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Full Length Research Paper

Contamination level variation of fecal Streptococci (Enterococci) in poultry slaughterhouse premises with hazard analysis and critical control point (HACCP) method

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Enterococci bacteria category that were previously named fecal streptococcus or group D Lancefield's streptococcus mostly are in human and animal intestinal tract and are among the most important indicators of fecal contamination of food. In addition, they cause food poisoning and diarrhea in humans (particularly children). In this study after validation of principles of system in poultry slaughter house, process premises diagram designed and hygiene hazards of poultry in slaughter line were studied and critical points detected. Then, 10 samples were taken from slaughtering stages such as (1) rectal swab (2) breast swab (3) after defeathering (4) after eviscerating (5) after spray cold water (6) after the chiller (7) before packaging and water samples of: (8) scalder (9) Chiller. Prevalence rate of Enterococci and contamination level were studied using standard methods. Paired t-test for qualitative data was used for statistical analyses. The results obtained from the 9 stages of sampling showed that logarithmic average contamination load of Enterococci were 2.82, 1.03, 1.19, 2.26, 1.64, 0.95, 093, 1.63 and 1.32%, respectively. Enterococci logarithmic average contamination load of breast swab as compared with rectal swab showed significant difference ($p<0.05$). Finally, the results obtained showed that poultry intestine is a seriously critical point in slaughter house.

Key words: Enterococci, poultry, slaughterhouse.

INTRODUCTION

The Enterococci as a group were first described by Thiercelin and the genus Enterococci was proposed by Thiercelin and Jouhaud for Gram-positive diplococcic of intestinal origin (Thiercelin, 1899; Thiercelin and Jouhaud, 1903). Andrewes and Horder (1906) classified potentially pathogenic bacteria from a patient with endocarditis as *Streptococcus faecalis*. Because of their close resemblance with strains isolated from the human intestine the species epithet 'faecalis' was suggested. Lancefield (1933) developed a serological typing system for streptococci in which those of 'faecal origin' possessed the group D antigen. This correlated with the grouping of Sherman (1937) who proposed a new classification scheme for the genus Streptococcus that separated it into four divisions designated: pyogenic, viridans, lactic and enterococci. The Enterococci group included *S. faecalis, Streptococcus faecium,*

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Streptococcus bovis and *Streptococcus equinus* as the enterococcal or group D strains (Sherman, 1937; Robinson, 2000). '*Streptococcus durans'* assumed various levels of acceptance, either as a separate species or as a subspecies of *S. faecium* within this group. It was regarded important in food microbiology because it was considered to be of non-faecal origin (Robinson, 2000). Strains of *S. durans* could be differentiated from *S. faecalis* and *S. faecium* by carbohydrate fermentation tests (Robinson, 2000). The classical taxonomy of the enterococci is vague because there are no phenotypic characteristics that unequivocally distinguish them from other Gram-positive, catalase-negative, cocci-shaped bacteria (Devriese et al., 1993). The majority of Enterococci species, however, can be distinguished from other Gram-positive, catalase-negative cocci by their ability to grow at 10 and 45°C, in 6.5% sodium chloride, at pH 9.6 and to survive heating at 60°C for 30 min (Hardie and Whiley, 1997; Morrison et al., 1997). Not all Enterococci species possess the group D antigen (Lancefield, 1933). The current Streptococcus spp., *S. bovis, S. suis* and *S. saccharolyticus*, as well as pediococci and certain Leuconostoc strains also react with Lancefield's group D antiserum (Lancefield, 1933). Some strains of lactococci, pediococci, aerococci and leuconostocs grow in the presence of 6.5% sodium chloride, but *Enterococci cecorum, Enterococci columbae* and *Enterococci avium* do not (Devriese et al., 1993). Pediococci and some lactococci grow at 45°C, while most lactococci, leuconostocs and some streptococci grow at 10°C, but *E. avium* generally does not (Murray, 1990; Devriese et al., 1993). Moreover, the phylogenetically distinct species or 'species groups' differ to some extent in their cell wall chemistry, physiology, growth and biochemical activity (Devriese et al., 1993). Correct species identification is of great importance to both medical and food microbiologists. For example, a clinical isolate would need to be correctly identified for appropriate antibiotic treatment, because susceptibility patterns differ considerably between species (Murray, 1990; Ruoff, 1990; Morrison et al., 1997). Correct species identification is useful for epidemiologic surveillance in hospitals (Murray, 1990). For the food microbiologist, correct identification may be important for selecting a starter strain and labeling of the product to which the starter is added. With the development of more sophisticated starter culture systems and the rapid changes in the taxonomy of lactic acid bacteria (LAB), it is of utmost importance for food microbiologists to be aware of current nomenclature (Stiles and Holzapfel, 1997). As regular inhabitants of the intestine, enterococci may serve as indicators of faecal contamination, and are therefore of particular importance in food and public health microbiology. *E. faecalis* and *E. faecium* have been suspected, but remain unconfirmed, as causative agents of food borne illness (Dack, 1956; Stiles and Holzapfel, 1997). Several strains are used as probiotics and others are involved in a number of food fermentations for the production of certain cheeses and other fermented milk products (Robinson, 2000). They are associated with natural fermentations such as occur in olives and fermented African products (Olasupo et al., 1994; Franz and Von Holy, 1996) and Enterococci may become the predominant population of in-package, heat-treated meats (Houben, 1982; Bell and DeLacey, 1984; Andre Gordon and Ahmad, 1991).Enterococci are considered as emerging pathogens of humans, and their role and importance have been reviewed by Lewis and Zervos (1990); Murray (1990) and Morrison et al. (1997). They have become of major importance in community-acquired and in hospital-acquired (nosocomial) infections and super infections such as endocarditis, bacteraemia, urinary tract, neonatal, central nervous system (CNS), intra abdominal and pelvic infections (Andrewes and Horder, 1906; Chenoweth and Schaberg, 1990; Murray, 1990). In poultry, Enterococci species have been associated with local and systemic infections, including septicemia and endocarditis (Wages et al., 2003). According to the health food control systems in modern processed foods, relies on the correct application of the principles of hazard analysis and critical control point (HACCP) and other systems is that the risks identified during the various stages of food processing and control are defined (USDA FSIS, 1996b; CFIA, 1997).

MATERIALS AND METHODS

Ten samples were taken in each of slaughtering stages of classy poultry slaughter house in Tabriz city, Iran: (A) Rectal swab (B) 25 cm^2 breast swab (C) skin and meat after defeathering (D) skin and meat after eviscerating (E) skin and meat after spray cold water (F) skin and meat after the chiller (G) 50 ml water Askaldr (H) 50 ml water chiller (I) skin and meat before packaging. Standard methods of Institute of standards and Industrial Research of Iran, no: 356, 1810 for preparation, culture and detection of Enterococci spp. in samples were used (ISIRI, 1985; ISIRI, 1993). Paired t-test for qualitative data was used for statistical analysis.

RESULTS AND DISCUSSION

Variations of Enterococci logarithmic average contamination load in different stages of poultry slaughter had been shown in Figure 1.

Logarithmic average contamination load of Enterococci in rectal swab samples 2.82 log cfu / g and breast swab samples were determined 1.03 log cfu / g. In samples of after defeathering and after eviscerating this amount increased to 1.19 and 2.26 log cfu / g. In samples of after spray cold water, after the chiller and before packaging, logarithmic average contamination load was 1.64, 0.95, 0.93 log cfu / g, respectively. 1.63 log cfu / g of samples of water scalder and 1.32 of samples of water chiller also have been contaminated with Enterococci (Figure 1).

The presence of Enterococci in the gastrointestinal

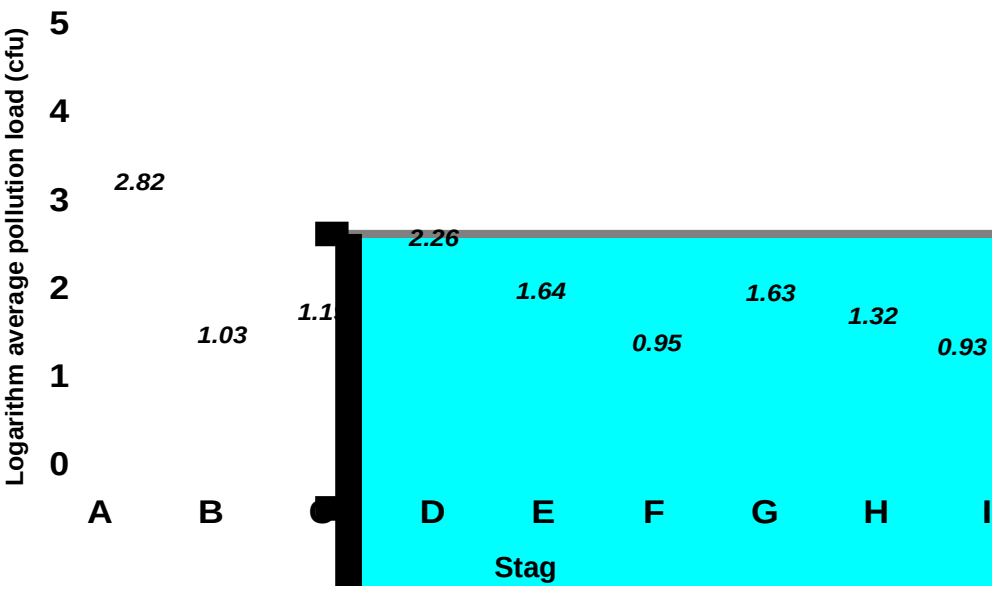

Figure 1. Variation of Enterococci logarithmic average contamination load in (A) rectal swab, (B) breast swab, (C) after defeathering, (D) after eviscerating, (E) after spray cold water, (F) after the chiller, (G) water scalder, (H) Water Chillers, (I) before packaging.

tracts of animals lead to high potential for contamination of meats at the time of slaughter, particularly, if the intestines are cut or broken (MAF, 2000; Robinson, 2000). This is likely to result in an increase in contamination by mesophilic bacteria, including intestinal pathogens such as Enterococci, Salmonella, Campylobacter, Clostridium perfringens, and Listeria (Bremner, 1996; CFIA, 1997). Enterococcus logarithmic average contamination load after breast swab compared with rectal swab were showed significant difference ($p<0.05$), (Figure 1). This result has obtained because the main habitat of Enterococci is gastrointestinal in poultry and also it is the microflora of intestine (Ruoff, 1992; Hirsh and Biberstein, 2004). Logarithmic average contamination load after eviscerating compared with defeathering were showed no significant difference ($p>0.05$), (Figure 1) and this can be due to the skills of workers in work, sanitation and accurate washing of facilities at this stage (Ann Marie, 1997). The data show that the rate of intestinal cut in the form of mechanical systems that eviscerating is 0/5 to 40%, however it increases to 80 to 90% in traditional systems because Enterococci is normal intestinal flora. Naturally, at this stage, amount of these organisms will increase until some extent that makes this stage one of the highest rates during the slaughter process (MAF, 2000). Of course, cleaning surfaces and equipment with high pressure water and chlorinated (minimal amount of 20 ppm) can reduce the increasing of contamination (MAF, 2000) .And in another study on poultry carcass sites in an organized slaughterhouse Vaidya and colleagues reported that the percent prevalence of indicator organisms, viz. enterococci, fecal coliforms, was 24.41 and 18.74, respectively. Among operations, bleeding and evisceration were noted as critical. Legs showed the maximum level of contamination and high prevalence of indicator organisms, denoted as a critical site. Statistical analysis of data revealed that enterococci and fecal coliforms showed highly significant difference ($p < 0.01$). Proper monitoring of each unit operation is important for production of quality meat (Vaidya et al., 2005). In another study systematic monitoring of fecal bacterial indicators as well as some classic pathogens was performed by Voidarou et al. (2007) at selected critical points in a water chiller ecosystem and Clostridium perfringens (100%) was found in all samples. Fecal coliforms (100%), Enterococci sp. (100%), and Streptococcus sp. (100%) were also found in all samples. *Escherichia coli* (40%), *Proteus mirabilis* (25%), Salmonella sp. (10%), *Proteus vulgaris* (5%) and *Morganella morganii* (5%) were also present. Devriese et al. (1991) reported that the intestinal microflora of young poultry contained principally *E. faecalis* and *E. faecium*, but *E. cecorum* predominated in the intestine of chickens over 12 weeks old. In another study Leclerc et al. (1996) reported that *E. fecalis* and *E. faecium* were the most frequently isolated species and this is in agreement with their predominance in the intestine and faeces of human and animals. Holzapfel and Steyn (unpublished results) noted a predominance of *E. faecalis* and *E. faecium* in the lower intestinal tract of the ostrich. In another study Turtura and Lorenzelli (1994) reported that *E. faecalis* predominated the Gram-positive coccal species isolated from chicken samples collected at poultry abattoirs. In

Czech Republic in 2010, Radimersky et al. (2010) studied on rectal swabs of feral pigeons and they totally isolated 143 Enterococci: *E. faecalis* (36 isolates), *E. faecium* (27), *E. durans* (19), *E. hirae* (17), *E. mundtii* (17), *E. gallinarum* (12), *E. casseliflavus* (12) and *E. columbae* (3).

Conclusion

Finally results were showed that, poultry intestine is seriously critical point in slaughter house and with proper implementation of management, sanitation and most importantly proper implementation of HACCP patterns in poultry slaughter can control the critical points.

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