

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

### SAFE FOOD, FAIR FOOD FOR CAMBODIA PROJECT Rortana Chea National of Animal Health and Production Research Institute (NAHPRI)

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### 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),
- Annul 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.



















### **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 supermarketsin 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
Total specimen	204	62	62	204
		Total spec	imen = 532	

<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,









# 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.

		Total	Total		Number of S	almonella Positi	ve Samples (	%)	Ν	Number of S. a	<i>ureus</i> Positive	Samples (%)	
Provinces/Municipalities	Markets <sup>1</sup>	Sample Collected <sup>2</sup>	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)		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)
Total <sup>3</sup>	52	496	214 (43.1)	84 (45.2)	26 (41.9)	19 (30.6)	85 (45.7)	23.2	155 (31.3)	78 (41.9)	12 (19.4)	7 (11.3)	58 (31.2)

<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 (%))	<i>p</i> -Value <sup>4</sup>
Traditional Market						
Dry season $^1$ (n = 416)		n = 156	n = 52	n = 156	n = 52	
Salmonella spp. & S. aureus	68	32 (20.5)	5 (9.6)	30 (19.2)	1 (1.9)	0.006
Salmonella spp.	169	63 (40.4)	22 (42.3)	70 (44.9)	14 (26.9)	0.150
S. aureus	144	72 (46.2)	11(21.2)	54 (34.6)	7 (13.5)	< 0.001
Wet season $^2$ (n = 80)		n = 30	n = 10	n = 30	n = 10	
Salmonella spp. & S. aureus	9	6 (20.0)	0	3 (10.0)	0	-
Salmonella spp.	45	21 (70.0)	4 (40.0)	15 (50.0)	5 (50.0)	-
S. aureus	10	6 (20.0)	0	4 (13.3)	0	-
Supermarkets <sup>3</sup> (n = 36)		n = 18	-	n = 18		
Salmonella spp. & S. aureus	1	0	-	1 (5.6)	-	-
Salmonella spp.	10	3 (16.7)	-	7 (38.9)	-	-
S. aureus	1	0	-	1 (5.6)	-	-
Overall (n = 532)		n = 204	n = 62	n = 204	n = 62	
Salmonella spp. & S. aureus	78	38 (18.6)	5 (8.1)	34 (16.7)	1 (1.6)	0.166
Salmonella spp.	224	87 (42.6)	26 (41.9)	92 (45.1)	19 (30.6)	0.249
S. aureus	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, Battambong 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 contaminationand co-contamination in samples from Cambodian markets

Pathogens	Variables	Odds Ratio	95% CI	Coefficient	S.E.	<i>p</i> -Value
	Species (chicken compared to pork)	1.28	0.78-2.1	0.25	0.25	0.32
Calmonalla area C	Sample (meat compared to cutting board)	4.66	1.97-11.03	1.54	0.44	< 0.001
Saimonella spp. &	Market type (supermarket compared to traditional market)	0.11	0.01-0.84	-2.18	1.02	0.034
Suphylococcus uureus	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
	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
Salmonella spp.	Market type (supermarket compared to traditional market)	0.51	0.24-1.1	-0.67	0.39	0.09
11	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
	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
Staphylococcus aureus	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





**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	<i>p</i> -Value
Market type (supermarket compared to traditional market)	-1.054	-1.4710.638	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) Constant	-0.590 0.927	-0.8800.300 0.516-1.338	0.147 0.209	<0.001 <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).







### **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.







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

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

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### Methods

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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%.

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### FEEDIFUTURE

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### Introduction

Fresh meat can be contaminated with microonganisms 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 (Grace, 2015)

### Objectives

1. To assess the prevalence of Salmonella and S. aureus in animalsource foods (chicken and pork) sold

at Cambodian traditional markets

2. Quantify Solmonella in the collected specimen



### Materials and methods

Sampling was conducted probabilistically from retail markets for pork and chicken mea in 25 provinces/municipal of Cambodia between October 2018 and August 2019, including repeat sampling in wet season in 4 provinces (Phnom Penh, Shanoukylle Battambang and Siem Reap) after approximately 5 months the 456 specimens were collected aseptically in retail wet market at about 9-11 am of

each day: chicken meat (n=185), chicken ratting board (n=62), park (n=186) and park cutting board (h=62). All spectreers were tested for presence of Salmooella (ISO SD29-1\_2002).

Waphylamicrus aurous (ISO 66881-1-1999), while one third of specimen were tested for most probable number (MPN) Solmonvilla using traditional procedure (Pasic et al., 2010).



Fig.2: Traditional market in Kampong Cham, children and park must up for participated in interview and empling meat and cutting board

45.2%, cuttingboard chicken 41.9%, cuttingboard park 30.6% and park

The prevalance of 5. oureus was 31.3% in all sample (chicken 41.9%, cuttingboard chicken 19.4%, cuttingbord pork 11.3% and pork 31.2%). Majority of specimen showed MPN less than 30 as presented in Table 1. According to Table 3 and Table 4, the prevalance of Salmonello trend higher than S. oureus which mean, the higher risk of Solmonello among chicken and pork in Cambodian tradiational markets

Table 3: Provalence of Solveose/Iz and S. ources in chicken pork, chicken and cutting board

Sample type	N. Specimen	N. positive both Solmoselic and S. consur	Salmonella positivo	S. awate positive
Chicken	150	38 (20.4%)	54 (4s.2%)	78 (41.9%)
Cuttingheard chicken	67	5 (9.7%)	26 (41.9%)	12 (19, 6%)
Cuttingboard park	62	1 (1.6%)	19 (30.75)	7 (11 3%)
Pork	1.86	33 (17.7%)	85 (45.7%)	58 (81.2%)
Grand Total	406	75 [15.7%]	214 43.1%	135 (51.3%)

### 0.3-3.0 3.1-30.0 30.1-110 Sample types 10.5











Results



Steung Treng Bratie Kampong Ther Conclusions

This study has detected two major foodborne bacteria, among the most common causes of foodborne illness in Cambodia. This indicates the importance of food safety and improved hygiene for public health.

Table 3. Number of positive Solmonei/o from difference sampling seasons, where first

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Table 4. Positive samples collected in all different province Number of

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This study is made possible by the generous support of the American people through the United Vates Agency for International Development (USAID) and its Leed the Lutur Innovation Lab for Evestock Systems managed by the University of Florida and the International Investock Research Institute. The contents are the responsibility of the presenter and do not necessarily reflect the views of USND or the United States

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Fig.3: Becterial isolation-35g mean + 335 mill of PRW wa

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### Thank you for your attention



# FEEDIFUTURE

The U.S. Government's Global Hunger & Food Security Initiative

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