in the study estimates.

Our results are consistent with recent abattoir studies which found that liver fluke infection has a negative impact on beef cattle performance (Bellet et al., 2016; Charlier et al., 2009; Sanchez-Vazquez and Lewis, 2013). These studies used regression models and adjusted for factors that they considered confounders, including age at slaughter and found that infected cattle have lower carcase weights. However, in all cases, the weight reductions were much less than the differences demonstrated in this work. A Scottish study examining slaughter age, estimated that cattle classified as having liver fluke damage were 10 days older than animals with no evidence of fasciolosis (Mazeri et al., 2017). The outcome of interest in this study, ΔLWG_{819}, is a measure of average daily gain which encompasses both time taken to reach slaughter age and weight at slaughter. If weight at slaughter alone is used as the outcome, it can bias differences towards zero because farmers may send animals to slaughter at a given weight and may wait for a slower growing steer to achieve that target. A Directed Acyclic Graph (DAG) (Greenland et al., 1999) is used here to examine whether age at slaughter should be considered a confounder for the liver fluke infection and average daily gain relationship (Fig. 8). The DAG (Fig. 8) shows that age at slaughter, (that is time to slaughter) does not fit the criteria for a confounder (McNamee, 2003) because it could be affected by the exposure (ie liver fluke infection). In other words infection with liver fluke could cause animals to take a longer time to reach slaughter. Therefore age was not considered to be a confounder in this study. It is reasonable to believe that in some way age does confound the relationship between liver fluke and average daily gain (or lifetime weight gain at 819 days) because age is related to both liver fluke infection (older animals are more likely to be infected) (Mazeri et al., 2017) and average daily gain. It is likely that age exerts its confounding effects through the age at which the animal is exposed to liver fluke, but this information was not available to take into consideration. We also acknowledge that there are other, unmeasured factors (breed, time of year of birth, farm management or co-infections with other pathogens) that could confound the relationship between liver fluke and weight gain and thus have an impact on our results (Fig. 8). For example, it is reasonable to assume that breed would affect LWG_{819} (Chewning et al., 1990) and different breeds may have differences in susceptibility to liver fluke (Pleasance et al., 2011). However, precise breed information was not available to us.

We recognise that weather has an effect on exposure to liver fluke (Selemetas and de Waal, 2015). The period that cattle in the study population could have been exposed to fluke was approximately 2012 – 2014. Weather conditions varied regionally and from month to month but annual rainfall and temperatures were close to average in 2013 and 2014. Summer 2012 was particularly wet, which combined with a warm August provided ideal conditions for *Galba truncatula*, intermediate host for liver fluke. (AFBI, DAFM, 2014; AFBI, DAFM, 2013; AFBI, DAFM, 2012) Overall, conditions for fluke during the lifespan of the animals in the study were typical of those generally found in Ireland.

Values of Se = Sp = 0.99 and 0.95 were used to explore the impact of misclassification on ΔLWG_{819} and its economic impact. When compared to their values at Se = Sp = 1, the $\Delta LWG_{819(RE)}$ increased by approximately 5% (to 38 kg) and 28% (to 46 kg) for Se = Sp = 0.99 and 0.95, respectively. Based on these results, estimating the effect of liver fluke infection on weight gain based on post - mortem liver fluke status without adjusting for misclassification will lead to a substantial underestimation of the effect. Using a Bayesian non-gold standard approach, Scottish researchers estimated the sensitivity of post-mortem liver inspection to be 0.68 (95% Bayesian Credible Interval: 95% BCI: 0.61-0.75) and the specificity to be 0.88 (95% BCI: 0.85-0.91) (Mazeri et al., 2016). An earlier study estimated the diagnostic sensitivity of post – mortem meat inspection for liver fluke infection to be 63.2% (95% BCI: 55.6–70.6) (Rapsch et al., 2006). These estimates of sensitivity and specificity are lower than those used in this work to calculate the impact of misclassification, therefore, the impact of misclassification is likely to be even greater than estimated in this study.

The results equate to a financial impact of €77.01 (95% PI: -2.57, 156.37), €80.65 (95% PI: -3.43, 164.74) and €98.67 (95% PI: -5.15, 202.27) for Se = Sp = 1, 0.99 and 0.95, respectively, given market prices at the time of writing (Department of Agriculture Food and the Marine, 2019). The point estimates and the majority of values within the intervals are above the value considered to be important. Both these results and threshold could also be compared to the cost of treatment of liver fluke. Depending on the product used, the cost of one treatment for a 400 kg steer could equate to 4–25% of the monetary value of the 15 kg threshold or less than 5% of the value of the summary $\Delta LWG_{819(RE)}$ estimate assuming Se = Sp = 1. This economic impact of liver fluke infection is substantial and these comparisons provide farmers and veterinary practitioners with an appreciation of the cost of the disease and the benefits of implementing control and prevention strategies.

Strengths and limitations

The main limitation of this work is that only association between liver fluke infection status and ΔLWG_{819} can be established. Limited information on potential confounders was available and, therefore, unadjusted estimates are provided. Additionally, the two processors providing data for the study used different definitions to record liver fluke in the abattoirs. This could be a source of misclassification due to a lack of comparability of definitions. However, a simple binary interpretation was employed to minimise misclassification in the analysis.

The immediate target population in this analysis are Irish steers which remain in their birth herd for their entire lifespan. The environment from which the animals originated is more homogenous than if they moved from farm to farm and it can be certain that the findings relate to the given location. We find a summary difference of between 36 kg (95% PI -1, 73) and 46 kg (95% PI -2, 94) in lifetime weight gain between LFN and LFP steers and our study provides robust evidence of the negative association between liver fluke infection and weight gain in cattle. For the first time, the effect of the imperfect sensitivity and specificity of post-mortem liver inspection on the difference in weight gain is quantified, demonstrating the practical and financial implications of liver fluke infection status misclassification. The findings of the current study, generated using data from Irish cattle where production is based on grazing in a temperate environment will be of particular relevance to other countries with similar systems and conditions of production.

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