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Localization and detection of campylobacter jejuni using bio-molecular techniques like immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) in raw chicken meat

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Abstract

The current study was done to comprehend the prevalence of *Campylobacter* spp. in 100 raw chicken meat samples collected from the various retail shops and local butcher shops in Ludhiana, Punjab, to study the putative impact of contamination of meat during meat handling. *C. jejuni* was isolated from raw chicken meat and its localization was done by histopathology and various bio-molecular techniques like IHC and Fluorescence in situ hybridization. Prevalence of 8% was observed i.e. 08 out of 100 samples were positive and these samples were subjected to molecular identification using Fluorescence in situ hybridization technique to corroborate their presence in these meat samples. Histopathological analysis of the samples revealed changes in the tissues viz. heart, muscle, liver, kidney, and gizzard varying from degenerative changes, inflammation, and focal to multifocal areas of fibrosis. Bio-molecular techniques like immunohistochemistry (IHC) and FISH attested the presence of *C. jejuni* in meat samples.

Keywords: *Campylobacter jejuni*, retail meat, IHC, FISH

Introduction

Campylobacter is a Gram-negative slender, motile and microaerophilic organism belonging to *Campylobacteraceae* family. *Campylobacter* spp. is a curved bacteria that appear as a comma or s-shaped, measuring about 0.2 to 0.8 μm wide and 0.5 to 5 μm in length [1]. They are confirmed positive by the simple biochemical tests (oxidase and catalase test). The organism struggles to survive in dry conditions, low pH ($\text{pH} \leq 5.0$), freezing temperatures, and saline environment [2]. *C. jejuni* is commonly associated with many bird species, and it naturally colonizes the digestive tract of poultry. *C. jejuni* is mostly found in poultry faeces and grows optimally between 37- 42°C in a low oxygen environment. The survival of *C. jejuni* outside the digestive tract is difficult, and the replication rate is very low [3].

Campylobacter genus consists of 22 species and 6 subspecies, of which 12 species of *Campylobacter* are frequently reported to cause food poisoning in human beings, with *C. jejuni* and *C. coli* being the most common in human diseases [4]. *Campylobacter* species have a wide range of distribution in warm-blooded animals. Food animals such as poultry, sheep, goats, and pigs; and in pets, including cats and dogs are known to harbor them. However, the contribution of each of the above species to the relative burden of disease is unclear but the consumption of undercooked contaminated poultry meat is believed to be a major contributor to the infection. Foodborne transmission is generally believed to be the main route of spread through undercooked meat and meat products, as well as raw or contaminated dairy products mainly milk [5]. *Campylobacteriosis* is a zoonotic disease with its association mainly to animals or animal products i.e. carcasses or meat products contaminated by *Campylobacter* from faeces during slaughtering and unhygienic meat handling. Therefore, the present study was carried out to delve into the putative impact of contamination of chicken meat with *Campylobacter jejuni* during unhygienic processing and meat handling.

Materials and Methods

Experimental procedures

The laboratory work was primarily performed at the Department of Veterinary Pathology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University

(GADVASU), Ludhiana.

Collection and processing of Samples

In this study, a total of 100 raw samples of poultry meat were collected from different retail shops in and around Ludhiana. About 100 grams of each of the meat samples were collected in dry, clean, and sterile polythene bags and transported to the laboratory for analysis within one hour or refrigerated at 4°C till further analysis was carried out. These samples were then processed no later than 48 hours after purchase. The meat samples were then placed in 10% Neutral Buffered Formalin for 24-48 hours for histopathological/ molecular studies.

Histopathology

The paraffin blocks prepared were cut using a rotary type microtome to less than 5 micron thick sections. Sections were obtained on clean glass slides and then stained with Hematoxylin and Eosin (H&E) and any other special histochemical stain, if required, as per the staining protocols [6]. The tissue impressions that were obtained on slides were also subjected to H&E staining. The cytological and histopathological observations were made by examining the slides under the microscope.

Immunohistochemistry

The tissue sections for immunohistochemistry were taken on Poly-L-Lysine coated slides. Before the coating of the slides, the slides were first cleaned with acid alcohol (1% HCl in

70% alcohol), followed by placing the slides in Poly-L-Lysine 0.1% (w/v) solution which was diluted to 1:10 with deionized water for ten minutes. Then the slides were drained and dried in the oven at 60°C. These slides were used for immunohistochemical studies. The primary antibody used in the present study along with the dilution. The primary antibody was diluted to 1:150 for *C. jejuni* in 0.1 M PBS (pH 7.4) for standardizing working dilutions to localize the bacterial antigen in the tissue sections and meat samples.

Fluorescence *in-situ* hybridization

Fluorescence *in-situ* hybridization was used to determine the bacterial localization in the chicken meat samples. A few slides that were found to be positive for bacteria through immunohistochemistry, were selected for FISH. The probe used in this technique is complementary to the 16s rRNA of the bacteria used in this study and has been tagged with red fluorescent dye Cy3 at the 5' end. For fluorescent microscopy, the slides were examined with an epi-illumination fluorescence microscope (Nikon Eclipse CiL) equipped with a digital camera (NikonDS-Fi2).

Results and Discussion

A total of 100 meat samples were examined for the presence of *Campylobacter jejuni*. It was observed that *Campylobacter jejuni* was present in 03 out of 80 (3.75%) fresh chicken samples collected with an overall prevalence of 03% (Table no.1).

Table 1: Comparison of detection of *Campylobacter jejuni* in meat samples using various techniques

Techniques	Total fresh meat samples (80)		Frozen Samples (20)	
	<i>Campylobacter jejuni</i>	%	<i>Campylobacter jejuni</i>	%
Histopathology	03	3.75	nil	nil
IHC	03	3.75	nil	nil
ISH	03	3.75	nil	nil
Total	03	3.75	nil	nil
Overall Total	(03+00)= 03 (03%)			

Histopathological examination of tissues revealed various lesions along with the presence of bacillary organisms. Thus, various lesions reported could be due to the presence of bacteria or bacterial antigens in the tissues. Rod-shaped bacteria numerous in number were also observed in H&E staining of certain tissue samples. In muscles, there was mild to moderate infiltration of heterophils and monomorphonuclear cells along with degenerative changes in muscle fibers and connective tissue fascia. The muscle section(s) also revealed the presence of chronic inflammation characterized by the presence of lymphomononuclear cells. There was mild to moderate diffuse myositis. Zenker's necrosis was also observed in a few tissue section(s) along with the scarification of the muscle tissue. Fibrosis in some muscles was another lesion seen (Fig. 1).

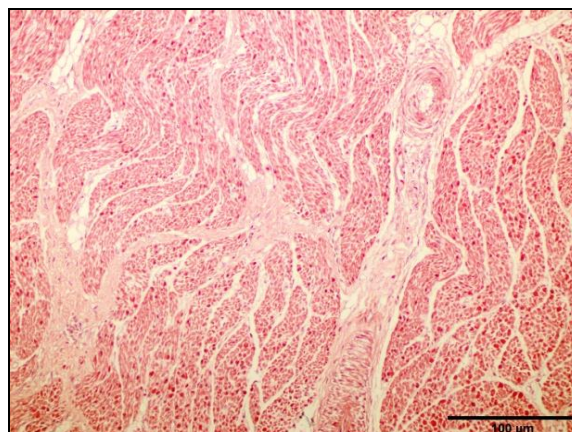


Fig 1: Section of muscle showing fibrosis. H&E. x20.

The liver section(s) revealed severe hepatitis with the presence of heterophils besides congestion and hemorrhage in certain liver sections along with the infiltration of lymphomononuclear cells. Certain section(s) of the liver also revealed mild to moderate fatty change.

The histopathology of the gizzard samples revealed a high degree of fibroplasia in the mucosal epithelium along with the loss of superficial kaolin layer with the underlying mucosal layer exhibited the presence of fibroplasia in the mucosal layer replacing the glandular part along with evidence of chronic focal infiltration of LMNs. A section of gizzard exhibited also reveals the proliferation of fibrous tissue confirmed by the presence of green-colored areas in the Masson's Trichrome Stain (Fig. 2).

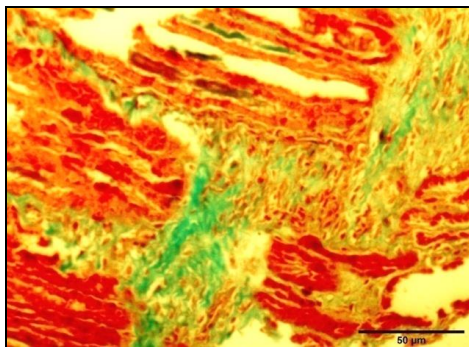


Fig 2: Section of gizzard showing diffuse fibrosis. Masson's trichrome stain. H&E. x100.

Kidney section(s) revealed severe fibrosis which was confirmed by special stain like Masson's Trichrome. In kidneys, degenerative changes and inflammatory lesions were evident as Glomerulitis along with the presence of inclusion bodies and characterized by Nephrosis/ Nephritis. There was degeneration of tubular epithelium and lumen was blocked partially or completely due to exfoliation of the lining of epithelial cells of tubules. There is a paucity of evidence to support the histopathological observations in meat samples.

The tissue sections of the heart revealed marked diffuse myocarditis with degenerative changes in the muscle fibers and the presence of heterophils in the myocardium. The areas in myocardium also showed the presence of hemorrhagic spots widely distributed. These changes observed in tissues are in concurrence with the findings of [7].

IHC was performed on fresh meat samples using the antibody (MBS534179/Biosource) for the immunolocalization of *Campylobacter jejuni* in the meat samples. There was the presence of *Campylobacter jejuni* (Fig. 3) in 03 out of 80 i.e. 3.75% prevalence.

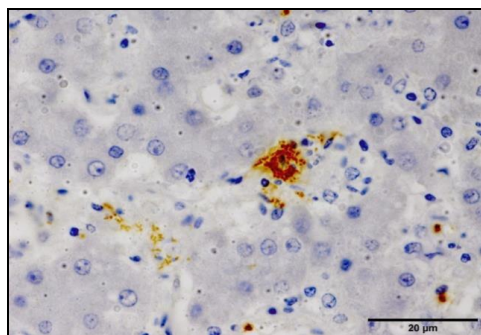


Fig 3: Liver section showing immuno-localization of *C. jejuni* as multifocal small to moderate-sized bacterial clusters in the liver. IHC. x40

Campylobacter jejuni could not be localized in the frozen meat sample. There is no evidence to support the findings of immunolocalization of *Campylobacter jejuni* due to scarcity and/or paucity of literature which indicate the demonstration of *Campylobacter jejuni* antigen in the meat samples, except a solitary report of localization of *Campylobacter jejuni* from frozen meat sample by [7].

In situ hybridization is a recent technique applied to the meat samples, there was very little literature available in annals to support the present findings. ISH was performed on fresh meat samples 03/80 and revealed a 3.75% presence of *Campylobacter jejuni*. The liver section showed *Campylobacter* species as characteristics comma-shaped organism emitting red fluorescence within the parenchyma and showed red fluorescence as an intact bacterial organism due to Cy3' labeled genus-specific oligonucleotide probe (Fig. 4).

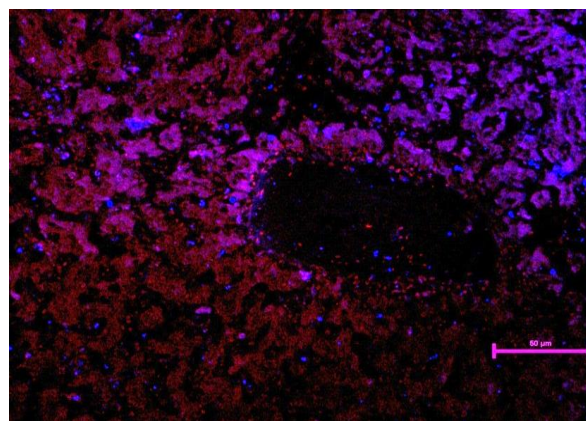


Fig 4: Liver, several *Campylobacter* organisms are seen near the central vein. x40. FISH. Red fluorescence showing intact bacterial organisms due to Cy3' labeled genus-specific oligonucleotide probe.

The sensitivity of fluorescent *in situ* hybridization technique was used to report thermotolerant *Campylobacter* spp. (*Campylobacter jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*) with high degrees of sensitivity for the identification of *C. jejuni* (90%), *C. coli* (97%), *C. lari* (81%), and *C. upsaliensis* (100%) to the species level by [8] with the same technique used in this study. Similarly, [9] evaluated the use of fluorescent *in situ* hybridization (FISH) technique for the detection of thermotolerant campylobacters in naturally contaminated chicken products and found the technique to be more sensitive, precise and rapid as compared to other molecular techniques of detection. Similar to the present study the use of 16S rRNA sequence was done for FISH analyses. But due to insufficient literature on ISH performed on chicken meat samples, the results of the present study provide valuable data to the new researchers in this field of study.

Conclusions

The present study revealed 3.75% *Campylobacter jejuni* in raw chicken meat samples which putatively might be a potential source of contamination for the corresponding carcasses. During the processing, the spread of *Campylobacter* spp. and the cross-contamination of broiler carcasses by the bacteria present in the intestinal content may be the potential source. The elucidation of *Campylobacter jejuni* in chicken meat is quite appurtenant as a common meat contaminant, which was buttressed by histopathological in countenance with IHC and FISH techniques giving credence

to the fact that meat should be handled with utmost care to avoid contamination.

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