MICROBIAL CONTAMINATION IN DIFFERENT MEAT TYPES AND HYGIENE LEVELS OF ABATTOIRS IN JOHOR FROM 2019 TO 2023

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ABSTRACT. Microbial contamination in meat, influenced by processing practices and hygiene standards in abattoirs and processing plants, presents public health risks. This study evaluated microbial contamination in various meat types and the hygiene levels of abattoirs in Johor from 2019 to 2023. A total of 1,413 samples were collected from processing plants and abattoirs, including 613 chicken, 568 pork, 42 ruminant meat (beef and lamb) and 190 environmental swab. Microbial tests including Aerobic Plate Count (APC), *Escherichia coli* (*E. coli*), and coliforms were conducted using the Petrifilm® method, *Sarcocystis* macrocysts by organoleptic test (examined visually), while *Yersinia enterocolitica*, *E. coli* O157 and *Salmonella* spp. by isolation and identification method. The results showed that ruminant meat had the highest average APC (4.50±0.98 log CFU/g), which was significantly higher than pork (4.06±0.82 log CFU/g) and chicken (3.91±0.75 log CFU/g), with less than 1% of samples exceeding acceptable limits. Coliform counts were notably higher in chicken meat (1.91±0.68 log CFU/g) compared to pork and ruminant meat (around 1.70 log CFU/g), with 4-10% of samples exceeding limits ($p>0.05$). Mean *E. coli* counts were similar across all meat types (1.57-1.67 log CFU/g), with 12% exceeding limits. *Salmonella* spp. was detected in 11% of samples, most prevalent in chicken (16%), followed by ruminant meat (7%) and pork (6%). *Yersinia enterocolitica* was detected once in pork, whereas ruminant meat was negative of *E. coli* O157 and sarcocysts. Environmental swabs showed average *E. coli* and coliform counts higher than limits, although *Salmonella* was not detected. Overall, the study suggests that while microbial contamination in meat was generally within acceptable limits, *Salmonella* contamination remains a concern, indicating possible cross-contamination during production activities.

Keywords: food safety, abattoir, meat, indicator organisms, pathogens

INTRODUCTION

Food safety is a shared responsibility across the entire food supply chain, from farm to fork. Microbial contamination in meat and meat products, influenced by processing practices and hygiene standards in abattoirs and processing plants, presents significant public health risks. Recognizing its global significance, the United Nations General Assembly, in collaboration with the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), established World Food Safety Day in 2018, with annual themes to highlight various aspects of food safety (WHO, 2024). The theme for 2023, 'Food standards saves lives,' emphasized the importance of food safety standards and the vital role governments play in ensuring: (i) the effectiveness of food control systems, (ii) the development of international food safety and quality standards, (iii) the implementation of policies to protect the food supply, and (iv) raising awareness among consumers and food industry workers about the consequences of neglecting food safety standards (WHO, 2023).

In Malaysia, the Department of Veterinary Services (DVS) along with other departments under many government' ministries are responsible for safeguarding food safety and quality. Through its national food safety monitoring program, DVS oversees the safety and quality of animal-based foods from abattoirs and processing plants certified under the Veterinary Health Mark (VHM) and Good Veterinary Hygiene Practice (GVHP) (DVS, 2024). This program also monitors the sanitary and hygiene levels of abattoirs (DVS, 2023).

Samples from monitoring programs are assessed in the safety & quality aspects, including microbiological tests (indicator and pathogen microorganisms). Indicator microorganisms (e.g.: aerobic plate count (APC), coliforms, *E. coli*) are important components in microbiological testing programs conducted both by regulatory agencies and the food industry. Coliform groups and *E. coli* for example, are widely applied in the food industry as indicators for sanitation, process integrity and for verification of Hazard Analysis Critical Control Point (HACCP) in food safety systems (Tortorello, 2003). Meanwhile, qualitative tests for pathogens like *Salmonella* spp., *Yersinia enterocolitica*, *E. coli* O157 and others are often associated with food safety aspects and could bring more severe adverse health effects to humans.

To date, most reports regarding microbiological contamination of meat in Malaysia focus only on pathogens, especially *Salmonella* spp. (Ismail *et al*., 2024, Sukri *et al*., 2021, Saira Banu *et al*., 2019 and Shafini *et al*., 2017). Notably, studies by Chong *et al*. (2017) and Zulfakar *et al*. (2019) have examined microbiological contamination in beef and meat contact surfaces, but comprehensive data on the environmental hygiene levels of abattoirs and the microbial safety and quality of different meat types remains scarce. This study aims to fill this gap by assessing the microbial contamination in meat from DVS certified processing plants and abattoirs in Johor, as well as evaluating the hygiene levels of the abattoirs. The findings may provide valuable insights into the effectiveness of the national food safety monitoring program and guide improvements to ensure a consistently safe and high-quality food supply for public consumption.

MATERIALS AND METHOD

Samples

From the year 2019 to 2023, around 1,413 samples under the national food safety monitoring program were collected in Johor state by DVS's meat inspectors and sent to the Veterinary Laboratory of Southern Zone (Johor Bahru). Samples were 613 raw chicken meat from four VHM-certified chicken processing plants, 568 raw pork samples from pig abattoirs (one DVS abattoir and two licensed private abattoirs), as well as 42 ruminant meat (beef and lamb) samples from ruminant abattoirs (two DVS and 17 licensed private abattoirs). Additionally, 190 swab samples were collected from the respective pig and ruminant abattoirs' environment (utensils, working area and other meat contact surface, after cleaning process and pre-operation of slaughter activities). Swabs were immersed in buffered peptone water as the transport media and kept in a cooler box at chilled temperature (4°C) during transportation to the laboratory.

Microbiological tests

Meat and swab samples were tested for Aerobic Plate Count (APC), *E. coli*, coliforms and *Salmonella* spp. Microbial enumeration of APC, *E. coli* and coliforms were carried out using the standard Petrifilm® (Neogen, USA) method. Meat samples were also tested for other microbes, such as *Sarcocystis* detection which was carried out by organoleptic test (visual examination on the presence of macrocytes on raw meat). For detection of *Yersinia enterocolitica* and *E. coli*

O157, conventional isolation and identification methods were used. *Salmonella* spp. was tested by enriching samples, streaking onto the selective agar, followed by biochemical tests and serotyping using antisera (DVS, 2016). *Salmonella* isolates other than Enteritidis and Typhimurium were sent to Veterinary Research Institute (VRI), for further serotyping work in the year 2022 onwards.

Statistical Analysis

Results for enumeration of bacterial loads were converted into log10 CFU/g or log10 CFU/cm^2, screened for the homogeneity and expressed as means ± standard deviation (SD). One-way ANOVA test was used to determine significant differences in bacterial counts between sample types. The percentage of positive samples (i.e., those exceeding microbial limit or testing positive for *Salmonella* pathogen) across sample types and years were compared using the Chi-square (X^2) test. All statistical analyses were performed using SPSS version 25 (IBM Corp., USA), with significant level set at $p<0.05$.

RESULTS AND DISCUSSION

Microbial Load and Contamination Across Different Meat Types and Environmental Swabs from Abattoirs

Table 1 shows that ruminant meat (beef and lamb) samples had significantly higher average

Table 1. Microbial load of samples (meat and swab of abattoirs environment), from 2019 to 2023.

Type of samples	Chicken meat	Pork	Ruminant meat (beef and lamb)	Limit[1] (Log CFU/g	Swab (abattoirs' environment)	Limit[2] (Log CFU/cm^2)
Number of samples, n	613	568	42		190	
APC, (mean±SD)*	$3.91^a \pm 0.75$	$4.06^a \pm 0.82$	$4.50^b \pm 0.98$	6.397	1.08 ± 1.05	3.0
E. coli, (mean±SD)	$1.67^a \pm 0.59$	$1.63^a \pm 0.56$	$1.57^a \pm 0.70$	2.0	1.17 ± 0.75	1.0
Coliforms, (mean±SD)	$1.91^b \pm 0.68$	$1.71^a \pm 0.61$	$1.70^a \pm 0.87$	3.041	1.56 ± 0.53	1.0
Salmonella spp. positive, n (%)	97 (16%)	32 (6%)	3 (7%)	ND	0	ND
Yersinia enterocolitica positive, n (%)	-	1(0.2%)	0	ND	-	-
E. coli O157 positive, n (%)	-	-	0	ND	-	-
Sarcocystic positive, n (%)	-	-	0	ND	-	-

Notes: *mean ± SD were average value in log CFU/g (meat) or log CFU/cm^2 (swab); SD = Standard deviation; a, b: Mean values with different superscripts in the same row indicates significant differences among microbial loads in various meat types ($p<0.05$); APC (p-value <0.0001), *E. coli* (p-value = 0.353), coliforms (p-value <0.0001); "– ": Not tested; ND: Not detected; Limit[1]: refers to microbiological guideline from APTVM 16(c):1/2011 (DVS, 2011); Limit[2]: refers to microbiological guideline from Manual of Monitoring Program for Sanitation and Hygiene of Abattoir (DVS, 2023)

APC load at 4.50±0.98 log CFU/g compared to pork (4.06±0.82 log CFU/g) and chicken meat samples at 3.91±0.75 log CFU/g ($p<0.0001$). The mean *E. coli* count for all samples were relatively similar, ranging from 1.57-1.67 log CFU/g ($p>0.05$). However, the mean coliform count was significantly higher in chicken meat (1.91±0.68 log CFU/g) than in pork and ruminant meat (both approximately 1.70 log CFU/g) ($p<0.0001$). These findings align with those of Chong *et al*. (2017), who reported quite similar mean APC load for beef samples from abattoirs in Selangor state at 4.00±0.934 log CFU/cm^2, *E. coli* load at 1.87 log CFU/cm^2 and 10% of samples contaminated with *Salmonella* spp. The average APC, *E. coli* and coliform counts in this study did not exceed the established limit of 6.397 log CFU/g, 2.0 log CFU/g and 3.041 log CFU/g respectively, according to guidelines by DVS (2011). Nevertheless, a certain percentage of samples did exceed these limits (Table 2) and the meat type with the highest average microbial load also had the highest percentage of samples exceeding microbial limits.

For swab samples in pig and ruminant abattoirs, the average APC load was 1.08±1.05 log CFU/cm^2, which is much lower than the limit at 3.0 log CFU/ cm^2. The DVS environmental swab limits (2023) were more stringent than those for raw meat (DVS, 2011), likely because the processing environment is a critical contamination pathway for produced goods (Bourdichon *et al*., 2021). The average of *E. coli* load (1.17±0.75 log CFU/cm^2) and coliform load (1.56±0.53 log CFU/cm^2) were slightly higher than their respective limits of 1.0 log CFU/cm^2. These higher *E. coli* and coliform loads were detected twice from the same pig abattoir in 2019 and 2020 but improved subsequently, no longer exceeding microbiological limit in subsequent years. No *Salmonella* spp. was detected in any of the swab samples. In contrast, another study of two ruminant abattoirs reported higher APC load at 4.77±1.14 log CFU/cm^2, *E. coli* at 2.91±1.00 log CFU/cm^2, and 25% of the total samples positive for *Salmonella* spp. (Zulfakar *et al*., 2019). This showed that abattoirs in the current study implemented good hygiene and sanitary procedures to produce microbiologically safe meat.

As shown in Table 2, only 0.3% (4/1,223) of all meat samples exceeded the APC limit, which is lower than the percentage for other bacterial contaminants. Ruminant meat samples had a significantly higher percentage of samples exceeding the APC limit (7%, 3/42) compared to pork (0.2%, 1/568), with none of the chicken meat samples exceeding the APC limit (X^2=62.260, $p<0.0001$). For coliforms, the exceedance percentage ranged between 4-10% across all meat samples ($p>0.05$), while 12% of samples exceeded the *E. coli* limit (147/1,223), and 11% were positive for *Salmonella* spp. (132/1,223). Chicken meat samples had the highest percentage of *Salmonella* contamination (16%, 97/613), followed by ruminant meat (7%, 3/42) and pork (6%, 32/568) (X^2=32.397, $p<0.0001$). These findings highlight the effectiveness of food safety procedures implemented by the processing plants or abattoirs from which the samples were taken, some of which followed strict Hazard Analysis Critical Control Point (HACCP) systems. Compared to meat from retail outlets such as supermarkets, butcher shops, and wet markets (which report contamination rates of 40% for chicken and 15% for beef (Shafini *et al*., 2017), the samples from these certified facilities showed better hygiene and less *Salmonella* contamination.

Table 2. Percentage of positive samples (exceeding microbial limits or positive pathogens) in meat samples (chicken, pig and ruminants) from 2019 to 2023.

Type of samples	Chicken meat	Pork	Ruminant meat (beef and lamb)	*p-value, (X^2 value)	Total
Number of samples, n	613	568	42	-	1,223
APC, n (%)	0 (0%)	1 (0.2%)	3 (7%)	<0.0001 (62.260)	4 (0.3%)
E. coli, n (%)	97 (16%)	43 (8%)	7 (17%)	<0.0001 (19.879)	147 (12%)
Coliforms, n (%)	36 (6%)	20 (4%)	4 (10%)	0.064 (5.843)	60 (5%)
Salmonella spp. positive, n (%)	97 (16%)	32 (6%)	3 (7%)	<0.0001 (32.397)	132 (11%)

Notes: *p-value, (X^2 value) refers to chi-square test on percentage of positive samples among each type of meat samples. Results within the same row were considered significant at p<0.05

Salmonella Serotypes

Among the 132 *Salmonella* isolates, more than half were classified as *Salmonella* spp. only, as isolates sent for serotyping at the Veterinary Research Institute started in the year 2022 onwards. Dominant serotypes included Brancaster (15/132), Enteritidis and Corvallis (8/132 samples each) in chicken meat, Schleissheim (3/132), Typhimurium and Stanley (2/132 samples each) in pork, and Weltevreden, Hindmarsh and Djugu in ruminant meat (Table 3). *Salmonella* Enteritidis was the most common serotype found in poultry meat or poultry processing environments in Malaysia. Other *Salmonella* serotypes frequently found included Corvallis, Indiana, Typhimurium, Albany, Hadar, Dublin, Anatum, Stanley, Gallinarum, Choleraesuis, and Brancaster. In pork meat, common serotypes were Rissen, Typhimurium, Typhi-Suis, and Weltevreden. Regarding ruminant meat (beef and lamb), notable serotypes included Jamaica, *Senftenberg* and Agona (Saira-Banu *et al*., 2019, Shafini *et al*., 2017, Nidaullah *et al*., 2017 and Roseliza *et al*., 2011).

Figures 1, 2, and 3 illustrate the trends in the percentage of positive samples (exceeding microbial limits or testing positive for *Salmonella*) over the five-year period from 2019 to 2023. Chi-square tests conducted to assess differences in the percentage of positive chicken meat samples across the years revealed significant variation for *E. coli* (p=0.029, X^2=10.757), coliforms (p=0.012, X^2=12.771), and *Salmonella* (p=0.002, X^2=17.463). The number of samples tested each year were 135 (2019), 134 (2020), 135 (2021), 140 (2022), and 69 (2023), as shown in Figure 1.

No significant variation was seen in positive pork samples across the years for total plate count (APC; p=0.523, X^2=3.213), *E. coli* (p=0.093, X^2=7.957), coliforms (p=0.202, X^2=5.960), and *Salmonella* (p=0.056, X^2=9.225). The number of samples tested each year was as follows: 2019 (135), 2020 (135), 2021 (135), 2022 (170), and 2023 (26), as shown in Figure 2. For ruminant meat samples across the years, no significant variation was found for APC (p=0.923, X^2=0.913), *E. coli* (p=0.868, X^2=1.263), coliforms (p=0.253, X^2=5.352), and

Table 3. Occurrence of *Salmonella* serotypes in meat samples (chicken, pig and ruminants) from 2019 to 2023.

	Meat types (n)			Total	%
***Salmonella serotypes**	**Chicken**	**Pork**	**Ruminant meat (beef and lamb)**		
***Salmonella* spp.**	59	22	0	81	61
Brancaster	15	0	0	15	11
Enteritidis	8	0	0	8	6
Corvallis	8	0	0	8	6
Typhimurium	1	2	0	3	2
Stanley	1	2	0	3	2
Schleissheim	0	3	0	3	2
Uganda	2	0	0	2	2
Albany	1	0	0	1	1
Weltevreden	0	0	1	1	1
Hindmarsh	0	0	1	1	1
Sandow	0	1	0	1	1
Saintpaul	1	0	0	1	1
Djugu	0	0	1	1	1
Typhi	1	0	0	1	1
***Salmonella* sp. (Group OME)**	0	1	0	1	1
***Salmonella* sp. (Group OMD)**	0	1	0	1	1
	97	32	3	132	

Note: *Serotyping of *Salmonella* isolates was done in the year 2022 onwards

Salmonella (p=0.418, X^2=3.911). The number of samples tested for ruminant meat were 1 (2019), 2 (2020), 2 (2021), 18 (2022), and 19 (2023), as shown in Figure 3.

For chicken meat, the highest percentage of positive samples for coliforms, *E. coli*, and *Salmonella* was observed in 2020, followed by a significant decline in 2021 and relatively stable levels in subsequent years. Notably, the percentage of *Salmonella* contamination decreased from 20% (27/134) in 2020 to 4% (6/135) in 2021, before rising again to 19% (27/140) in 2022 (Figure 1). This fluctuation may indicate variations in hygiene practices or environmental factors affecting contamination rates.

In pork samples, the percentage of positive *Salmonella* increased from 2020 to 2021, peaking at 9% (12/135) in 2021, while coliform counts decreased during the same period (Figure 2). For ruminant meat, an increasing trend in positive samples was observed after 2021. This rise is likely attributed to an increase in sample collection and testing efforts rather than an actual increase in contamination levels (Figure 3).

Other foodborne pathogens, such as Yersinia enterocolitica, were detected only once in the 2021 samples. Meanwhile, ruminant meat samples tested negative for *E. coli* O157:H7 and *Sarcocysts* (Table 1).

Correlation Between *E. coli* and *Salmonella*

A similar trend was observed across all meat types, where high percentages of samples exceeding *E. coli* limits were also highly contaminated with *Salmonella* spp. (Figures 1, 2 and 3). Although Buncic (2006) reported no proven correlation between indicator organisms and pathogen prevalence or levels,

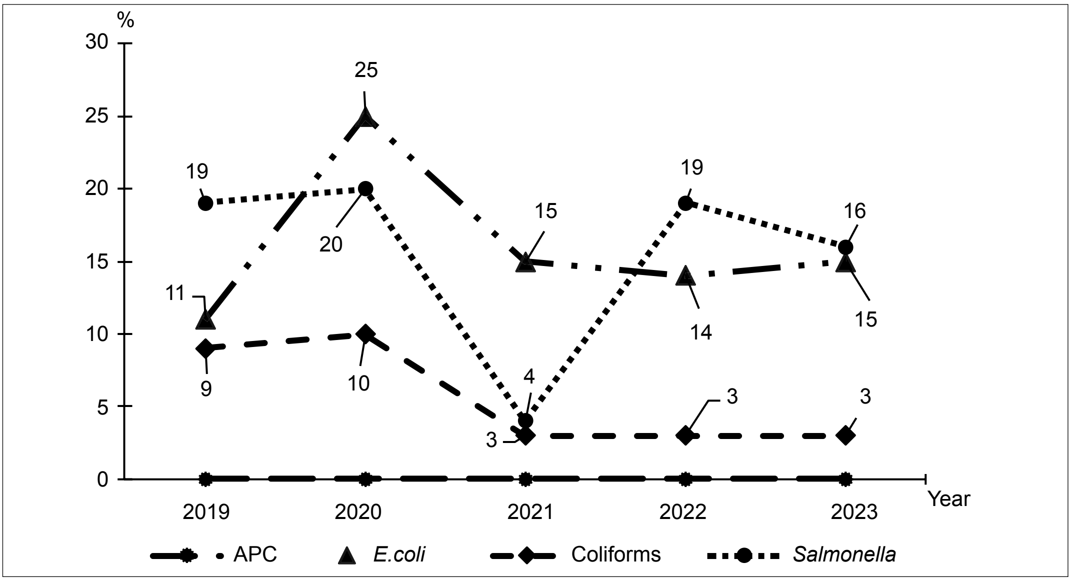

Figure 1: Percentage of positive samples (exceeding microbial limits or positive pathogens) in chicken meat samples over the year 2019 to 2023.

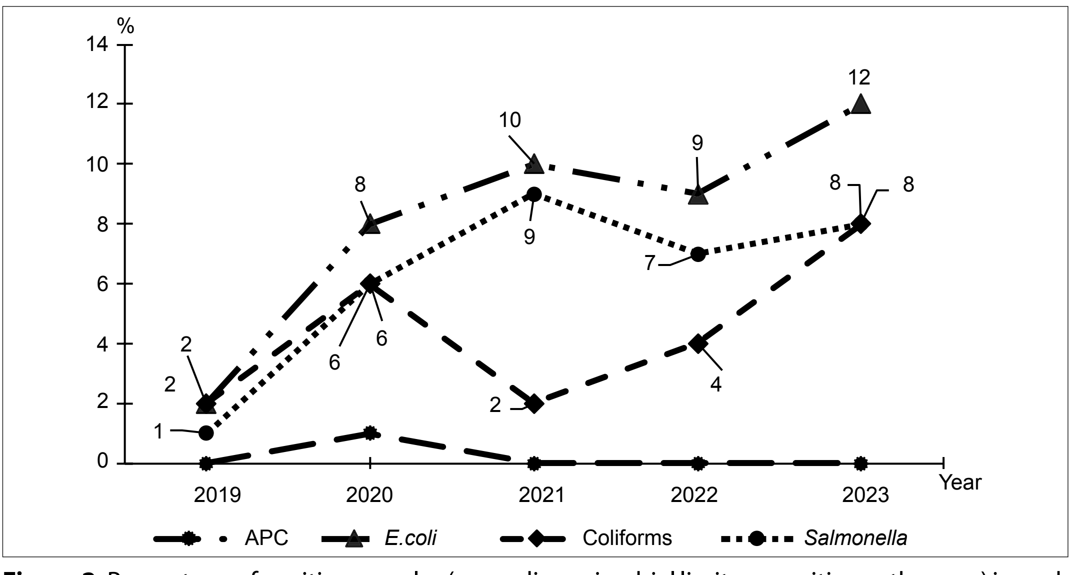

Figure 2. Percentage of positive samples (exceeding microbial limits or positive pathogens) in pork samples over the year 2019 to 2023

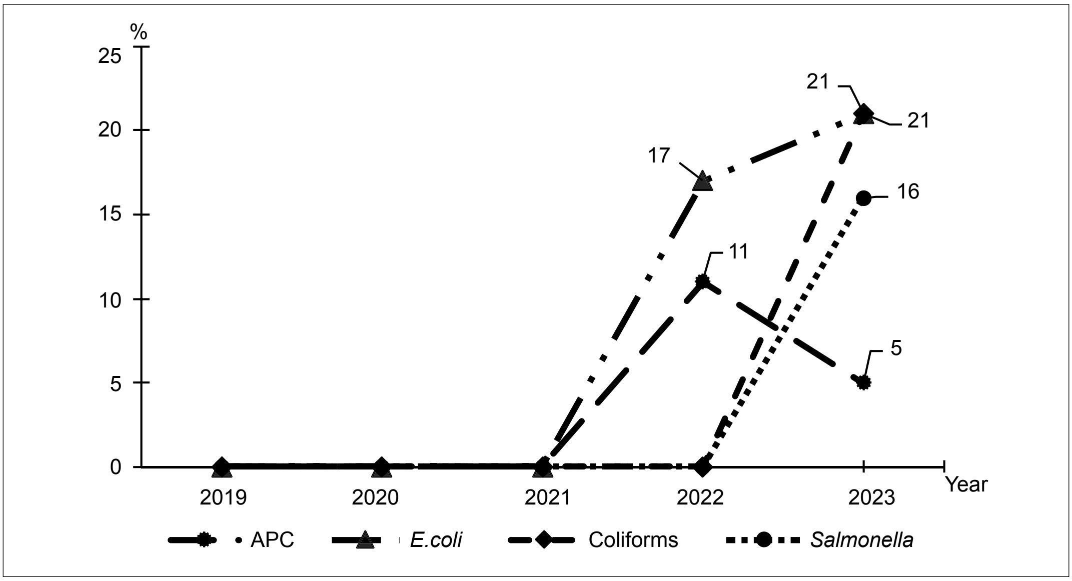

Figure 3. Percentage of positive samples (exceeding microbial limits or positive pathogens) in ruminant- meat samples (beef and lamb) over the year 2019 to 2023.

it was emphasized that indicator organisms should be interpreted to assess general trends in hygiene practices. Tortorello (2003) proposed that indicator organisms can suggest the potential presence of pathogens, highlight lapses in sanitation and good manufacturing practices (GMPs), indicate process failures, and reflect storage time and conditions, all factors that ultimately impact food quality and consumer acceptability. Furthermore, bacterial load and type help determine whether food meets acceptable standards, specifications, and guidelines, while guiding subsequent heat-processing parameters (Ray, 2001).

This study underscores the importance of ongoing monitoring of microbial contamination in meat and the processing environment to safeguard public health and enforce regulatory practices effectively.

CONCLUSION

Overall, meat produced in processing plants and abattoirs under the supervision of the DVS, as well as the production areas within these facilities, was in good hygiene level. However, a relatively high percentage of meat samples detected positive with *Salmonella* spp. suggest the potential for contamination from various sources during production, despite adherence to strict food safety procedures. These findings could assist the DVS to assess the effectiveness of its food safety monitoring programs in ensuring the supply of safe and quality food for public consumption.

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