ASSESSMENT OF IMS FOR SEPARATION OF PORCINE INTESTINAL SPIROCHAETES FROM FAECAL SAMPLES

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Introduction. Immunomagnetic separation (IMS) has been increasingly applied to enhance the detection of *E. coli* O157 or *Salmonella* Typhimurium from faecal samples or food samples (3, 9, 10). This study aimed to assess potential of IMS for isolation of *B. pilosicoli* and *B. hyodysenteriae* from pig faeces, and compare its sensitivity with existing methods as direct culture and PCR.

Material and Methods. Mab to *B. pilosicoli* BJL/AC1 (4), and Mab to *B. hyodysenteriae* BJL/SH1 (5) were used for coating magnetic beads (Dynabeads M-450 Rat anti-Mouse IgM, DYNAL, UK), and polyclonal antibody (Pab) from Veterinary Laboratory Agency, UK specific to *B. hyodysenteriae* was used for coating (Dynabeads M-280 Sheep anti-Rabbit IgG, DYNAL, UK). *Brachyspira* negative pig faeces (diluted 1:20) were spiked with known numbers of target cells *(B. pilosicoli* or *B. hyodysenteriae*), and IMS (direct and indirect methods) applied to separate them. Inocula from IMS and controls (without IMS) were cultured on TA plates at 42 °C for 3-4 days. PCR was applied to samples of IMS and controls.

Results

-IMS direct and indirect for B. pilosicoli. Controls (without IMS) were 10^3 and 10^1 more sensitive than IMS (direct and indirect), respectively. IMS (indirect) was 10^2 more sensitive than IMS (direct). IMS (direct without washing) was 10^2 more sensitive than IMS (direct with washing). *B. pilosicoli* was recovered from three of the washings.
-IMS direct and indirect for B. hyodysenteriae. Using Mabs, controls (without IMS) were 10^3 more sensitive than IMS (direct and indirect), and IMS (indirect) was 10-fold more sensitive than IMS (direct). Using Pabs IMS (direct) was 10^1 one 10^2 less sensitive than IMS using the Mab BJL/SH1.
-Comparison of PCR with culture. PCR for detection of *B. pilosicoli* on controls (without IMS) was 10^1 more sensitive than PCR on IMS (direct). PCR on IMS (direct without washing) for *B. pilosicoli* was 10-fold more sensitive than on IMS (with washings), correlating with the improvement of 10-fold on culture by IMS (without washing). However, culture of *B. pilosicoli* was still 10^2-fold more sensitive than PCR. PCR on IMS (indirect) for *B. hyodysenteriae* was 10-fold more sensitive than culture of IMS (direct). PCR on controls (without IMS) for *B. hyodysenteriae* had sensitivity as culture.

Discussion. In this study IMS did not enhance detection rates of *B. pilosicoli* or *B. hyodysenteriae* from pig faeces. Although target cells successfully adhered to beads coated with Mabs many cells became detached during the washing steps. This appears to be the key technical problem to address in the future, in relation to this technique. Excretion of *Brachyspira* spp. has been reported at 1×10^5 to 2×10^9 cells per g of faeces (7), however excretion rates of asymptomatic carrier pigs are thought to be lower than

that, so tests with high sensitivity are required for successful detection. Sensitivity of PCR for detection of intestinal spirochaetes has been reported elsewhere (1, 6, 8), and it is consistent with the sensitivity of PCR in this study. Sensitivity of culture of *B. pilosicoli* at 42 °C for 7 days has been reported at 15 cells per inoculum (2). In this study recovery of *B. pilosicoli* on culture was at 1.5 x 10^2 cfu, 10-fold higher than that reported by Fellström *et al* (1997). However, the target cells were laboratory-adapted, so results could be lower for attempting isolation of intestinal spirochaetes from field samples. In conclusion IMS using Mabs shows potential but further work is required to overcome loss of targeted cells during washings.

Table 1. Sensitivity of comparative methods for detection of porcine intestinal spirochaetes.

Assay	B. pilosicoli		B. hyodysenteriae	
	culture	PCR	culture	PCR
IMS (direct)	10^3 (4.8×10^5 cfu)	10^3 (4.8×10^5 cfu)	10^1 (2.5×10^4 cfu)	10^2 (2.5×10^3 cfu)
Controls	10^6 (4.8×10^2 cfu)	10^4 (4.8×10^4 cfu)	10^4 (2.5×10^1 cfu)	10^4 (2.5×10^1 cfu)
IMS (indirect)	10^5 (1.5×10^3 cfu)	10^3 (1.5×10^3 cfu)	10^2 (9.9×10^3 cfu)	ND
Controls	10^6 (1.5×10^2 cfu)	ND	10^5 (9.9 cfu)	ND
IMS (with washings)	10^3 (9.9×10^3 cfu)	ND	ND	ND
Washing 1	10^5 (9.9×10^1 cfu)	ND	ND	ND
Washing 2	10^5 (9.9×10^1 cfu)	ND	ND	ND
Washing 3	10^3 (9.9×10^3 cfu)	ND	ND	ND
IMS (without washing)	10^5 (9.9×10^1 cfu)	10^3 (9.9×10^3 cfu)	ND	ND

References

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