

## **4. RESULTS**

Results were divided into 3 parts in relation to the objectives of this study as follows:

### **4.1 Investigation on porcine cysticercosis**

The first objective of this study was to establish the current prevalence status of porcine cysticercosis in slaughter pigs, which came from different areas of Cambodia, and to conduct studies on predilection sites as well as the microscopic examination of cysts for confirmation of cysticercus species.

#### **4.1.1 Slaughterhouse survey results**

Visits were made to 4 slaughterhouses in Phnom Penh during 2004-2005 where a total of 432 pigs were examined. Among them, 220 were local pigs and 212 were commercial pigs. They came from 29 districts in 9 provinces and from 3 intensive farms.

##### **4.1.1.1 Distribution of prevalence for porcine cysticercosis by breeds of slaughter pigs in Cambodia**

In 4 slaughterhouses, 220 local pigs were examined by tongue palpation. After slaughter, all 432 pigs were examined for the presence of cysticerci by meat inspection. The results are summarized in Table 4. It can be seen that ante mortem (tongue) inspection yielded 29 positive pigs out of 220 local pigs examined (13.2%), while the number of positive cases increases to 47 pigs (21%) by meat inspection. None of the pigs from intensive production farms was positive.

Table 4. Distribution of prevalence for porcine cysticercosis by breeds of slaughter pigs in Phnom Penh/ Cambodia, 2004-2005

Type of pigs	No. pigs	Tongue palpation		Meat inspection	
		No. examined	No. positive (%)	No. examined	No. positive (%)
Local	220	220	29(13.2%)	220	47 (21%)
Commercial	212	0		212	0 (0%)

#### 4.1.1.2 Distribution of prevalence of porcine cysticercosis by slaughterhouses

The distribution of the prevalence of porcine cysticercosis by slaughterhouses is showed in Table 5. The overall prevalence of porcine cysticercosis in slaughterhouses was 6.7% by tongue palpation and 10.9% by meat inspection. Moreover, there were no significant differences (KW= 3.06, df=3, p=0.382) among slaughterhouse prevalence obtained by tongue palpation. Similarly, it was not significant (H= 4.27, df=3, p=0.233) among slaughterhouse prevalence by meat inspection.

Table 5. Prevalence of porcine cysticercosis by four slaughterhouses in Phnom Penh/ Cambodia, 2004-2005

Sl.*** code	Total examined	Tongue palpation			Meat inspection		
		No. of positive	Preval* (%)	95% CI**	No. of positive	Preval* (%)	95% CI**
1	108	11	10.18	5.19-17.5	17	15.74	9.44-24.0
2	108	7	6.48	2.64-12.9	12	11.11	5.87-18.6
3	108	6	5.55	2.06-11.7	8	7.40	3.25-14.07
4	108	5	4.62	1.52-10.5	10	9.25	4.52-16.36
Total	432	29	6.71	4.54-9.48	47	10.87	8.10-17.2

\*Prevalence, \*\* Confidence interval, \*\*\* Slaughterhouse

The comparison between tongue palpation and overall meat inspection gave a Kappa statistic of 0.717 (71.7%). Thus, it showed substantial agreement (0.6-0.8; Dohoo *et al.*, 2003) between tongue and meat inspection. This Kappa value was statistically significantly better than that expected (0.06) due to chance. However, the confidence interval was wide, reflecting considerable uncertainty about the estimate.

#### 4.1.1.3 Distribution of prevalence for porcine cysticercosis by farm types

Table 6 shows that the prevalence of porcine cysticercosis as determined by tongue palpation was 16.1% and 25.5% by meat inspection in carcasses of outdoor-reared pigs, while it was 8.43 % by tongue palpation and 14.45 % by meat inspection in carcasses of pigs raised in mixed types of farms. Between farm-type specific prevalences, by procedure of inspection, showed significant differences by tongue palpation (Kruskal Wallis:  $H = 34.75$ ,  $df = 4$ ,  $p = 0.0001$ ) and by meat inspection (Kruskal Wallis:  $H = 57.37$ ,  $df = 4$ ,  $p = 0.0001$ ). None of the indoor-raised pigs and their carcasses was found infected.

Table 6. Distribution of prevalence for porcine cysticercosis by farm types, Cambodia, 2004-2005

Types of farms	Total examined	Tongue palpation			Meat inspection		
		No. of positive	Preval* (%)	95% CI**	No. of positive	Preval* (%)	95% CI**
Outdoor	137	22	16.05	10.34-23.3	35	25.54	18.5-33.7
Mixed	83	7	8.43	3.45-16.6	12	14.45	7.69-23.93
Intensive	212	0	0	0	0	0	0

\* Prevalence, \*\* confidence interval

#### 4.1.1.4 Distribution of prevalence of porcine cysticercosis by districts of provinces

The detailed distribution of the prevalence of porcine cysticercosis was arranged by districts where pigs were. Positive cases were from 19 out of 29 districts as summarized in Table 7.

Table 7. Distribution of prevalence for porcine cysticercosis by districts/Cambodia, 2004-2005

Districts	Total examined	Tongue palpation			Meat inspection		
		No. of positive	Preval* (%)	95% CI**	No. of positive	Preval* (%)	95% CI**
1	13	1	7.69	0.19-36	4	30.76	9.09-61.44
2	8	2	25	3.18-65	2	25	3.18-65
3	10	1	10	0.25-44.5	3	30	6.67-65.24
6	7	3	42.85	9.89-81.59	3	42.85	9.89-81.59
7	7	1	14.28	0.36-57.87	2	28.57	3.66-70.95
8	7	1	14.28	0.36-57.87	2	28.57	3.66-70.95
9	10	2	20	2.52-55.6	4	40	12.15-73.76
10	9	3	33.33	7.48-70	3	33.33	7.48-70
11	10	3	30	6.67-65.24	4	40	12.15-73.76
12	19	3	15.78	3.38-39.57	7	36.84	16.28-61.64
13	22	2	9.09	1.12-29.16	2	9	1.12-29.16
15	6	1	16.66	0.42-64.12	2	33.33	4.32-77.72
16	7	0	0	0	1	14.28	0.36-57.87
17	3	1	33.33	0.84-90.57	1	33.33	0.84-90.57
18	8	2	25	3.18-65	2	25	3.18-65
20	2	1	50	12.57-98.74	1	50	1.25-98.74
22	4	0	0	0	1	25	0.63-80.58
27	5	1	20	0.5-71.64	2	40	5.27-85.33
28	9	1	11.11	0.28-48.24	1	11.11	0.28-48.24

\* Prevalence, \*\* confidence interval

This table shows prevalences of porcine cysticercosis obtained by both tongue palpation and meat inspection. A prevalence range of 7.7% to 50% in 19 districts was recorded by tongue palpation and 9% to 50 % by meat inspection. Methodologically, the district-specific prevalences were compared and the outcomes showed that they were significantly (Kruskal Wallis:  $H= 80.93$ ,  $df=31$ ,  $P=0.0001$ ) different by tongue palpation and by meat inspection (Kruskal Wallis:  $H= 105.30$ ,  $df=31$ ,  $P=0.0001$ ).

#### 4.1.1.5 Distribution of prevalence of porcine cysticercosis by provinces

The Distribution of prevalence of porcine cysticercosis by provinces is shown in Table 8.

Table 8. Distribution of prevalence for porcine cysticercosis by provinces/ Cambodia

Province codes	Total examined	Tongue palpation			Meat inspection		
		No. of positive	Preval* (%)	95% CI**	No. of positive	Preval* (%)	95% CI**
1	80	4	5	1.37-12.30	9	11.25	5.27-20.28
2	33	8	24.24	11.0-42.25	12	36.36	20.4-54.87
3	58	10	17.24	8.59-29.42	15	25.86	15.25-39.0
4	20	2	10	1.23-31.69	4	20	5.73-43.66
5	11	2	18.18	2.28-51.77	2	18.18	2.28-51.77
6	27	1	3.7	0.09-18.97	2	7.40	0.91-24.28
7	164	0	0	0	0	0	0
8	20	1	5	0.12-24.87	2	10	1.23-31.69
9	19	1	5.26	0.13-26.0	1	5.26	0.13-26.0

\*Prevalence, \*\* confidence interval

It can be seen that infections were distributed in pigs from 8 provinces. Pigs from province 7 were negative by both tongue palpation and meat inspection. The highest prevalence was among pigs from province 2, which was 24.2%, and the lowest

prevalence was in province 6, which was 3.7%. The comparison of prevalence among nine provinces showed significant ( $H=14.81$ ,  $p=0.038$ ) results obtained by tongue palpation as well as meat inspection ( $H=17.82$ ,  $p=0.021$ ).

#### 4.1.1.6 Predilection sites of cysts for porcine cysticercosis by meat inspection.

To find out which organ of pigs was the predilection for cysticerci, each organ or muscle of infected pig carcasses was sliced, and the degree of infection was recorded as negative, low and high (Appendix 3). Results of organ involvement (%) in infected pigs are summarized in Table 9.

Table 9. Organ and muscle involvement (%) of cysts in slaughter pigs in Phnom Penh / Cambodia, 2004-2005 (n= 47)

Muscles/ Organs	Total of positive by organs	Degree of infection					
		Low*			High**		
		No.	%	95%CI	No.	%	95%CI
Tongue	31	19	61.3	42.18-78.15	12	38.7	21.84-57.81
Esophagus	37	28	75.7	58.8-88.22	9	24.3	11.77-41.19
Heart	47	27	57.4	42.17-71.74	20	42.6	28.25-57.82
Diaphragm	47	29	61.7	46.37-75.49	18	38.3	24.5-53.62
Brain	3	3	100	29.24-100	0	0	0
External masseter	44	13	29.5	16.76-45.2	31	70.5	54.79-83.23
Internal masseter	42	12	28.6	15.71-44.58	30	71.4	55.41-84.28
Triceps brachii	47	19	40.4	26.36-55.73	28	59.6	44.26-73.63
Fore limb	44	6	13.6	5.27-27.35	38	86.4	72.64-94.82
Hind limb	45	4	8.9	24.75-21.22	41	91.1	78.77-97.52

\*  $\leq 1-100$  cysts per organ, \*\*  $>100$  cysts per organ



This table shows percent levels of infections categorized by either a low or high degree of infections from the examined muscles or organs as follows: brain, esophagus, diaphragm, tongue, heart, triceps brachii, external masseter, internal masseter, fore limb and hind limb muscles, respectively. The organs as lungs, spleen, intestine, kidney, liver were not detected to harbor cysticerci and, in a very few case, they were found in the brain.

#### 4.1.2 Morphology of *T. solium* cysticerci hooks

In order to confirm the species of cysticerci, 235 cysts randomly were selected (5 cysts per sample) from tongue, esophagus, heart, muscle and others and examined microscopically for the presence of scolices and hooks. When present, the size of hooks was measured under the microscope at 100 and 400 magnifications and multiplied by 2.4 to yield micrometer ( $\mu\text{m}$ ). The data are summarized in Table 10. Two hundred and thirty five (235) cysts were collected from 47 infected carcasses during meat inspection. Based on scolices and morphology of hooklets, all 235 cysts were identified as cysticerci of *Taenia solium*. The large rostellum hooks had a mean of 139  $\mu\text{m}$  and a range of 120-156  $\mu\text{m}$ , whereas the small rostellum hooks had a mean of 94  $\mu\text{m}$  and a range of 84-108  $\mu\text{m}$ .

Table 10. Morphology of *T. solium* cysticerci hooks obtained from pig carcasses

Type of hooks	Mean ( $\mu\text{m}$ )	SD* ( $\mu\text{m}$ )	Range of hook ( $\mu\text{m}$ )
Large rostellum hooks	139	12	120-156 (50-65)**
Small rostellum hooks	94	9	84-108 (35-45)

\*Standard deviation, \*\* = Size was measured under 400 magnifications.

## 4.2 Investigation on trichinellosis

The second objective of this study was to establish the current seroprevalence status of trichinellosis in slaughter pigs from different areas of Cambodia. The 440 pigs examined were from 9 provinces, 29 districts and from the only three intensive (commercial) farms. Of these pigs, 224 pigs were of local breeds and 216 were commercial (exotic/crossbreeds) pigs.

### 4.2.1 Distribution of sero-positive samples for trichinellosis by breeds of slaughter pigs

Pig sera were tested for antibodies against *T. spiralis*. The results are presented in Table 11. This table shows that by AB-ELISA test and endpoint titration, 5 sera out of 440 serum samples positive for *Trichinella* antibodies. Two positive serum samples were from outdoor-raised pigs, 1 from mixed-raised pigs and 2 from indoor-raised pigs. Overall, five serum samples produced doubtful results.

Table 11. Distribution of sero-positive samples (by AB-ELISA) for trichinellosis by breeds of slaughter pigs in Cambodia, 2004-2005

Breeds of pigs	Total of samples	Serological examination			
		AB-ELISA		Endpoint titration	
		No. positive	No. doubtful	No. positive	No. doubtful
Local breeds (native)	224	3	3	3	3
Commercial breeds	216	2	2	2	2
Total	440	5	5	5	5



#### 4.2.2 Summary results of AB-ELISA reading of *Trichinella* of slaughter pigs

Ten sera, 5 positive and 5 doubtful were re-examined by AB-ELISA. The results are shown in Table 12. This table shows that in the first AB-ELISA two samples were positive and 8 samples gave doubtful results (+/-). Of the two positive samples, one was from indoor-farmed pigs and the other from outdoor-raised pigs. The first of the two positive samples had  $OD^{a1} = 0.403$  and an  $Index^{a1}$  (cut-off) = 34% while, the second one had  $OD^{a8} = 0.352$  and an  $Index^{a8}$  (cutoff) = 30%. The second AB-ELISA revealed three more positive sera with the following readings:  $OD^{b6} = 0.232$ ,  $Index^{b6} = 22\%$ ,  $OD^{b7} = 0.225$ ,  $Index^{b7} = 21\%$ ,  $OD^{b9} = 0.275$  and  $Index^{b9} = 25.6\%$ . In this second test five serum samples gave doubtful results.

#### 4.2.3 Endpoint titration of serum samples positive by single dilution AB-ELISA.

The positive samples were tested by endpoint titration for confirmation and the results of the test are shown in Table 13. This table shows that the five samples were confirmed to be positive by endpoint titration.

#### 4.2.4 Sero-prevalence of trichinellosis of slaughter pigs by husbandry and geographical distribution

Distribution of sero-positive pigs and prevalence of sero-positivity are shown in Table 14. Table 14 shows that the overall sero-prevalence of trichinellosis in slaughtered pigs was found 1.13%. One or two positive pigs were found in each slaughterhouse.

By farm type, 1.40% of outdoor-raised pigs, 1.21% of mixed-reared and 0.92% of indoor-reared pigs were positive, respectively. The comparison between farm-types specific sero-prevalence, by AB-ELISA, showed no significant differences (Kruskal

Wallis:  $H = 7.16$ ,  $df = 4$ ,  $p = 0.127$ ). Doubtful test results were 0.70% in outdoor-raised pigs, 2.43% in mixed-reared and 0.9% in indoor-raised farms.

Positive pigs were from four out of 29 district and 3 intensive farms. Sero-positive rates ranged from 2.66 to 16.66%. The district-specific prevalences were compared and the outcomes showed that they were not significantly (Kruskal Wallis:  $H = 10.97$ ,  $df = 31$ ,  $P = 0.268$ ) different by AB-ELISA.

From 9 provinces, sero-positive pigs were distributed in 4 provinces. The percentage of positive ranged from 1.19% in province 9 to 5.26% in province 7. The doubtful test results ranged from 1.19% of pigs from province 7 to 5% in province 4. The comparison of prevalence among 9 provinces showed no significant (Kruskal Wallis:  $H = 14.81$ ,  $df = 8$ ,  $p = 0.592$ ) results obtain by AB-ELISA.

Table 12. Summary results of AB-ELISA reading of *Trichinella* of slaughter pigs (n=440)

Specimens No.	First test							Second test			
	Slaughterhouse ID	Farm ID	Breed	Age month	OD <sup>a</sup> *	Index <sup>a</sup> (cut- off) (%)	Results	OD <sup>b</sup> *	Index <sup>b</sup> (cut- off) (%)	Border- line titer	Results
1	1	indoor	exotic	6	0.403	34	++++	-	-	-	-
2	4	mixed	local	6	0.164	13.7	+/-	0.115	11	1: 160	+/-
3	1	outdoor	local	7	0.105	8.8	+/-	0.141	13	1:160	+/-
4	2	indoor	exotic	6	0.272	17	+/-	0.112	10	1:80	+/-
5	2	indoor	exotic	6	0.227	14	+/-	0.140	12	1:80	+/-
6	3	outdoor	local	7	0.243	15	+/-	0.232	22	1:160	+++
7	4	mixed	local	6	0.175	11	+/-	0.225	21	1:320	+++
8	3	outdoor	local	7	0.352	30	+++	-	-	-	-
9	3	indoor	exotic	6	0.135	12	+/-	0.275	25.6	1:160	+++
10	3	mixed	local	6	0.263	14.8	+/-	0.143	12.9	1:80	+/-

\*Optical density.

OD<sup>a</sup>\* = Optical density of the first AB-ELISA, OD<sup>b</sup>\* = Optical density of the second AB-ELISA.Index<sup>a</sup> = cut off of point of the first AB-ELISA, Index<sup>b</sup> = cut off of point of the second AB-ELISA.

Table 13. Endpoint titration of *Trichinella* positives of slaughter pigs by single dilution AB-ELISA

Specimens No.	ELISA index % (1:10)	Border titer	Result
1	83	1:640	+++
6	78	1:160	++
7	76	1:320	++
8	73	1:320	++
9	81	1:320	+++

Table 14. Sero-prevalence of trichinellosis of slaughter pigs by husbandry and geographical distribution in Cambodia, 2004-2005

Items	Total examined	AB-ELISA				
		No. positive	Preval* (%)	95% CI**	No. doubtful	Doubtful (%)
At farm types						
Outdoor	142	2	1.40	0.17-4.99	1	0.70
Mixed	82	1	1.21	0.03-6.60	2	2.43
Indoor	216	2	0.92	0.11-3.30	2	0.9
At district level						
District 1	13	0	0	0	1	7.69
District 5	6	1	16.66	0.42-64.12	0	0
District 11	10	1	10	0.25-44.50	1	10
District 16	7	0	0	0	1	14.28
District 29	10	1	10	0.25-44.50	0	0
District 31	75	2	2.66	0.32-9.30	2	2.66
At province level						
Province 1	80	1	1.25	0.03-6.76	1	1.25
Province 3	62	1	1.61	0.04-8.66	1	1.61

Table 14 (Continued). Sero-prevalence of trichinellosis of slaughter pigs by husbandry and geographical distribution in Cambodia, 2004-2005

Items	Total examined	AB-ELISA				
		No. positive	Preval* (%)	95% CI**	No. doubtful	Doubtful (%)
Province 4	20	0	0	0	1	5
Province 7	168	2	1.19	0.14-4.23	2	1.19
Province 9	19	1	5.26	0.13-26.02	0	0
Total	440	5	1.13	0.36-2.63	5	1.13

\* Prevalence \*\* Confidence interval

### 4.3 Results from the questionnaire surveys

The questionnaire surveys were administered to interviewees in three sites, namely four slaughterhouses, farms in three provinces and four hospitals around Phnom Penh city. All were conveniently selected. A summary of the responses is as follows:

#### 4.3.1 Responses of slaughterhouse questionnaire survey

The responses are summarized in Appendix 4. Twenty-three percent of respondents were in slaughterhouse 1, 24% in slaughterhouse 2, 29% in slaughterhouse 3 and 24 % in slaughterhouse 4. Sixty percent of slaughterhouse workers had a low level of education. Eighty nine percent of respondents said that demand of pork in Phnom Penh was higher (average 500-2000 slaughter pigs per day) than that of other meats such as beef, fish and seafood. They indicated that the demand fluctuates (increase or decrease) by seasons. All (100%) the responses indicated that the environmental and hygienic conditions were poor. Most (40%) of the pigs delivered to the slaughterhouses were raised in a household environment while, 33% and 27% were produced at small and medium farms (intensive production farms) respectively.

Of all the interviewees, 36 % and 61% of respondents had not heard about porcine cysticercosis and trichinellosis, respectively. Thus, only 57% of porcine carcasses were inspected for cysticercosis using tongue palpation (13%), by meat inspection (31%) and by using both (tongue palpation and meat inspection) (56%). The answers further showed that 63 % of respondents found parasitic diseases, mainly porcine cysticercosis. Respondents indicated that they detected 1-3 infected carcasses per month. They (74 %) speculated that the infected carcasses were from pigs that were raised extensively. Concerning trichinellosis, they have never inspected pigs for the infection.

Respondents observed infected carcasses irrespective of age, i.e., young or old as either having heavy (57 %) or light (43 %) infection. Twenty two percent of the heavily infected carcasses were destroyed whereas, 64% were processed for animal feeds or for human consumption in special institutions and only 14 % (lightly infected cases) went straight for human consumption. Most people, 57%, prefer well-cooked food for consumption, while the remainders ate uncooked or both cooked and uncooked foods.

#### 4.3.2 Responses to farm questionnaire survey

The responses are summarized in Appendix 5. Thirty percent of respondents were in province 1, 34% in province 2 and 36% in province 3. The appendix shows that seventy nine percent of farmers had a low level of education. Most (85%) of the pigs in these areas were reared in the household environment, while 15% were produced in the small and medium farms (intensive production farms). The stages in pig production were as follows: fattening 45%, piglets (31%) and both (24%) stages. These pigs were sold depending on family needs and income. For example, 32 % of pigs were at the age of 2-4 months, 22% at 6 months, 36 % at 7-12 months and 10 % at more than 12 months. But, it is worth noting that sixty five percent of the farms brought in piglets from outside. The pig breeds commonly found in the study provinces were local pigs (native) (89%), while 11% of the respondents reared exotic and cross breeds.



Fifty four percent of the respondents used traditional feeds, 17% used leftovers, 5% used formula feeds and 24% used mixed rations. In addition, 55 % of respondents produced their own feeds, while 45% of them bought from outside. Water sources for both humans and pigs were as follows: 66% from ponds, underground and 17% from river waters (17%). Ninety three percent of respondents indicated that they used the water from its sources directly without treatment.

Only 4% of respondents had concrete floors in pens. All the responses indicated that the environmental conditions and waste management were poor. Most (83%) of the people in the rural areas encountered parasitic diseases. Of all the interviewees, 40%, 54% and 83% had not heard about porcine cysticercosis, *Taenia solium* and trichinellosis, respectively. Of all live pigs, only 66% were inspected for cysticercosis using tongue palpation and the rest (34%) were examined using both (tongue palpation and meat inspection). Respondents (28%) indicated that they detected 1-3 infected pigs per month, while 18% reported more than 5 infected pigs per month. Most (61%) of respondents sent infected pigs for proper processing and only 14% referred infected pigs directly for human consumption. Proper food cooking was indicated by 59% of the respondents while, 35% preferred uncooked pork and 6% well cooked or mixed. In addition, 84% of respondents said that there were not enough communal toilets. Seventy nine percent of respondents stated that control and prevention were still very poor and still not applied to these areas due to underdeveloped public health infrastructures within the country.

#### 4.3.3 Responses to hospital questionnaire survey

Health personnel in two hospitals, one public health institute and the Pasteur Institute in Phnom Penh, Cambodia, were visited and questionnaires administered to them. In all these institutions, there were no records of human neurocysticercosis and medical staff had very little knowledge of neurocysticercosis. None of the medical centres visited had the capacity for diagnosing neurocysticercosis.