DOMESTIC ANIMAL RESERVOIRS OF *CAMPYLOBACTER JEJUNI*, *CAMPYLOBACTER COLI* AND *YERSINIA ENTEROCOLITICA* AS POSSIBLE SOURCES OF HUMAN INFECTION

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SUMMARY

Acute diarrhoeal diseases are of great public health importance in tropical developing countries and are most prevalent during the first two years of life. Gastroenteritis is a major cause of morbidity and mortality especially in children in the tropics. Many enteropathogens have been incriminated as causative agents but more common are rotaviruses, *Shigella*, *Salmonella*, *Escherichia coli* and *Vibrio cholerae*. Recently, attention has been focused on infections by thermophilic *Campylobacter* species and *Yersinia enterocolitica*.

Campylobacter species are now recognised as important causal agents of diarrhoea in man. In many developing countries, campylobacteriosis is the third most common cause of acute diarrhoea after rotaviruses and enterotoxigenic *E. coli*. The thermophilic *Campylobacter* species, also called *Campylobacter fetus* subsp. *jejuni* or *Campylobacter jejuni/coli* has now been divided into *C. jejuni*, *C. coli* and *C. laridis* and certain "new *Campylobacters*". *Campylobacter* species have been isolated from the intestinal contents of a variety of warm-blooded animals, including domestic as well as certain wildlife species. This bacterial group has been convincingly shown to be one of the most important causal agents of acute

enteritis in man. In the majority of other mammalian species, *Campylobacter* spp.are present in apparently healthy carriers. However, it is becoming evident that thermophilic *Campylobacter* species may cause enteritis in certain species of animals other than man. Moreover, epidemiological data have provided strong indications that animals and food products of animal origin may be major reservoirs for human infection. The possible zoonotic nature of campylobacter infections is of major concern in public health and campylobacteriosis has been shown to be a greater problem than salmonellosis in certain countries. This study was aimed at the isolation and identification of *Campylobacter* species and *Yersinia enterocolitica* from apparently healthy or diarrhoeic domestic animals in an attempt to elucidate the role of animals as potential sources of human infections in Kenya.

In the study presented here, a total of 1089 fecal samples from human and animal sources were examined for thermophilic *Campylobacter* species and *Yersinia enterocolitica*. *C. jejuni* and *C. coli* were isolated from 55.1% (27/49) of diarrhoeic pigs, 44% (66/150) of healthy pigs, 51.5% (137/266) of healthy chicken, 47.2 (42/89) of diarrhoeic dogs, 29.4% (28/85) of healthy ducks, 6.3% (8/128) of healthy goats, 5.8% (7/121) of healthy cattle and 2% (2/98) of healthy

sheep. *C. jejuni* was isolated from 1.3% (1/75) of diarrhoeic children and from 9.1% (2/22) of diarrhoeic adult patients. The most common biotype was *C. jejuni* (51.4%), followed by *C. coli* (40.1%) and untypable *Campylobacter* strains (8.5%). *C. jejuni* was the only biotype isolated from goats, sheep, cattle and man, and constituted 85.7%, 68% and 56% of the strains from dogs, ducks and chicken, respectively. In pigs, 68% of the strains were *C. coli*. Similar findings have been reported by other investigators. The strains from diarrhoeic animals and man comprised of *C. jejuni* (68.1%), *C. coli* (25%) and untypable *Campylobacter* strains (6.9%). Of the isolates from healthy animals, 46.5% were *C. jejuni*, 44.5% were *C. coli* and 9% were untypable *Campylobacter* strains. In all, the untypable *Campylobacter* strains could be divided into three categories on the basis of biochemical reactions. Two of these categories had previously been reported, but have as yet not attained species status. The category not yet reported elsewhere contained strains which showed the following characteristics: catalase positive, sensitivity to 30µg nalidixic acid, hippurate hydrolysis negative, and H_2S production in iron medium.

The most common biotyping scheme is that proposed by Skirrow and Benjamin. This was able to type 91.5%

of 317 isolates. Another scheme, proposed by Lior, is an extension of the scheme of Skirrow and Benjamin. Using Lior's scheme, 91.8% of 73 isolates of *Campylobacter* species were typable. The advantage of Lior's scheme over that of Skirrow and Benjamin was the provision of sub-divisions. Otherwise, the ability of the two schemes to type isolates was similar.

C. laridis was the only known species resistant to 30µg nalidixic acid and this was used to differentiate the organism from other *Campylobacter* species. Ninety-five percent of the *Campylobacter* strains isolated in this study were sensitive to nalidixic acid. The resistant strains were not *C. laridis* but belonged to the group of untypable *Campylobacter* strains. Some of the resistant strains shared characteristics with *C. jejuni*, while others were similar to certain strains isolated by other workers and called "untypables".

Sensitivity to metronidazole has been used to differentiate strains obtained from different animal species. In certain farming systems where nitroimidazoles have been used extensively, a higher proportion of the isolates have shown resistance to 5µg concentration of metronidazole. In the study presented here, all 7 strains from cattle, 6 of 8 strains from goats, 85.4% (117/137) of poultry strains, 68.8% (64/93) of

pig strains, 64% (16/25) of duck strains and 55% (23/42) of dog strains were found to be resistant to 5µg of metronidazole. These results indicate that unrestricted use of various antimicrobial and antiprotozoal agents in Kenya, might have contributed to a high prevalence of *Campylobacter* strains that are resistant to metronidazole.

C. coli is able to grow in the presence of 400µg/ml concentration of 2,3,5-triphenyltetrazolium chloride (TTC), while *C. jejuni* is inhibited. This property has been suggested as a differential test for the two species. In this study, 91% (148/163) of *C. jejuni* and 92% (117/127) of *C. coli* grew in the presence of TTC. In the untypable group, 85% (23/27) of the strains were able to grow. Since the difference between the proportions of resistant *C. jejuni* and *C. coli* was insignificant, it is concluded that the ability to grow in the presence of TTC cannot be used as a distinguishing feature.

Certain investigators have asserted that reduction of selenite may be used to differentiate the various *Campylobacter* species. The findings in this study do not support this assertion since 52% of *C. jejuni* or *C. coli* reduced selenite. This test appears to have no value for differentiation of

C. jejuni from *C. coli*. It was, however, observed that differences existed between strains from avian and certain mammalian sources. The percentage of human and avian strains that reduced selenite were relatively lower than those in the other mammalian strains.

When the tests for the ability to grow in the presence of TTC and reduction of selenite were combined, 51% (83/163) of *C. jejuni* and 48% (61/127) of *C. coli* exhibited both properties. This implies that a combination of the two tests does not improve the discriminatory capabilities.

Certain *C. jejuni* strains have been found to produce a heat-labile enterotoxin immunologically similar to cholera toxin. One hundred and thirty strains of thermophilic *Campylobacter* species from cattle (7), chicken (48), dogs (14), ducks (25), pigs (34) and man (2) were examined, using the double antibody sandwich enzyme-linked immunosorbent assay, for the ability to produce such a toxin. No toxin producers were found. It is concluded that a heat-labile toxin similar to cholera toxin is not produced by thermophilic *Campylobacter* species. This conclusion agrees with genetic probe findings but casts doubts on the reports of partial identity between *C. jejuni* "toxin" and cholera toxin as observed in immunodiffusion tests by some investigators.

Yersiniosis is more common in temperate countries than in the tropics. Although the zoonotic nature of yersinial enteritis has been doubted, animals have occassionally been implicated as sources of infection for man.

One thousand and eighty nine fecal samples from human and animal sources were examined and only one strain of *Yersinia enterocolitica* was isolated from a healthy piglet. The isolate was a Nilehn biotype 1 strain. This biotype was initially considered non-pathogenic but recently it was isolated in a case of septicaemia. In view of the low isolation rate of *Y. enterocolitica* in this study, it is unlikely that animals play any significant role in the epidemiology of human yersiniosis in Kenya.

This study has shown that diarrhoeic and apparently healthy animals may harbour biotypes of *Campylobacter* species identical to those known to be pathogenic to man and may therefore play a significant role in the epidemiology of human campylobacteriosis by serving as reservoirs. The role of animals as reservoirs for human yersiniosis in Kenya is likely to be insignificant.