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362

Identification of *Cryptosporidium parvum* Oocysts Isolated from Swine by PCR Technique

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ABSTRACT. *Of the 72 faecal samples collected from a swine farm located in the swine-belt area, 7 apparently healthy swine were excreting Cryptosporidium oocysts at an average intensity of 429 oocysts per gram of faeces as has been demonstrated by salt flotation, followed by staining with the modified Ziehl Neelsen technique. To confirm the species of Cryptosporidium in the observed swine, a representative isolate was purified and then subjected to simple freeze-thaw procedure in order to release the nucleic acids from oocysts. The DNA was extracted using DNAzol® (Life Technologies, USA). The amplification reaction included a set of primers (5' CCTGATCCTGTACCACCTCC 3' and 5' GTCATTTCTGATGAGCACGG 3') designed from β-tubulin gene of Cryptosporidium parvum. PCR products were subjected to electrophoretic separation in 1% agarose gel stained with ethidium bromide. Expected band size of 460 bp in the PCR product appeared on agarose gel, confirming the presence of C. parvum in swine.*

INTRODUCTION

The association of *Cryptosporidium* species in causing childhood diarrhoea in Sri Lanka has been identified (Perera and Lucas, 1990). The zoonotic *C. parvum* has been recorded in goats (Noordeen *et al.*, 2000), calves and buffaloes (Senasighe *et al.*, 2002) in Sri Lanka. The objective of this study was to identify the occurrence of *Cryptosporidium* species in swine and to determine whether the oocysts recovered from infected animals represent the species *C. parvum*.

MATERIALS AND METHODS

Seventy two direct faecal samples were collected from a swine farm located in the swine-belt area and screened for the presence of *Cryptosporidium* oocysts by salt flotation, followed by staining with modified Ziehl Neelsen technique (Noordeen *et al.*, 2000). To confirm the species of *Cryptosporidium* present in the observed swine, a representative isolate which had the highest oocysts count was purified and then subjected to simple freeze-thaw procedure to release the nucleic acids from oocysts. The sample debris was pelleted by centrifugation and supernatants were used as the template in the PCR assay

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(Widmer *et al.*, 1998). The amplification reaction included a set of primers (5' CCTGATCCTGTACCACCTCC 3' and 5' GTCATTTCTGATGAGCACGG 3') designed from β-tubulin gene of *C. parvum* and are universal to *C. parvum* of all host animal species. After initial denaturation for 2 min at 95°C, amplification was done for 40 cycles at 94°C for 30 seconds, 61°C for 1 min, and 72°C for 1 min with a 5 min final extension at 72°C. The PCR products, size markers and negative control were electrophoresed on 1% agarose gel stained with ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

Seven swine (9%) were shedding *Cryptosporidium* oocysts in their faeces at an intensity of 429 (200-1200) oocysts per gram of faeces. The DNA of *Cryptosporidium* oocysts isolated from swine was identified as *C. parvum* and agarose gel visualization of PCR products is shown in Fig. 1.

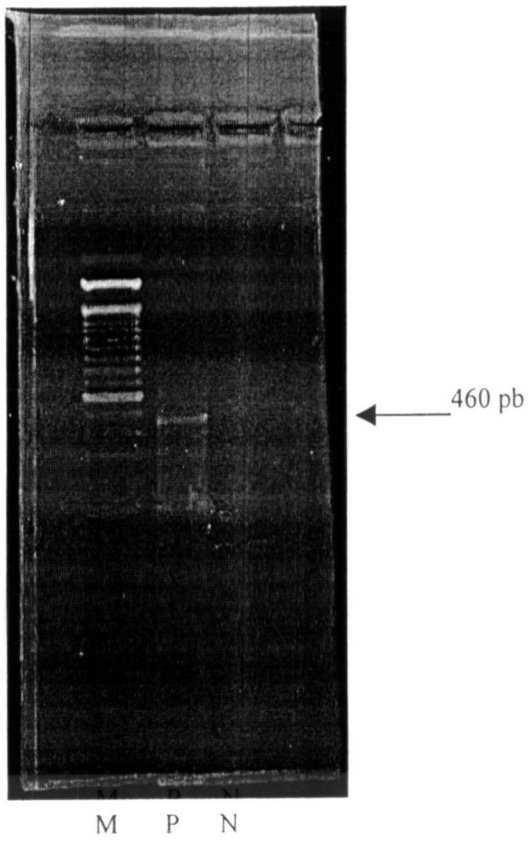

Fig. 1. Agarose gel analysis of PCR amplification of *Cryptosporidium parvum* DNA. [Note: *Cryptosporidium* oocysts isolates originated from swine (lane 'P'). Lane 'N' shows negative control. Expected *C. parvum* specific band size is 460 base pairs. Lane 'M' represents the molecular size marker (100 bp). Numbers in left/right sides show DNA fragment sizes in base pairs.

This is the first record of the occurrence of *C. parvum* in swine in Sri Lanka. The occurrence of zoonotic *C. parvum* in goats (Noordeen *et al.*, 2000) and cattle and buffaloes (Senasinghe *et al.*, 2002) in Sri Lanka has also been identified. However, further analysis is needed to conclude the zoonotic potential of *C. parvum* isolated from swine in the present study, since it can be originated from two distinct genotypes: a 'porcine' genotype exclusive to swine and a 'bovine' genotype, that is common to other livestock species and human being (Morgan *et al.*, 1999).

CONCLUSIONS

Some of the observed swine were excreting *Cryptosporidium* oocysts which represented the *C. parvum*. Further analysis is needed to establish its zoonotic potential.

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