2016

Detection of Yersinia enterocolitica serotype O:9 in the faeces of cattle with false positive reactions in serological tests for brucellosis in Ireland

Don O'Grady

Kevin Kenny

Seamus Power

See next page for additional authors

Authors

Don O'Grady, Kevin Kenny, Seamus Power, John Egan, and Fergus Ryan

The Veterinary Journal 216 (2016) 133–135

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Short Communication

Detection of *Yersinia enterocolitica* serotype O:9 in the faeces of cattle with false positive reactions in serological tests for brucellosis in Ireland

Don O'Grady [a,b,*], Kevin Kenny [a], Seamus Power [c], John Egan [a], Fergus Ryan [b]

[a] Department of Agriculture, Food, and the Marine, Central Veterinary Research Laboratory, Backweston, Celbridge, Co. Kildare, Ireland
[b] School of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland
[c] Department of Agiculture, Food, and the Marine, Blood Testing Laboratory, Model Farm Road, Cork, Ireland

ARTICLE INFO

Article history:
Accepted 28 July 2016

Keywords:
Yersinia enterocolitica serotype O:9
Faecal culture
Serology
False positive
Brucellosis

ABSTRACT

Intestinal infection by *Yersinia enterocolitica* serotype O:9 (YeO9) in cattle has been linked to false positive serological reactivity (FPSR) in diagnostic tests for brucellosis. Although eradicated in Ireland, brucellosis monitoring still identifies seropositive animals, usually one or two (termed singletons) per herd, which are classed as FPSR. To investigate a link between FPSR and YeO9, faeces and blood were collected from singleton FPSR cattle, and from companion animals, in eight selected herds with more than one FPSR animal, for YeO9 culture and Brucella serology. YeO9 was isolated from 76/474 (16%) FPSR singletons in 309 herds, but not from any of 621 animals in 122 control non-FPSR herds. In the FPSR herds 52/187 (27.8%) animals were culture positive, and 17% of the isolates were from seronegative animals. Seropositive animals were more likely to have a rising antibody titre when culture positive.

Brucella abortus causes high rates of abortion and infertility in cattle, with major economic losses if not controlled. Brucellosis was eradicated from Ireland in 2009 after a programme of screening the national herd using the micro-serum agglutination test (MSAT) to identify suspect herds, followed by disease confirmation using the indirect ELISA (iELISA) and the complement fixation test (CFT), and the subsequent removal of seropositive animals and herds. Serological screening now continues at a reduced level, with approximately 300 animals reacting positively in the annual test in the absence of any evidence of brucellosis (Dr J. Egan, personal communication). Usually, only one or two animals (termed singletons) are seropositive in herds with false positive serological reactivity (FPSR) (Gerbier et al., 1997), but the required investigation and quarantine of affected and contiguous herds is costly (Hayes et al., 2009).

The standard brucellosis tests detect antibodies to the smooth lipopolysaccharide O-antigen, but *Yersinia enterocolitica* serotype O:9 (YeO9) is known to possess a similar O-antigen, which could give rise to cross-reacting antibodies (Caroff et al., 1984). Experimental inoculation of cattle with YeO9 has produced a self-limiting infection without clinical signs, while also giving rise to positive reactions in brucellosis tests (Godfroid et al., 2002). FPSR due to YeO9 infection has been reported in countries with a low prevalence of brucellosis (Hilbink et al., 1995; Gerbier et al., 1997). Previously, a three-year study of reactors in Ireland (Hayes et al., 2009) demonstrated the presence of FPSR on the basis of the absence of evidence of brucellosis from surveillance data, but did not investigate the specific causes of FPSR. In the present study, a bacteriological survey for YeO9 was carried out on FPSR animals and companion herd animals, as well as animals in non-FSPR herds, supporting a possible role of YeO9 in FPSR.

During the FPSR singleton survey, faeces and clotted blood were collected over 3 years from 474 cattle identified as typical FPSR, as defined by Hayes et al. (2009), from all areas of Ireland, following an annual herd brucellosis test (see Appendix: Supplementary Fig. S1). The samples were collected 1–2 weeks after the initial positive test. Blood samples were tested for brucellosis using the CFT and iELISA according to the OIE Manual of Diagnostic Standards and Tests (Corbel and MacMillan, 1996) and the annex C of the Council Directive 64/432/EEC (1999). Faeces were collected from the rectum of cattle and stored at 2–8 °C for 1–5 days before testing.

Herds were selected for the FPSR herd study following the identification of several (up to four) reactors during the annual test, and faeces and clotted blood were collected from these animals and a random selection of companion animals. A total of 187 animals from eight of these herds were sampled. To estimate the background prevalence of YeO9, cattle arriving at 23 abattoirs representing 80% of national production were sampled over a 9 month period. Rectal swabs were collected from five animals in each of 122 herds and the five swabs from each herd were pooled for testing.

* Corresponding author.
E-mail address: don.ogrady@agriculture.gov.ie (D. O'Grady).

http://dx.doi.org/10.1016/j.tvjl.2016.07.016
1090-0233/

Table 1
Detection of *Yersinia enterocolitica* serotype O:9 in the faeces of false positive serological reactor (FPSR) singleton survey, FPSR herd and background prevalence survey animals.

	Number of animals	Number of animals positive (% positive)[a]	Number of herds	Number of herds positive (% positive)[a]
FPSR singleton survey	474	76 (16.0)	307	51 (16.6)
Background prevalence survey	610[d]	0 (0)	122	0 (0)
FPSR herd animals	187	52 (27.8)	8	NA[e]
FPSR herd animals seropositive[b]	75	33 (44.0)	NA[e]	NA[e]
FPSR herd animals seronegative[c]	112	19 (17.0)	NA[e]	NA[e]

[a] *Yersinia enterocolitica* serotype O:9 was isolated by faecal culture.
[b] The *Brucella* complement fixation test (CFT) and/or the indirect ELISA (iELISA) were positive in the initial screening (first) and/or the subsequent (second) blood test.
[c] The *Brucella* CFT and iELISA were negative in both the first and second blood tests.
[d] These samples were tested as a combined sample from five animals in each of 122 herds.
[e] NA, not applicable.

For YeO9 culture (see Appendix: Supplementary Fig. S2) 10% W/V and 1% W/V suspensions of faeces, or the material pelleted from five rectal swabs, were prepared in phosphate buffered saline (PBS) pH 7.2. Both suspensions were inoculated on modified Cefsulodin-Irgasan-Novobiocin (mCIN) medium (Oxoid, product code CM0653) directly and following exposure to dilute alkali (Frederiksson-Ahomaa and Korkeala, 2003), and the 10% W/V suspension was stored at 2–8 °C. Following incubation at 28 °C for 48 h, suspect colonies were tested for agglutination using *Y. enterocolitica* serotype O9 antiserum (Sifin GmbH, catalogue number TS1703) and positive colonies were confirmed as *Y. enterocolitica* using API20E test strips (BioMerieux). Confirmed isolates were tested for virulence by PCR (see Appendix: Supplementary Fig. S3) targeting the *ail* virulence gene (Thoerner et al., 2003). When samples were culture negative, the stored suspension was retested as before after cold enrichment at 2–8 °C for 20–23 days (Pai et al., 1979).

The results of faecal culture are presented in Table 1. In the FPSR singleton survey, YeO9 was isolated from 76/474 (16%) animals and 51/307 (16.6%) herds, and from 52/187 (27.8%) animals in the FPSR herd study. All of the control samples (five animals from each of 122 herds) were negative, indicating a low background prevalence (<0.82% of herds). All isolates were *ail* positive by PCR.

These isolation rates may underestimate the true occurrence of YeO9. In spite of the selective and enrichment measures, the conventional culture method used is considered less effective for faeces due to culture overgrowth by other faecal flora (Frederiksson-Ahomaa and Korkeala, 2003), and the heavy background growth present in the majority (>50%) of negative cultures possibly prevented isolation of YeO9 in some cases. Also, experimental infection studies have shown that the peak YeO9 shedding period is brief and precedes seroconversion, with less likelihood of recovering YeO9 from seropositive animals (Godfroid et al., 2002). The isolation of YeO9 from the seronegative animals (19/52 isolates) contributed to the higher detection (27.8%) in the FPSR herd study, suggesting that these animals may have been in the shedding/pre-seroconversion stage.

The combined culture and serology profiles for the FPSR herd animals (Table 2) shows significant numbers in the culture positive/seronegative (10.2%), culture positive/seropositive (17.6%) and culture negative/seropositive (22.5%) categories. This suggests an active YeO9 infection, with a short duration of progression from culture positive only, to culture positive and seropositive, and finally to seropositive only. The association between a positive YeO9 culture and increasing antibody levels (Table 3), in contrast to the predominantly decreasing levels for the culture negative animals, further supports this infection model.

Table 2
Detection of *Yersinia enterocolitica* serotype O:9 in the faeces of seropositive and seronegative animals in eight herds (herds A–H) with false positive serological reactivity (FPSR) in *Brucella* serological tests.

	FPSR herds A–H (n = 187 animals)	
	Mean herd value	Range of values
Number of animals tested	23.4	11.0–32.0
% Culture positive	27.8	16.7–41.7
% Seropositive[a]	40.1	16.1–90.5
% Culture positive/seronegative[b]	10.2	4.4–27.3
% Culture positive/seropositive[a]	17.6	9.7–33.3
% Culture negative/seropositive[a]	22.5	6.5–66.7
% Culture negative/seronegative[b]	49.7	9.1–71.9

Herd selection was based on the detection of several (up to four) FPSR animals in each herd by the annual screening test.
[a] The *Brucella* complement fixation test (CFT) and/or the indirect ELISA (iELISA) were positive in the initial screening (first) and/or the subsequent (second) blood test.
[b] The *Brucella* CFT and iELISA were negative in both the first and second blood tests.

Table 3
Change in serological reactivity (positive to negative and vice versa) and in the complement fixation test (CFT) titre between the initial and subsequent serological tests[a] for individual animals in the false positive serological reactor (FPSR) singleton survey and the FPSR herd study, in relation to faecal culture for *Yersinia enterocolitica* serotype O:9.

	Number of animals	Number (%) of animals showing a change in serological reactivity				
		Transition from seronegative[b] to seropositive[c]	Transition from seropositive[c] to seronegative[b]	Increasing[d] CFT titre	No change[e] in CFT titre	Decreasing[d] CFT titre
FPSR singleton survey, culture positive	76	NA[f]	11 (14.5)	13 (17.1)	44 (57.9)	19 (25.0)
FPSR singleton survey, culture negative	398	NA[f]	132 (33.2)	14 (3.5)	142 (35.7)	242 (60.8)
FPSR herd study, culture positive	75	11 (14.7)	0	27 (36.0)	31 (41.3)	17 (22.7)
FPSR herd study, culture negative	112	5 (14.5)	11 (9.8)	11 (9.8)	34 (30.4)	67 (59.8)

Faeces were collected at the same time as the second blood sample. The figures denote the numbers of animals, and the figures in parentheses the percentage of the number of animals in the category.
[a] Interval between sample collection for the first and second tests was 1–2 weeks.
[b] Both the CFT and the indirect ELISA (iELISA) were negative.
[c] The CFT and/or the iELISA were positive.
[d] A change in CFT titre was recorded when there was an increase or decrease in the number of CFT international units corresponding to at least one full double dilution of the titration, or involving a change from a negative to a positive result (increasing) or vice versa (decreasing).
[e] This category also includes animals that were CFT negative on both occasions.
[f] NA, not applicable. These animals are by definition seropositive in the first test.

The detection of YeO9 in 16% of animals in the FPSR survey provides strong evidence of a causal link between YeO9 infection and brucellosis FPSR which has also been observed in other countries (Hilbink et al., 1995; Gerbier et al., 1997; Grattarola et al., 2006), although it does not preclude other causes. Whilst dual infection with YeO9 and *B. abortus* is possible, when other risk factors for brucellosis are absent, detection of YeO9 in seropositive or companion animals can provide additional evidence of the absence of the disease in FPSR herds.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of this paper.

Acknowledgements

The authors wish to thank the many staff of the Department of Agriculture, Food, and the Marine and the various meat processing plants for their invaluable assistance in the collection and testing of samples for the study.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2016.07.016.

References

Caroff, M., Bundle, D.R., Perry, M.B., 1984. Structure of the O-chain of the phenol-phase soluble cellular lipopolysaccharide of *Yersinia enterocolitica* O:9. European Journal of Biochemistry 139, 195–200.

Corbel, M.J., MacMillan, A.P., 1996. Bovine brucellosis. In: Manual of Standards for Diagnostic Tests and Vaccines, 4th Ed. Office Internationale des Epizooties, Paris, France, pp. 242–255.

Frederiksson-Ahomaa, M., Korkeala, H., 2003. Low occurrence of *Yersinia enterocolitica* in clinical, food, and environmental samples: A methodological problem. Clinical Microbiology Reviews 16, 220–229.

Gerbier, G., Garin-Bastuji, B., Pouillot, R., Very, P., Cau, C., Berr, V., Dufour, B., Moutou, F., 1997. False positive serological reactions in bovine brucellosis: Evidence of the role of *Yersinia enterocolitica* serotype O:9 in a field trial. Veterinary Research 28, 375–383.

Godfroid, J., Saegerman, C., Wellemans, V., Walravens, K., Letesson, J.-J., Tibor, A., McMillan, A., Spencer, S., Sanna, M., Bakker, D., et al., 2002. How to substantiate eradication of bovine brucellosis when aspecific serological reactions occur in the course of brucellosis testing. Veterinary Microbiology 90, 461–477.

Grattarola, C., Gennero, M.S., Zoppi, S., Bergagna, S., Chiavacci, L., Dondo, A., 2006. *Yersinia enterocolitica* and its role in bovine brucellosis monitoring: A statistical analysis. Epidemiologie et Sante Animale 50, 153–155.

Hayes, M., Ashe, S., Collins, D.M., Power, S., Kenny, K., Sheahan, M., O'Hagan, G., More, S., 2009. An evaluation of Irish cattle herds with inconclusive serological evidence of bovine brucellosis. Irish Veterinary Journal 62, 182–190.

Hilbink, F., Fenwick, S.G., Thompson, E.J., Kittelberger, R., Penrose, M., Ross, G.P., 1995. Non-specific seroreactions against *Brucella abortus* in ruminants in New Zealand and the presence of *Yersinia enterocolitica* O:9. New Zealand Veterinary Journal 43, 175–178.

Pai, C.H., Sorger, S., Lafleur, L., Lackman, L., Marks, M.I., 1979. Efficacy of cold enrichment techniques for the recovery of *Yersinia enterocolitica* from human stools. Journal of Clinical Microbiology 9, 712–715.

Thoerner, P., Bin Kingombe, C.I., Bogli-Stuber, K., Bissig-Choisat, B., Wassenaar, T.M., Frey, J., Jemmi, T., 2003. PCR detection of virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and investigation of virulence gene distribution. Applied and Environmental Microbiology 69, 1810–1816.