4. RESULTS

4.1 Characteristics of the slaughtered pigs

A total of 576 pigs were selected. Meat and serum samples were collected from 487 of those pigs whereas from 64 of those pigs only meat and from 25 of those pigs only blood samples were collected. The highest number of samples was collected from the Kathmandu valley (184), followed by Rauthat (98), Chitwan (92), Kavre (85), Pokhara (42) Dhading (40), and far-western (35). Based on the geographical location, 45.31% (261/576) were collected from the mountain region (Kathmandu, Pokhara and far-western), whereas 32.98% (190/576) were collected from the plain region (Rauthat and Chitwan) and 21.70% (125/576) from the hilly region (Kavre and Dhading) of Nepal.

Pigs selected for this study had characteristics of 4 different states of the farms: 26% of the pigs were from commercial farms, 17.2% from semi-commercial farms, 37.2% from scavenging and 19.6% from household raised pigs. The pigs from all three types *i.e.* indoor (44.4%), outdoor (37.8%) and mixed (17.7%) system of rearing were recorded. The breed characteristics of the sampled pigs were local 56.9% (Hurra, Bampudake, Wild), exotic 26.6% (Landrace, Yorkshire, Hampshire) and Pakharibas cross 16.5% (Dharane kalo). Regarding gender the pigs were 59% males and 41% females. Regarding the age, 37.8% samples were from pigs below 1 year of age, 56.1% pigs were between 1-2 years of age and 6.1% pigs were more than 2 years of age. It was found that both immature (<5 month) and senile (>3 years) pigs were slaughtered.

4.2 *Trichinella* investigation through Pepsin digestion

The meat samples from a total of 551 pigs were analyzed through the Pepsin digestion technique. Out of that 90 individual meat samples were also analyzed

through compressorium. *Trichinella* larvae were not found in any of those pork samples.

4.3 *Trichinella* investigation through indirect ELISA serology

A total of 344 randomly selected sera were tested for antibodies against *Trichinella spiralis*. Out of 344 sera, only 320 sera had corresponding meat samples that were already analyzed through the Pepsin digestion which was negative. All sera were tested by AB-ELISA. The ELISA OD indices of the 344 sera were shown graphically in appendix C. It was found that 14 samples were doubtful (12 ≤ ELISA-index <18) and 2 samples were positive (≥ 18 ELISA index). The detailed information regarding such doubtful and positive sera is presented in table 2. The clusture column comparing ELISA OD values with ELISA indices (appendix C), it was found that ELISA OD value of serum samples had positive correlation with ELISA index. The trend of the serum samples showed that if ELISA OD value of serum sample was more than 0.23 then the ELISA index can be expected arithmatically more than 12 which means either it was doubtful or positive through AB-ELISA.

These doubtful and positive samples were tested again for end-point titer single dilution AB-ELISA; from that all samples had ELISA index less than 70%, and border of titre less than 1:80. Based on the test evaluation criteria all serum samples failed to show antibodies against *Trichinella spiralis*, as summarized in table 3.

The precision of the ELISA-test was determined by testing at different times the same positive or negative control sera. The percentage of the coefficient of variation (%CV) was calculated which was used for assessing the reproducibility of the test according to Mahannop *et al.* (1995). The result showed that coefficient of variation of method increased with concentration of the sample. It means that if the test sera have higher concentration of the antibodies against *Trichinella spiralis* then the variation is higher in result output. The reproducibility of positive and negative control sera used for AB-ELISA test is shown in table 4.

Table 2: Results of the AB-ELISA of serum samples from pigs of different regions, breeds and management systems of Nepal

Sample no.	Site of collection	Status of pig			ELISA index (%)	Results
		Breed	Age/Sex	Rearing		
82	Kavre	Black Cross	3 Year Female	Commercial Indoor	14.05	Doubtful
92	Kathmandu	Local	2 Year Male	Commercial Indoor	16.84	Doubtful
178	Chitwan	Exotic	1 Year Male	Semi-comm Indoor	19.43	Positive
191	Chitwan	Exotic	3 Year Female	Semi-comm Indoor	13.44	Doubtful
202	Chitwan	Exotic	16 month Female	Commercial Mixed	13.79	Doubtful
213	Rauthat	Local	14 month Female	Scavenging Outdoor	17.75	Doubtful
264	Kathmandu	Exotic	1 Year Female	Household Indoor	15.60	Doubtful
269	Kathmandu	Black Cross	18 month Female	Commercial Indoor	17.15	Doubtful
376	Kavre	Black Cross	3 Year Female	Scavenging Outdoor	13.96	Doubtful
378	Kathmandu	Local	11 month Female	Commercial Indoor	12.58	Doubtful
443	Pokhara	Local	3 Year Female	Commercial Indoor	15.34	Doubtful
535	Far-western	Local	5 month Male	Scavenging Outdoor	17.13	Doubtful
540	Far-western	Local	1 Year Male	Scavenging Outdoor	17.20	Doubtful
542	Far-western	Local	1 Year Male	Scavenging Outdoor	12.58	Doubtful
551	Far-western	Local	5 month Female	Semi-comm Mixed	12.50	Doubtful
565	Far-western	Local	2 year Male	Semi-comm Mixed	18.78	Positive

Semi-comm = Semi-commercial

Table 3: ELISA indices and titration observations of serum samples from pigs of different regions, breeds and management systems of Nepal that were positive and doubtful through AB-ELISA

Sample no.	IgG 1:10 ELISA %	Border of titer	Result
82	19.71	1:10	Negative
92	21.10	1:10	Negative
178	34.61	1:20	Negative
191	27.17	1:10	Negative
202	22.34	1:10	Negative
213	24.11	1:10	Negative
264	49.97	1:40	Negative
269	48.15	1:40	Negative
376	39.26	1:40	Negative
378	32.59	1:20	Negative
443	54.57	1:40	Negative
535	34.02	1:20	Negative
540	46.64	1:40	Negative
542	21.96	1:10	Negative
551	35.45	1:20	Negative
565	47.19	1:40	Negative

Table 4: The reproducibility test of the *Trichinella* of control sera used for AB-ELISA

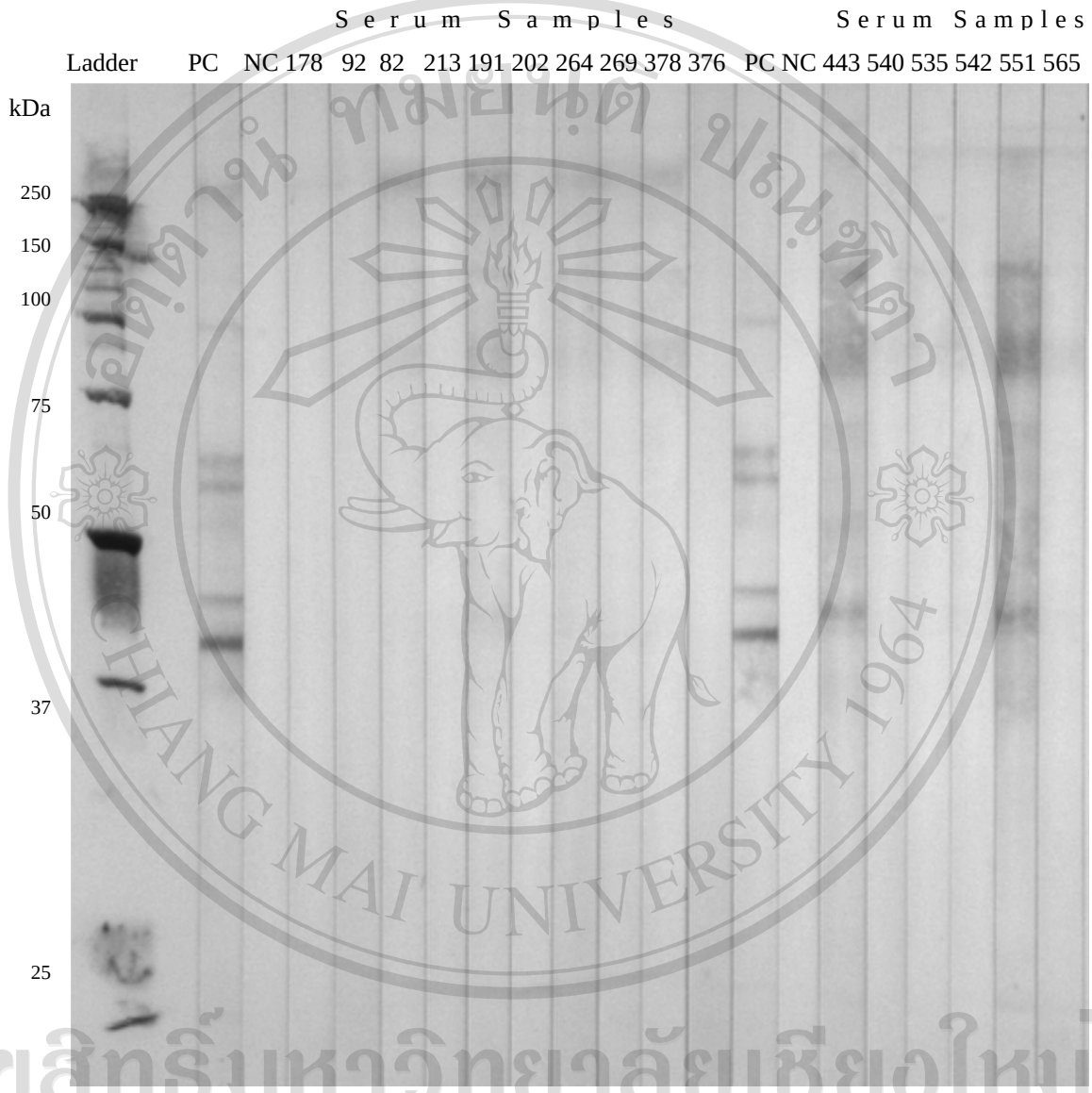
Control sera	1st	2nd	3rd	4th	5th	Mean (x)	S.D	%CV= (S.D/x)*100
Positive	1.127	2.203	1.253	1.537	1.921	1.6145	0.477066	29.54%
	0.945	2.295	1.255	1.415	2.074			
	0.942	2.214	1.202	1.449	1.974			
					2.026			
Negative	0.106	0.103	0.115	0.101	0.072	0.09736	0.014143	14.52%
	0.096	0.096	0.087	0.083	0.117			
	0.109	0.111	0.083	0.084	0.124			
	0.091	0.103	0.086	0.083				

S.D = Standard deviation

4.4 *Trichinella* investigation through western blot

16 serum samples were sent to the BfR for confirmatory diagnosis of trichinellosis. The molecular sizes of the bands were evaluated by a comparison with a molecular size ladder. The ladder used for comparison had well-recognized molecular weight patterns of 25, 37, 50, 75, 100, 150 and 250 kDa. Sera from animals with parasitic infections other than trichinellosis would produce very different patterns of molecular weight. The tested sera had not shown specific band on its ladder, as shown in figure 5.

The comprehensive details of each sample specific and non-specific bands patterns were summarized in appendix D. Based on the evaluation criteria, it had found that none of the sample had shown two types of specific bands on its ladder. This means all the tested sera samples were negative for *Trichinella* genotype through western blot. Thus it was concluded that all serum samples which were doubtful and positive through AB-ELISA were actually true negative samples.



PC = Positive control

NC = Negative control

kDa = Molecular weight of ladder in kilodalton

Figure 5: Western blot analyses of sera from pigs that were positive and doubtful through AB-ELISA

4.5 Possible maximum prevalence of *Trichinella*

It was found that from the all tested meat samples of pig that there was no positive result for *Trichinella* by the Pepsin digestion method. However firstly through AB-ELISA of randomly selected sera, 2 samples were positive and 14 were doubtful, but the confirmation of these samples through end-point titration single dilution ELISA and western blot had revealed that all samples were true negative for *Trichinella* spp. In that aspect the following mentioned formula was used based on win Episcope 2.0 to estimate the maximum number of possible positive animals in the central development region of Nepal.

$$D = [1- (1 – CL)^{1/n}] * [N – (n-1)/2]$$

Where, D = The maximum number of *Trichinella* positive animals

CL = Confidence limit as a fraction

n = Samples size that showed negative

N = Total number of pigs in central development region

$$D = [1-(1 – 0.95)^{1/576}] * [172949 – (576-1)/2]$$

$$= 896 \text{ Pigs}$$

Possible maximum prevalence = (896 / 172949)* 100 = 0.52% at 95% confidence interval and with sampling fraction of 0.33%.

4.6 Pig producer questionnaire survey

52.5% of the respondents had a farm in the urban area and were under 40 years of age. Most of the farms (60%) have no employee *i.e.* the owner and his/her family are involved in the pig husbandry. The surveyed farmers have low pig holding capacity (62.5%) and more precisely 77.5%, 97.5% and 52.5% have kept only 1-20 sows, boars and piglets, respectively. This questionnaire surveys had found that at the farms the *Trichinella* control measures were very negligible, as shown in table 5.

Table 5: Status of pig farms with hygienic measures for *Trichinella* control in Nepal (n = 40)

No.	Factors to be considered	Frequency	Percentage
1	Rodent control program exists in the farm	12	30%
2	Architectural barrier in the farm for wild life	29	72.5%
3	Feed containing leftovers (hotel, home)	26	65%
4	Waste food containing meat products was cooked to inactivate *Trichinella*	0	0%
5	Garbage dumping in the vicinity	33	82.5%
6	Bird access from dumping area to the farm	17	42.5%
7	Direct outlet of farm wastes due to lack of facility	33	82.5%
8	Information about pork diseases	18	45%
9	Awareness regarding *Trichinella* control	0	0%