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## Prevalence of *Salmonella* spp. and *Staphylococcus aureus* in chicken and pork meat from Cambodian markets

SAFE FOOD, FAIR FOOD FOR CAMBODIA PROJECT

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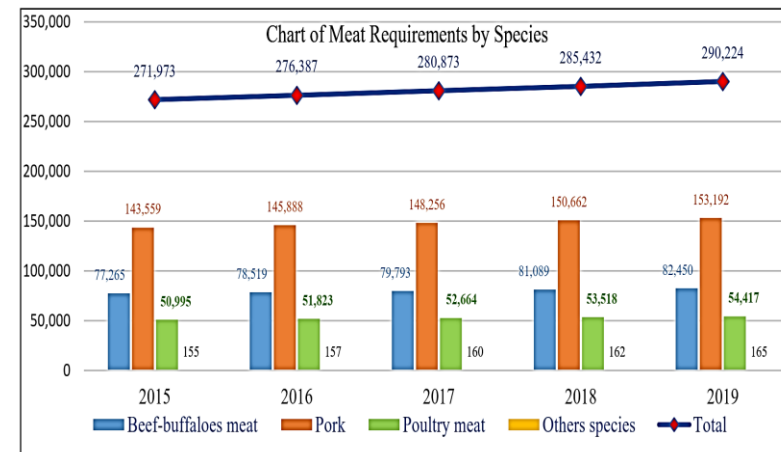
*Project final workshop*

*Phnom Penh, 21–22 June 2021*



## Key facts

- Foodborne diseases (FBDs) are the illness conditions caused by the ingestion of food containing biological, chemical, or physical hazards.
- *Salmonella* is 1 of 4 key global causes of diarrheal diseases.
- The recent estimation of cost of illness due to foodborne bacteria was about USD 92 per-episode and 63 USD\$ per day (SFFF, 2019),
- Annual net of chicken meat consumption in 2019: 290,224 ton/year (GDAHP, 2019).
- Basic food hygiene practices, such as "cook thoroughly", can help to prevent FBDs.





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## Objective of this study

- To determine the prevalence of *Salmonella* spp. and *S. aureus* in chicken and pork meat and cutting boards for chicken and pork in Cambodian traditional markets and supermarkets .





## Materials and methods

- This cross-sectional study was carried out between October 2018 and August 2019.
- Markets comprised 532 samples from 52 traditional markets and 6 supermarkets in 25 provinces/municipalities of Cambodia.
- Specimen: pork, chicken and swab of cutting boards
- Bacteria pathogen: *Salmonella* and *Staphylococcus*
- Protocols:
  - Detection *Salmonella* species (ISO 6579-1\_2017)
  - Most Probable Number of *Salmonella* (Pavic et al., 2010)
  - Enumeration and Detection *Staphylococcus* (ISO-6888-1, 1999)



## Sampling and Sample collection

**Table 1.** Number of samples collected from traditional markets and supermarkets in Cambodia

Sampling round	Chicken meat	Chicken cutting board	Pork cutting board	Pork
Traditional market <sup>1</sup>	156	52	52	156
Repeat sampling <sup>2</sup>	30	10	10	30
Supermarkets <sup>3</sup>	18	-	-	18
<b>Total specimen</b>	<b>204</b>	<b>62</b>	<b>62</b>	<b>204</b>
<b>Total specimen = 532</b>				

<sup>1</sup>Three markets were included in Phnom Penh and Siem Reap, while two markets were included in the other 23 provinces,

<sup>2</sup>The total 80 sample were re-sampling from Battambang, Phnom Penh, Siem Reap, and Sihanoukville.

<sup>3</sup>Four supermarkets in Phnom Penh and two supermarkets in Siem Reap,



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# Results





**Table 2. Prevalence of *Salmonella* spp. and *S. aureus* in chicken, cutting board of chicken, pork and cutting board of pork in Cambodian markets by province.**

Provinces/Municipalities	Markets <sup>1</sup>	Total Sample Collected <sup>2</sup>	Total Positive Samples	Number of <i>Salmonella</i> Positive Samples (%)					Number of <i>S. aureus</i> Positive Samples (%)				
				Chicken	Cutting Board Chicken	Cutting Board Pork	Pork	Average <sup>4</sup> MPN/g	Total Positive Samples	Chicken	Cutting Board Chicken	Cutting Board Pork	Pork
Phnom Penh	3 (2 times)	48	13 (27.1)	8 (44.4)	1 (16.7)	0 (0%)	4 (22.2)	16.1	12 (25.0)	5 (27.8)	1 (16.6)	1 (16.6)	5 (27.8)
Siem Reap	3 (2 times)	48	31 (64.6)	14 (77.8)	3 (50.0)	1 (16.7)	13 (72.2)	2.6	12 (25.0)	8 (44.4)	0	0	4 (22.2)
Battambang	2 (2 times)	32	14 (43.8)	4 (33.3)	2 (50.0)	2 (50.0)	6 (50.0)	5.9	10 (31.3)	5 (41.7)	2 (50.0)	0	3 (25.0)
Preah Sihanouk	2 (2 times)	32	18 (56.3)	9 (75.0)	1 (25.0)	2 (50.0)	6 (50.0)	25.4	11 (34.4)	7 (58.3)	0	0	4 (33.3)
Takeo	2	16	8 (50.0)	3 (50.0)	1 (50.0)	1 (50.0)	3 (50.0)	15.7	5 (31.3)	2 (33.3)	1 (50.0)	0	2 (33.3)
Kampong Cham	2	16	5 (31.3)	1 (16.7)	1 (50.0)	0	3 (50.0)	15.0	10 (62.5)	5 (83.3)	1 (50.0)	1 (50.0)	3 (50.0)
Tboung Khmum	2	16	7 (43.8)	2 (33.3)	1 (50.0)	1 (50.0)	3 (50.0)	8.3	6 (37.5)	3 (50.0)	0	0	3 (50.0)
Kep	2	16	10 (62.5)	3 (50.0)	1 (50.0)	0	6 (100)	58.6	4 (25.0)	1 (16.7)	0	0	3 (50.0)
Kampot	2	16	10 (62.5)	3 (50.0)	1 (50.0)	1 (50.0)	5 (83.3)	55.2	5 (31.3)	4 (66.7)	0	0	1 (16.7)
Kampong Speu	2	16	6 (37.5)	3 (50.0)	0	0	3 (50.0)	3.5	11 (68.8)	6 (100)	0	0	5 (83.3)
Kandal	2	16	6 (37.5)	1 (16.7)	1 (50.0)	2 (100)	2 (33.3)	107.5	3 (18.8)	3 (50.0)	0	0	0
Kampong Chhnang	2	16	9 (56.3)	4 (66.7)	2 (100)	0	3 (50.0)	51.5	10 (62.5)	3 (50.0)	2 (100)	1 (50.0)	4 (66.7)
Oddor Mean Chey	2	16	7 (43.8)	3 (50.0)	0	1 (50.0)	3 (50.0)	1.28	0	0	0	0	0
Koh Kong	2	16	0	0	0	0	0	0	3 (18.8)	2 (33.3)	0	0	1 (16.7)
Paillin	2	16	5 (31.3)	3 (50.0)	1 (50.0)	1 (50.0)	0	4.4	4 (25.0)	2 (33.3)	0	0	2 (33.3)
Bantheay Mean Chey	2	16	2 (12.5)	0	1 (50.0)	1 (50.0)	0	0.29	4 (25.0)	2 (33.3)	1 (50.0)	0	1 (16.7)
Pursat	2	16	5 (31.3)	1 (16.7)	2 (100)	1 (50.0)	1 (16.7)	8.6	2 (12.5)	2 (33.3)	0	0	0
Prey Veng	2	16	6 (37.5)	1 (16.7)	0	1 (50.0)	4 (66.7)	1.3	4 (25.0)	4 (66.7)	0	0	0
Svay Rieng	2	16	3 (18.8)	1 (16.7)	1 (50.0)	0	1 (16.7)	15.0	9 (56.3)	3 (50.0)	0	1 (50.0)	4 (66.7)
Mundulkiri	2	16	13 (81.3)	5 (83.3)	2 (100)	2 (100)	4 (66.7)	2.6	6 (37.5)	2 (33.3)	0	1 (50.0)	3 (50.0)
Ratanakiri	2	16	7 (43.8)	4 (66.7)	0	0	3 (50.0)	2.0	5 (31.3)	2 (33.3)	0	0	3 (50.0)
Steung Treng	2	16	4 (25.0)	1 (16.7)	0	0	3 (50.0)	10.1	8 (50.0)	3 (50.0)	1 (50.0)	1 (50.0)	3 (50.0)
Kratie	2	16	6 (37.5)	2 (33.3)	0	1 (50.0)	3 (50.0)	5.2	8 (50.0)	3 (50.0)	1 (50.0)	1 (50.0)	3 (50.0)
Kampong Thom	2	16	8 (50.0)	3 (50.0)	2 (100)	0	3 (50.0)	106.1	0	0	0	0	0
Preah Vihear	2	16	11 (68.8)	5 (83.3)	2 (100)	1 (50.0)	3 (50.0)	76.6	3 (18.8)	1 (16.7)	1 (50.0)	0	1 (16.7)
<b>Total <sup>3</sup></b>	<b>52</b>	<b>496</b>	<b>214 (43.1)</b>	<b>84 (45.2)</b>	<b>26 (41.9)</b>	<b>19 (30.6)</b>	<b>85 (45.7)</b>	<b>23.2</b>	<b>155 (31.3)</b>	<b>78 (41.9)</b>	<b>12 (19.4)</b>	<b>7 (11.3)</b>	<b>58 (31.2)</b>

<sup>1</sup> Three markets were included in Phnom Penh (PP) and Siem Reap (SR), regarded as having the highest population, while two were included in the other 23 provinces. <sup>2</sup> The total number of each specimen was different in Phnom Penh and Siem Reap (18 chicken, 6 chicken cutting boards, 18 pork, and 6 pork cutting boards); Battambang (BB) and Preah Sihanouk (PSH) (12 chicken, 4 chicken cutting boards, 12 pork, 4 pork cutting boards), compared to other provinces (6 chicken, 2 chicken cutting boards, 6 pork, 2 pork cutting boards). <sup>3</sup> The total 496 samples included the 80 repeated samples of the 4 provinces/municipalities (PP, SR, BB, PSH) and excluded 36 samples from supermarkets. <sup>4</sup> Samples with MPN/g < 0.3, negative with *Salmonella* spp. were counted as 0, and not included in the average. MPN/g > 110 was assigned randomly between 111 and 250 MPN/g for the calculation.



**Table 3.** The prevalence of *Salmonella* spp. and *S. aureus* in chicken, pork, cutting board pork and cutting board chicken from traditional markets, supermarkets, and variation within one year.

Market Types	Total Positive Sample	Chicken (No. of Positive (%))	Chicken Cutting Board (No. of Positive (%))	Pork (No. of Positive (%))	Pork Cutting Board (No. of Positive (%))	p-Value <sup>4</sup>
<b>Traditional Market</b>						
<b>Dry season <sup>1</sup> (n = 416)</b>		n = 156	n = 52	n = 156	n = 52	
<i>Salmonella</i> spp. & <i>S. aureus</i>	68	32 (20.5)	5 (9.6)	30 (19.2)	1 (1.9)	0.006
<i>Salmonella</i> spp.	169	63 (40.4)	22 (42.3)	70 (44.9)	14 (26.9)	0.150
<i>S. aureus</i>	144	72 (46.2)	11(21.2)	54 (34.6)	7 (13.5)	<0.001
<b>Wet season <sup>2</sup> (n = 80)</b>		n = 30	n = 10	n = 30	n = 10	
<i>Salmonella</i> spp. & <i>S. aureus</i>	9	6 (20.0)	0	3 (10.0)	0	-
<i>Salmonella</i> spp.	45	21 (70.0)	4 (40.0)	15 (50.0)	5 (50.0)	-
<i>S. aureus</i>	10	6 (20.0)	0	4 (13.3)	0	-
<b>Supermarkets <sup>3</sup> (n = 36)</b>		n = 18	-	n = 18	-	
<i>Salmonella</i> spp. & <i>S. aureus</i>	1	0	-	1 (5.6)	-	-
<i>Salmonella</i> spp.	10	3 (16.7)	-	7 (38.9)	-	-
<i>S. aureus</i>	1	0	-	1 (5.6)	-	-
<b>Overall (n = 532)</b>		n = 204	n = 62	n = 204	n = 62	
<i>Salmonella</i> spp. & <i>S. aureus</i>	78	38 (18.6)	5 (8.1)	34 (16.7)	1 (1.6)	0.166
<i>Salmonella</i> spp.	224	87 (42.6)	26 (41.9)	92 (45.1)	19 (30.6)	0.249
<i>S. aureus</i>	155/532	78 (38.2)	11 (17.7)	59 (28.9)	7 (11.3)	<0.001

<sup>1</sup> The samples were from 2 markets in each of 23 provinces and 3 markets in Phnom Penh and Siem Reap. <sup>2</sup> The 80 repeated samples in the wet season were only from 4 provinces/municipalities, including Phnom Penh, Siem Reap, Battambang and Preah Shihanouk. <sup>3</sup> The samples were from 4 supermarkets in Phnom Penh and 2 supermarkets in Siem Reap and collected only in the dry season. <sup>4</sup> Chi-square test.

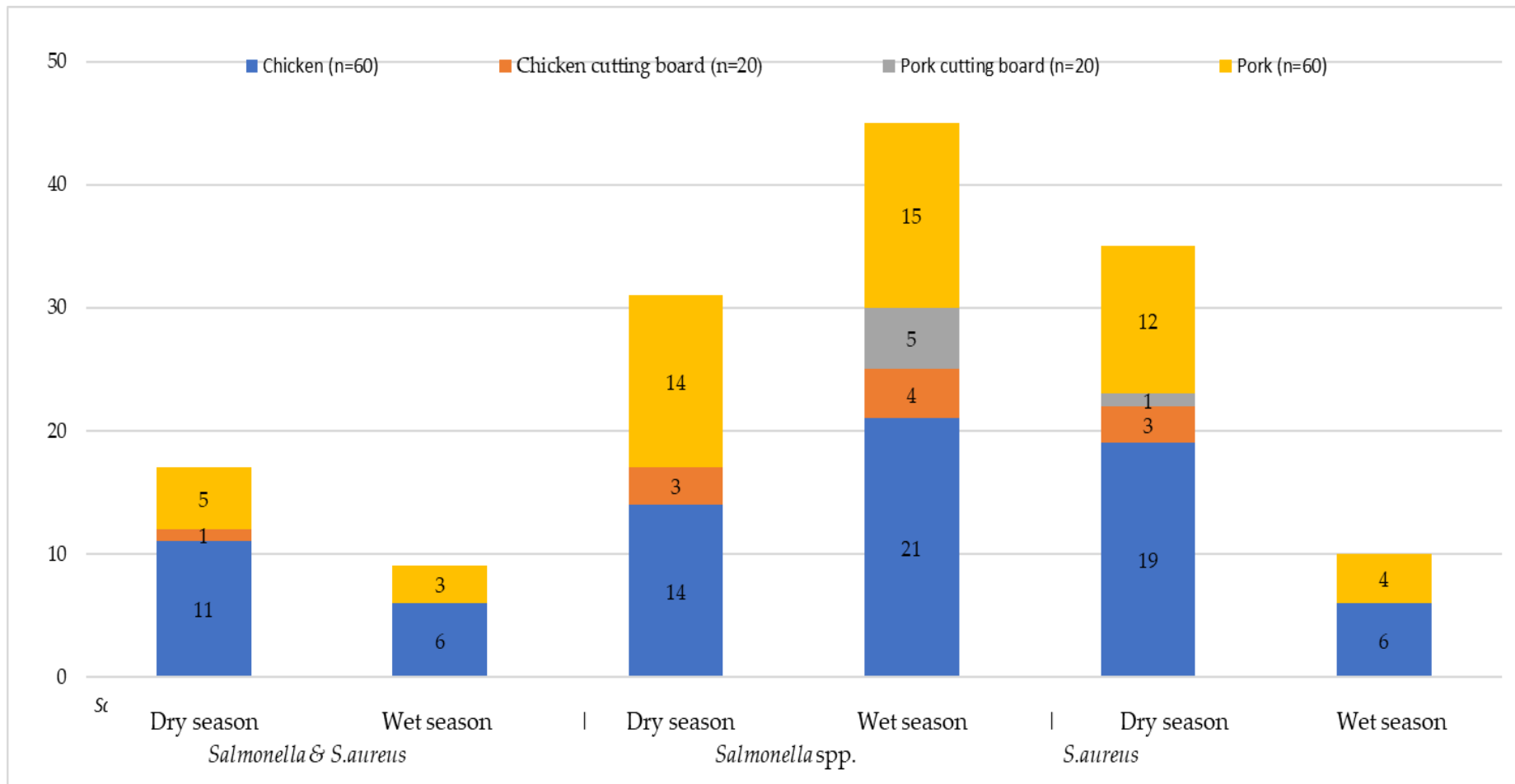
In total, **30.6 to 45.1%** of the samples were positive for *Salmonella* spp. and **11.3 to 38.2%** were positive for *S. aureus*.

The prevalence of both bacteria in meat samples (chicken and pork) was significantly higher than that on cutting boards used for chicken and pork (*p*-value < 0.001).





The prevalence of *Salmonella* spp. increased during the wet season, while the prevalence of *S. aureus* was the opposite





**Table 4.** Multivariable logistic regression of *Salmonella* spp. and *S. aureus* contamination and co-contamination in samples from Cambodian markets

Pathogens	Variables	Odds Ratio	95% CI	Coefficient	S.E.	p-Value
<i>Salmonella</i> spp. & <i>Staphylococcus aureus</i>	Species (chicken compared to pork)	1.28	0.78–2.1	0.25	0.25	0.32
	Sample (meat compared to cutting board)	4.66	1.97–11.03	1.54	0.44	<0.001
	Market type (supermarket compared to traditional market)	0.11	0.01–0.84	–2.18	1.02	0.034
	Season (dry compared to wet season)	0.64	0.3–1.36	–0.45	0.38	0.24
	Constant			–3.05	0.44	<0.001
<i>Salmonella</i> spp.	Species (chicken compared to pork)	1.03	0.72–1.46	0.03	0.18	0.86
	Sample (meat compared to cutting board)	1.47	0.96–2.24	0.38	0.22	0.07
	Market type (supermarket compared to traditional market)	0.51	0.24–1.1	–0.67	0.39	0.09
	Season (wet compared to dry season)	1.89	1.16–3.06	0.63	0.25	0.01
	Constant			–0.69	0.21	0.001
<i>Staphylococcus aureus</i>	Species (chicken compared to pork)	1.60	1.07–2.37	0.47	0.2	0.021
	Sample (meat compared to cutting board)	3.55	2.05–6.15	1.27	0.28	<0.001
	Market type (supermarket compared to traditional market)	0.04	0.01–0.3	–3.2	1.02	0.002
	Season (wet compared to dry season)	0.26	0.12–0.51	–1.37	0.36	<0.001
	Constant			–1.89	0.28	<0.001

The prevalence of *Salmonella* was not significantly different between these two market types ( $p$ -value = 0.09).

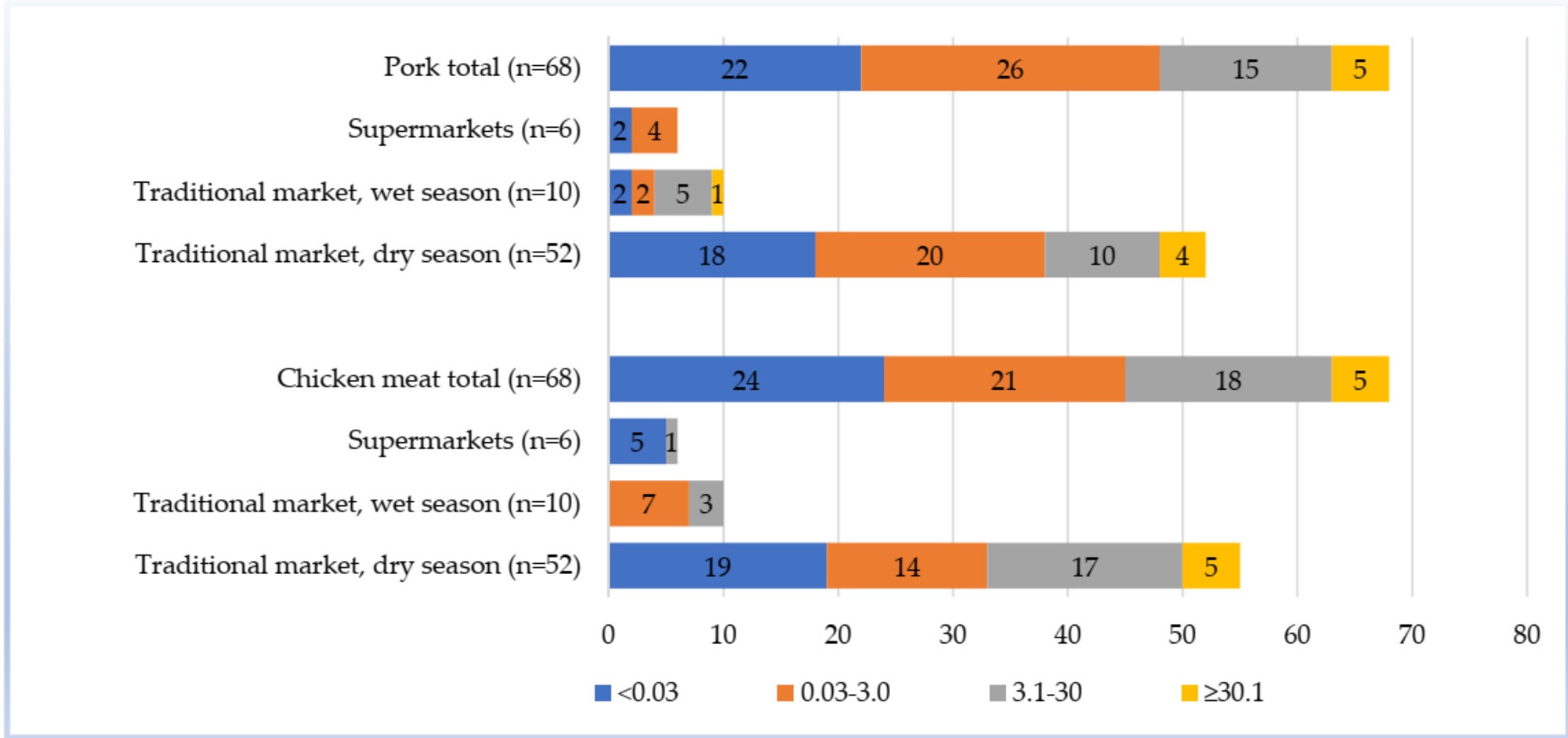
High prevalence in traditional markets regarding of both *Salmonella* spp. and *S. aureus* ( $p$ -value = 0.034) and with only *S. aureus* ( $p$ -value = 0.002).

The prevalence of *S. aureus* was significantly higher ( $p$ -value < 0.001) in meat samples than in cutting boards



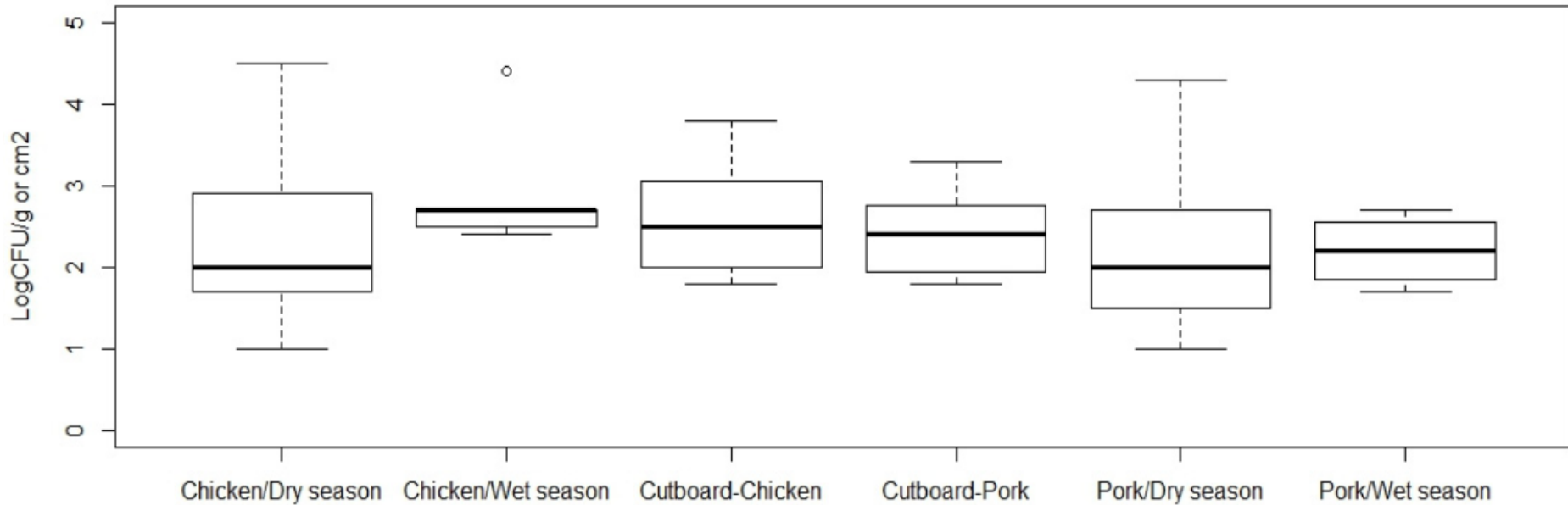
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**Figure 2.** Frequency of *Salmonella* spp. most probable number (MPN/g) ranges in meat samples (n = 136) collected from Cambodian markets.





**Figure 3.** Contamination of coagulase-positive staphylococci (Log CFU/g or cm<sup>2</sup>) in samples collected from Cambodian traditional markets in dry and wet seasons. Cutting board samples in chicken and pork shops were only collected in the dry season.

An average Log CFU/g of CPS from chicken meat and pork samples was higher in wet season compared to dry season, 2.3 (SD 1.0) versus and 2.8 (SD 0.7) in chicken, and 2.1 (SD 0.9) versus 2.2 (SD 0.4) in pork.



**Table 5.** Variables associated with Log CFU/g of coagulase-positive staphylococci in samples collected from Cambodian markets.

Variable	Coefficient	95% Confidence Interval	Std Error	p-Value
Market type (supermarket compared to traditional market)	-1.054	-1.471--0.638	0.212	<0.001
Meat type (chicken compared to pork)	0.250	0.044-0.456	0.105	0.017
Sample type (meat compared to cutting board)	0.648	0.402-0.894	0.125	<0.001
Season (dry compared to wet)	-0.590	-0.880--0.300	0.147	<0.001
Constant	0.927	0.516-1.338	0.209	<0.001

Results from linear regression showed that the CPS contamination in meat in supermarkets was lower than in traditional markets ( $p$ -value < 0.001; Table 5).

Regarding meat types, the load of CPS in chicken was significantly higher than in pork ( $p$ -value = 0.017), whereas the load of CPS in meat was significantly higher than in cutting board ( $p$ -value < 0.001, Table 5).



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## Conclusions

- In conclusion, this study found a high prevalence of both *Salmonella* spp. and *S. aureus* in chicken meat and pork samples, which could cause serious FBD in humans.
- Vulnerable people who consume fresh chicken meat and pork purchased from the traditional market might be at risk of contracting FBD.
- The pathogens may exist and contribute to common foodborne illness in Cambodia, with limited of reports accessed.







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## Recommendations

- Interventions to improve hygienic standards in Cambodian markets are strongly recommended on the traditional market from provinces at higher prevalence of *Salmonella* spp. and/or *S. aureus*.
- The further studies were suggested on how *Salmonella* spp. and/or *S. aureus* could cross-contaminated to ready-to eat food or any common food in Cambodian household.





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## pathogens

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### Prevalence of *Salmonella* spp. and *Staphylococcus aureus* in Chicken Meat and Pork from Cambodian Markets

Chea Rortana; Hung Nguyen-Viet; Sothya Tum; Fred Unger; Sofia Boqvist; Sinh Dang-Xuan; Sok Koam; Delia Grace; Kristina Osbjer; Theng Heng; Seng Sarim; Or Phirum; Roern Sophia; Johanna F. Lindahl

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### Prevalence of *Salmonella* and *Staphylococcus aureus* in meat in Cambodian markets

Chea Rortana<sup>1</sup>, Delia Grace<sup>2</sup>, Hung Nguyen-Viet<sup>3</sup>, Sothya Tum<sup>4</sup>, Fred Unger<sup>5</sup>, Sofia Boqvist<sup>6</sup>, Sinh Dang-Xuan<sup>7</sup>, Sok Koam<sup>8</sup>, Kristina Osbjer<sup>9</sup>, Theng Heng<sup>10</sup>, Seng Sarim<sup>11</sup>, Or Phirum<sup>12</sup>, Roern Sophia<sup>13</sup>, Johanna F. Lindahl<sup>14</sup>

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#### Introduction

Fresh meat can be contaminated with microorganisms during harvest, slaughter or processing and handling (Xuan et al., 2019). Foodborne diseases are important in low-middle-income countries, because of their high health burden and huge economic cost (Baird, 2015).

#### Objectives

- To assess the prevalence of *Salmonella* and *S. aureus* in animal source foods (chicken and pork) sold at Cambodian traditional markets
- Quantify *Salmonella* in the collected specimen.



Fig. 1: Sampling locations in Cambodia.

Materials and methods  
Sampling was conducted ambulatorily from retail markets for pork and chicken meat in 23 provinces/municipal of Cambodia between October 2018 and August 2019, including repeat sampling in wet season in 4 provinces (Choeung Chh, Sihanoukville, Kampong Speu and Kampong Speu) after approximately 6 months. The 200 specimens were collected aseptically in retail and market at about 10-15 km of each day: chicken meat (n=100), chicken cutting board (n=50), pork (n=50) and pork cutting board (n=50).

All specimens were tested for presence of *Salmonella* (SI) (SI-045-2002) and *Staphylococcus aureus* (SI) (SI-005-2002), while one third of specimens were tested for most probable number (MPN) *Salmonella* using traditional procedure (Datta et al., 2013).



Fig. 2: Retail market in Kampong Speu, chicken and pork meat for sale for *Salmonella* and *S. aureus* detection.

#### Results

Among the total isolates, the prevalence of *Salmonella* was 43.1% (chicken 45.2%, cuttingboard pork 30.6% and pork 45.7%).

The prevalence of *S. aureus* was 31.3% in all sample (chicken 41.9%, cuttingboard chicken 13.4%, cuttingboard pork 11.3% and pork 31.2%). Majority of specimen showed MPN less than 30 as presented in Table 1. According to Table 1 and Table 4, the prevalence of *Salmonella* trend higher than *S. aureus* which mean, the higher risk of *Salmonella* among chicken and pork in Cambodian traditional markets.

Table 1: Prevalence of *Salmonella* and *S. aureus* in chicken, pork, chicken and cutting board meat, in 14 provinces/municipal of Cambodia.

Sample type	Specimen	No. positive item		<i>Salmonella</i> positive		<i>S. aureus</i> positive	
		Number	%	Number	%	Number	%
Chicken	138	62	44.9	75	54.4	45	32.6
Chicken cutting board	107	44	40.9	16	14.9	11	10.3
Chicken pork	67	31	46.3	19	28.4	7	10.4
Pork	136	62	45.6	45	33.1	52	38.2
<b>Grand Total</b>	<b>456</b>	<b>199</b>	<b>43.7</b>	<b>115</b>	<b>25.2</b>	<b>115</b>	<b>25.2</b>

Table 2: The MPN profiles of *Salmonella* from the 114 tested samples.

Sample types	<0.5	0.5-1.0	1.1-2.0	2.1-2.9	3.0-10	>10
Chicken	62	21	27	2	3	0
Pork	76	32	5	1	4	0



#### Prevalence of *Salmonella* and *Staphylococcus aureus* in meat in Cambodian markets

Chea Rortana, Delia Grace, Hung Nguyen-Viet, Sothya Tum, Fred Unger, Sofia Boqvist, Sinh Dang-Xuan, Sok Koam, Kristina Osbjer, Theng Heng, Seng Sarim, Or Phirum, Roern Sophia, Johanna F. Lindahl

#### Introduction

Foodborne diseases represent a global public health problem because of their high burden and huge economic cost.

Food safety and food security are important for the development of a country.

Here we assessed the prevalence of *Salmonella* and *Staphylococcus aureus* in meat and pork sold at Cambodian traditional markets.

#### Methods

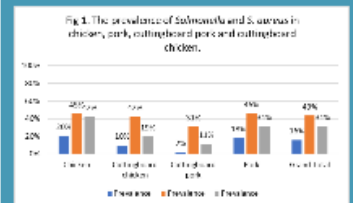
Sampling was conducted ambulatorily from retail markets for pork and chicken meat in 23 provinces/municipal of Cambodia between October 2018 and August 2019, including repeat sampling in wet season in 4 provinces (Choeung Chh, Sihanoukville, Kampong Speu and Kampong Speu) after approximately 6 months.

The 456 specimens were collected aseptically in retail and market at about 10-15 km of each day: chicken meat (n=100), chicken cutting board (n=50), pork (n=50) and pork cutting board (n=50).

All specimens were tested for presence of *Salmonella* and *S. aureus*.

A nationwide multi-hazard survey in markets in Cambodia found the prevalence in meat (pork and chicken) of *Salmonella* was 43% and of *Staphylococcus* was 31%.

The prevalence of *Salmonella* found in chicken 45.2%, cutting board of chicken 41.9%, pork 45.7% and cutting board of pork 31.2%. The prevalence of *S. aureus* found in chicken 41.9%, cutting board of chicken 13.4%, pork 45.7% and cutting board of pork 10.6%.



#### Results

Fig. 1: The prevalence of *Salmonella* and *S. aureus* in chicken, pork, cutting board of chicken and cutting board of pork.

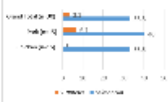


Table 1: Prevalence of *Salmonella* and *S. aureus* in chicken, pork, chicken and cutting board meat, in 14 provinces/municipal of Cambodia.

Sample type	Specimen	No. positive item		<i>Salmonella</i> positive		<i>S. aureus</i> positive	
		Number	%	Number	%	Number	%
Chicken	138	62	44.9	75	54.4	45	32.6
Chicken cutting board	107	44	40.9	16	14.9	11	10.3
Chicken pork	67	31	46.3	19	28.4	7	10.4
Pork	136	62	45.6	45	33.1	52	38.2
<b>Grand Total</b>	<b>456</b>	<b>199</b>	<b>43.7</b>	<b>115</b>	<b>25.2</b>	<b>115</b>	<b>25.2</b>

#### Recommendations

- The study found that 43.7% of specimen were contaminated by *Salmonella* and 25.2% by *S. aureus* in meat and pork.

#### Research gaps or future opportunities

- The study was a cross-sectional study, so we cannot determine the prevalence of *Salmonella* and *S. aureus* in meat and pork over time.



# Thank you for your attention



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