

THE ISOLATION OF *CAMPYLOBACTER HYOINTESTINALIS* FROM A PIG IN SOUTH AFRICA

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ABSTRACT

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The isolation of a strain of *Campylobacter hyointestinalis* from a piglet is described. The animal originated from a farm where another animal showed signs of intestinal adenomatosis. The animal from which the isolation was made had diarrhoea, and an enteropathogenic *Escherichia coli* was also isolated. No pathological changes indicative of intestinal adenomatosis were detected in this animal.

INTRODUCTION

Campylobacter hyointestinalis was first identified in 1983 from the intestines of swine with enteric disease (Gebhart, Ward, Chang & Kurtz, 1983). Ileal sections were examined bacteriologically for the presence of various *Campylobacter* species. Colonies of *C. hyointestinalis* were first discovered as secondary growth on plates used for the isolation of *C. jejuni/coli* and *C. mucosalis*. As *C. hyointestinalis* was catalase-positive it was immediately distinguishable from the catalase-negative *C. mucosalis*. Biochemical tests differentiated it from the catalase-positive *C. coli/jejuni* (Gebhart *et al.*, 1983).

In that study *C. hyointestinalis* was isolated from swine with intestinal disease together with other common aerobic enteropathogens, as well as from swine with intestinal adenomatosis, either together with *C. mucosalis* (Roop, Smibert, Johnson & Krieg, 1985) or on its own. Since the first identification of *C. hyointestinalis* by Gebhart *et al.* (1983), other workers were also able to identify some of their catalase-positive campylobacters isolated from swine with enteric disease as *C. hyointestinalis* (Lambert, Jones & Lister, 1984). Isolates of *C. hyointestinalis* have been made from healthy as well as from calves with enteric disease (Morgan & Bland, 1985). The significance of the presence of *C. hyointestinalis* in association with other enteropathogens is unknown, but of special interest is the association of *C. hyointestinalis* with porcine intestinal adenomatosis (PIA). PIA is a disease complex (Rowland & Lawson, 1981), from which *C. hyointestinalis* and *C. mucosalis* are the only 2 infective agents isolated thus far (Lawson & Rowland, 1974; Gebhart *et al.*, 1983).

At the Veterinary Research Institute (VRI), Onderstepoort, samples for the isolation of *C. hyointestinalis* and *C. mucosalis* are routinely taken from pigs with PIA symptoms. The first known isolation of *C. hyointestinalis* in South Africa is described.

MATERIALS AND METHODS

History of the case

An outbreak of diarrhoea with occasional deaths occurred in 5-6-week old piglets from a large pig production unit near Warmbad, Transvaal. These piglets were weaned at the age of 3 weeks. Clinical signs included a yellowish watery diarrhoea, mass loss and occasional coughing. Three diseased piglets were referred to the Section of Pathology, VRI, for necropsy, but no definite diagnosis could be made. No samples were taken for *Campylobacter* isolation. In 1 piglet however, adenomatous changes, consistent with PIA, were seen with light microscopy. Two further piglets (C/28-1 and C/28-2) with the same clinical symptoms were submitted for pathology and bacteriology a week later.

Pathology

Necropsies were performed on the 2 piglets (C/28-1 and 2). Specimens of small and large intestine, including terminal ileum and a range of other tissues were collected in 10 % buffered formalin, routinely processed and stained with haematoxylin and eosin (HE) for light microscopy. Selected sections of terminal ileum and colon were stained by Young's modification of the Warthin-Starry method (Young, 1969).

Bacteriology

Specimens of liver, brain, lung, kidney, mesenteric lymph node and terminal ileum were collected and submitted for routine aerobic bacterial isolations. In addition, freshly collected portions of the terminal ileum of both animals were submitted for *Campylobacter* isolation.

Campylobacter-isolation procedures

The method used was essentially that described by Lawson & Rowland (1974). Immediately after the animal was killed, a section of the ileum was cut off and taken to the laboratory. The gut section was cut open and rinsed with phosphate buffered saline (PBS, pH 7.4) to get rid of all visible material. The gut mucosa was scraped off and a 1 g aliquot was homogenized in 20 ml of reinforced clostridial broth¹. Further dilutions of 1:40 and 1:80 were done and 0.1 ml of each of the dilutions were plated out onto Columbia blood agar (CBA)¹ and CBA containing antibiotics (Lawson & Rowland, 1974). The plates were incubated at 37 °C for 48 h in anaerobic jars¹ containing an H₂-microaerophilic atmosphere. This was achieved by evacuating the jars to -560 mm Hg, and replacing the evacuated air by a gas mixture of 15 % CO₂ and 85 % H₂. This resulted in an atmosphere of 12.75 % CO₂, 3 % O₂, 72.25 % H₂ and 12 % N₂.

The plates were examined for colonies resembling *C. hyointestinalis* and/or *C. sputorum*. No catalase-negative *Campylobacter*-like colonies appeared, but catalase-positive colonies were found. These colonies were further characterized.

Characterization tests

The tests described by Roop, Smibert, Johnson & Krieg (1984) were used. Incubation was in the H₂-atmosphere as described at 37 °C except for examination of growth at 25 °C on CBA-plates. All tests were read after 24-72 h.

Sensitivity to antimicrobials was examined on Difco tryptose agar² with 10 % horse blood which contained

¹ Oxoid, Protea Laboratory Services, P.O. Box 784978, Sandton 0146

² Laboratory & Scientific Equipment Co. (Pty) Ltd., P.O. Box 45125, Mayfair, Johannesburg 2108

³ BDH, Merck (SA) (Pty) Ltd., P.O. Box 3497, Johannesburg 2000

⁴ Sigma, Labretoria (Pty) Ltd., P.O. Box 20295, Alkantrant, Pretoria 0005

⁵ Lilly Laboratories (S.A.) (Pty) Ltd., Short Street, Isando

TABLE 1 Characteristics of strain C/28-2 and differential characteristics of catalase-positive campylobacters¹

Characteristics	Strain C/28-2	<i>C. hyointestinalis</i> ²	<i>C. jejuni/coli</i>	<i>C. fetus</i>	<i>C. lari-dis</i>	<i>C. fecalis</i>
H ₂ S on TSI	+ ³	+	+/- ⁴	- ⁵	+	+
Growth at 25 °C	+	+	-	+	-	-
Sensitivity to—						
Tetrazolium	S ⁶	S	R/S ⁷	S	S	NK ⁹
Cephalothin	S	S	R/S	S	R ⁸	S
Nalidixic acid	R	R	S	R	R	R
Nitrate reduction	+	+	+	+	+	+
Growth in the presence of—						
1 % glycine	+	+	+	+	+	+
1 % oxgall	+	+	+	+	+	+
3,5 % NaCl	-	-	-	-	-	+
(Plates) 1,5 % NaCl	-	-	-	+	+	-
Control	+	+	+	+	+	+

¹ Roop *et al.*, 1984² Characteristics of strain used as reference³ + = growth⁴ +/- = variable⁵ - = no growth⁶ S = sensitive⁷ R/S = variable⁸ R = resistant⁹ NK = not known

either 0,4 g % triphenyl tetrazolium salt³, 0,003 g % nalidixic acid⁴ or 0,003 g/l sodium cephalothin⁵ (Section of Reproduction, Veterinary Research Institute, working document).

The ability to grow in the presence of 1,5 % NaCl was examined on agar plates (Lawson & Rowland, 1974). *Brucella*-broths² with 0,16 % agar and containing either 1 % glycine, 3,5 % NaCl or 1 % oxgall² were used. A *Brucella*-broth without additives was inoculated as control. Nitrate reduction was examined in a fluid medium (MacFaddin, 1980).

Some of the tests were repeated, using a predominant N₂-atmosphere, used for the routine cultivation of *C. jejuni/coli* in this laboratory. This atmosphere was achieved by the evacuation of an anaerobic jar to -480 mm Hg. The evacuated air was replaced by a mixture of 16 % CO₂ and 84 % N₂, which resulted in an atmosphere of 5 % O₂, 12 % CO₂ and 83 % N₂.

A *C. hyointestinalis* strain obtained from the Central Veterinary Laboratory, Weybridge, U.K., was included in all the tests as a reference. All plates and tubes were incubated in the H₂-microaerophilic atmosphere and examined after 48-72 h.

RESULTS

Pathology

The small and large intestines of both animals were filled with a yellowish watery content and the mesenteric lymph nodes were oedematous. In addition, there was a moderate purulent pneumonia with abscessation in case No. C/28-1. No macro- or microscopical lesions indicative of porcine intestinal adenomatosis were noticed in the intestines. In addition, bacteria with a morphology typical of *Campylobacter* could not be demonstrated microscopically in sections stained with Warthin-Starry.

Aerobic bacteriology

Staphylococcus aureus was isolated from all the organs except the ileum of case No. C/28-1, and *E. coli* 0149:K91 from the ileum. *E. coli* 09:K34 was isolated from the ileum of case No. C/28-2.

Campylobacter bacteriology

The plates of case No. C/28-1 yielded no colonies showing bacteria with *Campylobacter* morphology. However, the CBA plates and CBA plates containing antibiotics of the 1:20 dilutions of case No. C/28-2, yielded numerous small, yellowish, translucent, round colonies 1,5 mm in diameter after 72 h incubation. These

colonies were catalase-positive and consisted of gram negative organisms. The organisms were either long, loosely spiralled or shorter, looking like curved bacilli. In older cultures no evidence of pleomorphism was detected.

The biochemical tests used to characterize isolate C/28-2 are given in Table 1. The isolate was consistent in its growth at 25 °C, its resistance to nalidixic acid and in H₂S production in TSI media as early as after 24 h incubation in the predominantly H₂ atmosphere used. In the predominantly N₂ atmosphere, the isolate grew at 37 °C, but was inconsistent in growth at 25 °C and in H₂S production on TSI.

DISCUSSION

C. hyointestinalis can easily be differentiated from the other catalase-positive campylobacters in terms of its growth at 25 °C and H₂S production from TSI agar (Table 1). *C. jejuni/coli* is the only other catalase-positive *Campylobacter* species which occurs in pigs. *C. hyointestinalis* differs from *C. jejuni/coli* in its ability to grow at 25 °C and its resistance to nalidixic acid. The isolate made from pig No. C/28-2 was easily identified as *C. hyointestinalis*.

No *C. jejuni/coli* was isolated from either animal. From animal No. C/28-1 no *Campylobacter* was isolated, and it is believed that the *S. aureus* was the cause of the animal's poor condition.

The biochemical characterization of strain C/28-2 was carried out in the predominantly H₂ atmosphere. When a predominantly N₂ atmosphere with no H₂ was used to examine H₂S production, variable results occurred. Lambert *et al.* (1984) and Rüdiger-Bösch, Schmidt, Mumme, Nienhoff & Kaup (1986) also found that in a predominantly N₂ atmosphere, H₂S production was variable. When Lambert *et al.* (1984) implemented a predominantly H₂ atmosphere, H₂S production was a constant feature. H₂S production is an important characteristic to differentiate *C. hyointestinalis* from *C. jejuni/coli*. The atmosphere chosen for the incubation of tests would seem to be important.

The ability of *C. hyointestinalis* to grow at 25 °C is another important differential characteristic between it and *C. jejuni/coli*. None of the isolates of Lambert *et al.* (1984) grew at 25 °C, while most (11/14) of the isolates of Gebhart *et al.* (1983) did. Isolate C/28-2 grew consistently at 25 °C in the predominantly H₂ atmosphere but sporadically in the N₂ atmosphere. Rüdiger-Bösch *et al.* (1986) found that some strains grew less well when using

an incubation temperature of 20–25 °C. When this characteristic of *C. hyointestinalis* was examined, an incubator whose temperature does not fluctuate below 25 °C, as well as the atmosphere used, would seem important.

The pathogenic nature of *C. hyointestinalis* is still unclear. In swine, it has been isolated only from cases of enteric disease (Gebhart *et al.*, 1983; Lambert *et al.*, 1984; Rüdiger-Bösch *et al.*, 1986). In this study, the *C. hyointestinalis* isolate C/28–2 was isolated in conjunction with an enteropathogenic *E. coli* 09:K34. This is in agreement with the findings of other workers (Lambert *et al.*, 1984). *C. hyointestinalis* can also be isolated together with other enteropathogens such as *Treponema hyodysenteriae* (Lambert *et al.*, 1984). As a result of its presence together with these well known enteropathogens in enteric disease, *C. hyointestinalis* would appear not to be a primary enteropathogen, but rather an opportunistic secondary pathogen.

However, in PIA, whose aetiology has not been proved beyond doubt, *C. hyointestinalis* alone (Gebhart *et al.*, 1983; Lambert *et al.*, 1984; Rüdiger-Bösch *et al.*, 1986) or together with *C. mucosalis* (Gebhart *et al.*, 1983; Wilson, Chang, Gebhart, Kurtz, Drake & Lintner, 1986) are the only infective agents isolated thus far. Both campylobacters can be demonstrated intracellularly in the ileal mucosa of PIA cases (Chang, Kurtz, Ward & Gebhart, 1984). Both of these organisms appear to be directly associated with the disease, but as reproduction of the disease remains unsuccessful (Boosinger, Thacker & Armstrong, 1985; Wilson *et al.*, 1986) the exact pathogenic role of these organisms remains unclear.

C. hyointestinalis could not be demonstrated in the gut of healthy swine when a fluorescent antibody technique was used (Chang *et al.*, 1984). Additional information on the presence of this organism in healthy swine or in other animals is limited. Morgan & Bland (1985) isolated *C. hyointestinalis* from as many healthy calves as from calves with enteric disease. As a result of these findings it is expected that *C. hyointestinalis* would occur in other animals too.

This *C. hyointestinalis* isolate (C/28–2) is the first known isolate of this organism in South Africa. It is possible that *C. hyointestinalis* could have been isolated earlier by other workers, but as the differentiation between *C. hyointestinalis* and *C. coli/jejuni* has only been recognized since 1983 (Gebhart *et al.*, 1983), earlier *C. hyointestinalis* isolates would have been identified as *C. jejuni/coli* with atypical reactions.

In South Africa, PIA occurs sporadically among pig producing units (Monthly reports, Regional Veterinary Laboratories; Spencer, personal communication, 1986; Loveday, personal communication, 1986). A *C. mucosalis* was isolated from a case of PIA recently (Van der Walt, Laboratory data), but *C. hyointestinalis* has still to be isolated in this country from a pig with PIA.

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