CHAPTER II

RESEARCH DESIGNS AND METHODS

This study combines a field and a laboratory-component. The investigations were designed to determine the proportions of *Salmonella*-infected pigs at farm levels, the rate of pre-slaughter infections and the contamination rate of slaughter carcasses and to identify the predominant *Salmonella* serogroups involved. Results further served to analyze the level of agreement between bacteriological and serological results and to provide an assessment of risk factors for infection of pigs with *Salmonella.*

Study subjects

The study was executed on groups of pigs' destinated for slaughter. Slaughter pigs were regarded as "related" by origin, when they came from the same farm, and/or were purchased from several farms and delivered by one trader to the slaughterhouse. From each pig 1 fecal sample, 1 lymph node sample, 2 surface swabs (for bacteriological investigation) and 1 serum sample and meat sample (for serological investigation) were taken (Figure 6).

Testing :

The microbiological investigation followed the guidelines given in the ISO 6579 (1993) for the detection of *Salmonella* bacteria. Figure 7 summarizes the diagnostic protocol followed.

Pre-enrichment

Inoculum: 25 g sample in 225 ml buffered peptone water (1%)

Incubation: 37 °C, 16-20 hrs

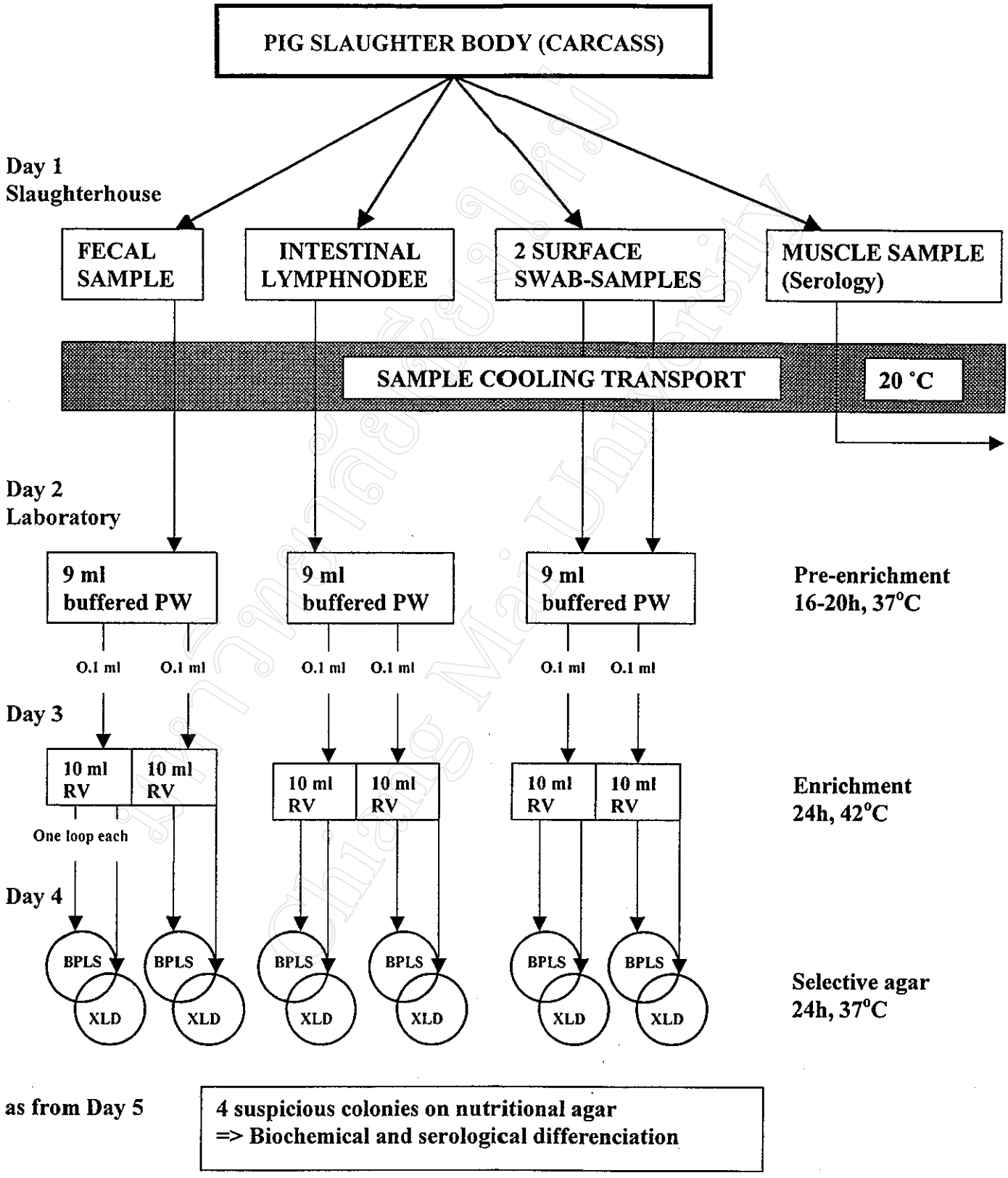

Figure 6. Flow diagram of investigation of samples for the prevalence of *Salmonella* in pigs

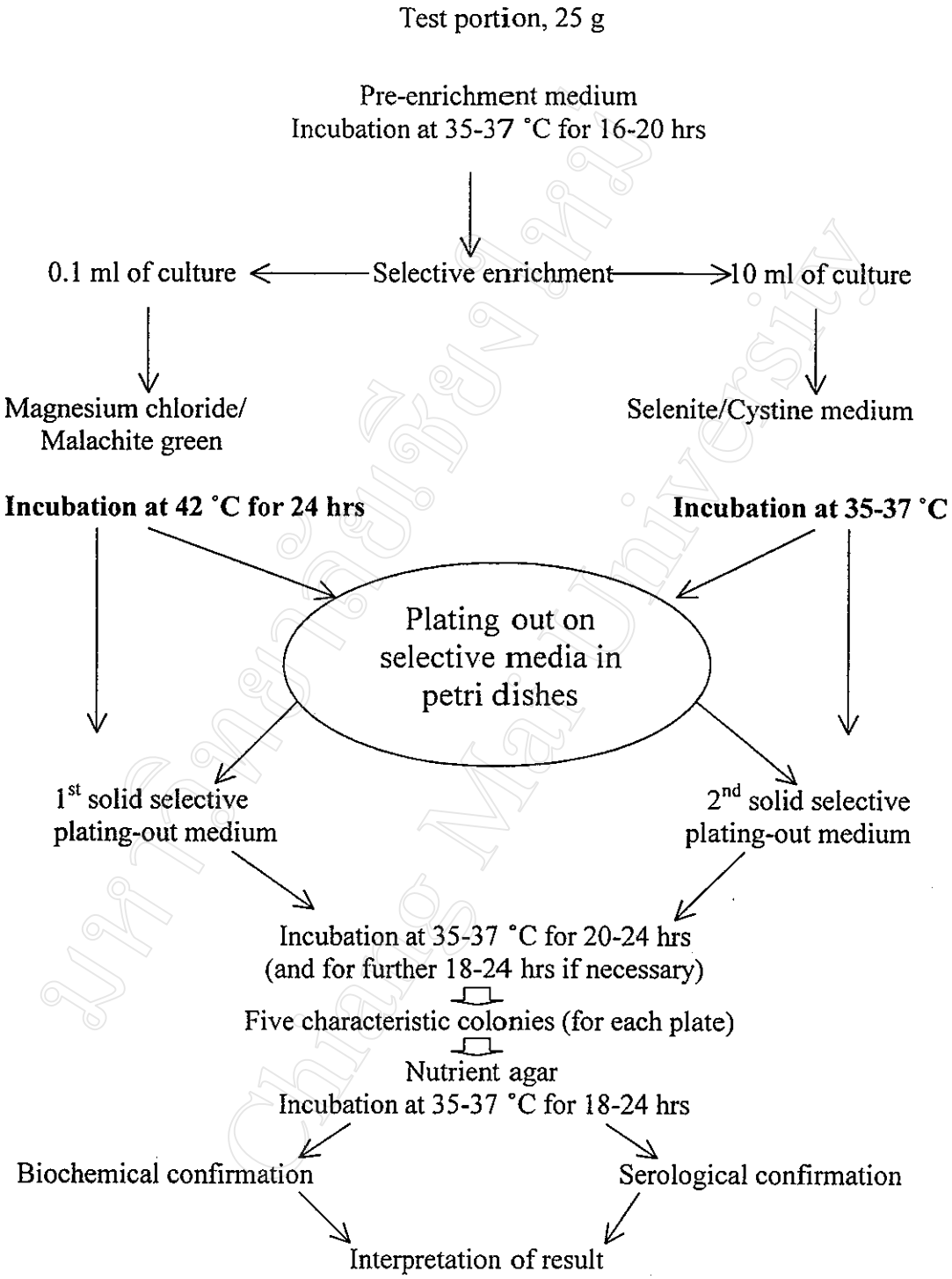

Figure 7. Bacteriological procedure for *Salmonella* detection (ISO 6579)

Selective enrichment

Inoculum: 0.1 ml pre-enrichment to 10 ml Rappaport-Vasiliadis medium

Incubation: 42 °C, 24 hrs

Isolation

Solid medium with brilliant-green

Inoculum : Loop strike from each selective enrichment media on BPLS-agar

Incubation: 37 °C, 24-48 hrs

Solid medium without brilliant-green

Inoculum: Loop strike from each selective enrichment media on XLD-agar

Incubation: 37 °C, 18-24 hrs

Confirmation

Selective of five characteristic colonies from each selective media

Pure culture

Inoculum: Loop strike on nutrient agar of the selected colonies

Incubation: 37 °C, 18-24 hrs

Biochemical confirmation

1. Glucose (+ gas formation) : positive
2. Lactose : negative
3. Sucrose : negative
4. Hydrogen sulfide : positive
5. Urea spliting : negative
6. Lysine decarboxylation : positive
7. β-Galactosidase reaction : negative
8. Voges-Proskauer reaction : negative
9. Indole : negative

Serological confirmation

Aggutination with

1. *Salmonella* polyvalent somatic (O) antiserum: A-E
2. *Salmonella* polyvalent somatic (O) antiserum: F-67
3. *Salmonella* somatic group (O) antiserum : B (O 4,5,27)
4. *Salmonella* somatic group (O) antiserum : C (O 6,7,8,20)
5. *Salmonella* somatic group (O) antiserum : D (O 9,46,Vi)
6. *Salmonella* somatic group (O) antiserum : E (O 3,10,15,19,34)

Samples:

Fecal sample

2 possible techniques:

-Swab sample out of the cut posterior portion of intestine (rectum)

-Swab sample above anus if carcass is elevated

Lymphnode

Several, if possible posterior located jejunum-lyphnodes. For sampling, the lymphnode was harvested by hand and placed in plastic bag.

Serum sample

V. Jugulars (pre-slaughter) or heart puncture (at slaughter)

Meat sample

Sample of a piece of meat of the diaphragm (at least the size of a walnut). Storage in clearly labeled plastic bag.

Surface sample

Per carcass 1 swab sample from the interior part of the thigh and 1 swab sample of the breast interior and exterior. Each sampled area to be 20 x 20 cm in size. Both samples will be pooled for microbiological investigation.

Slaughterhouse sampling:

Selection of two slaughterhouses in Chiang Mai province: Muang and Sansai districts (slaughter unit). For each slaughterhouse, information on the the number of pigs slaughtered in each slaughter house and the area of origin of pig herds (farms) delivered to the slaughter houses was collected prior to the investigations. This information was used to set up the sampling frame and to determine the sample size. The total of samples will be distributed proportional between the 2 slaughterhouses and proportional to the 5 slaughter-unit size strata within each slaughterhouse.

Sample size determination

95% (= 2 SE) confidence, 5% assumed prevalence (p), Standard Error (SE): 3%:

$n = p \times (1-p)/SE^2$

$n = 5 \times (100 - 5)/1.5^2$

$n = 211$

Sample period: 1 month

Samples were collected over 1 month at 4 fixed sampling days for each slaughterhouse. Selection of sample pigs per stratum (delivery farm) was done using systematic random sampling. For this, all animals per farm were marked at arrival at the slaughterhouse. For ecological reasons (effect of temperature on *Salmonella* prevalence), the investigation was repeated once in the cool and once in the hot season.

Sampling Frame

1. Muang District Slaughterhouse

Farm of origin	Pig slaughtered/night	Transportation(hrs)	Type of operation
A	25	3-4	Farrow to finishing
B	6	5-6	Farrow to finishing
C	11	0.5-1	Farrow to finishing
D	10	5-6	Farrow to finishing
E	34	1-2	Farrow to finishing
F	19	0.5-1	Farrow to finishing
G	3	1-2	Farrow to finishing
TOTAL	108		

2. Sansai District Slaughterhouse

H	64	3-4	Farrow to finishing
I	19	0.5-1	Farrow to finishing
J	27	4-5	Farrow to finishing
K	10	2-3	Farrow to finishing
Other	37	overnight	Not consistent
TOTAL	157		

Slaughter unit size strata (No pigs slaughtered/night)

1	1-10	4 Farms	= 33.3%
2	11-20	3 Farms	= 25%
3	21-30	2 Farms	= 16.7%
4	31-40	2 Farms	= 16.7%
5	> 40	1 Farm	= 8.3%

Sample distribution (1 month sampling period)

1. Chiang Mai Municipal Slaughterhouse

	Calculation base: 40% of total of 211 samples = 85 samples		
Farm size strata	Strata group	Group: % of total	Samples/month
A	3	16.7	14
B	1	33.3	14
C	2	25.0	11
D	1	33.3	10
E	4	16.7	14
F	2	25.0	11
G	1	33.3	14
			Total: 88 samples

2. Sansai District Municipal Slaughterhouse

	Calculation base: 60% of total of 211 samples = 126 samples		
H	5	8.3	11
I	2	25.0	32
J	3	16.7	21
K	1	33.3	42
Other	4	16.7	21
			Total: 127 samples

Information on farm of origin gathered on each group of slaughter pigs and information of pre-slaughter risk factors

For each study farm (farm delivering pigs for slaughter to one of the two slaughter houses), information was systematically collected on husbandry characteristics, the feeding system used and on pre-treatments (antibiotics?) of pigs. Information on pre-slaughter risk factors included time for transport from the farm of origin to the slaughterhouse and waiting period at the slaughterhouse until slaughtering. Information details are contained in Appendix 2.

Places of investigations and data collection

At the slaughter houses available in Chiang-Mai province (Muang and Sansai Slaughterhouse)

Evaluation of the Danish Mix-ELISA

The applicability of the Danish Mix-ELISA for local use was assessed the by comparing the O antigens identified by serogroup testing of samples with those contained in the Danish Mix-ELISA. Results are expressed as a proportion of positive samples that can be identified by this Mix-ELISA.

Data management and analysis

The data files were managed in SPSS (SPSS^{R} Version 10.0.1, 1999). Prior to analysis, the data files were screened for any errors. The analyses, such as the computation of sample-specific prevalences of *Salmonella* and their differences, were performed using the same computer software. Specifically, the McNemar Technique was used to determine the existence of differences of the sample-specific prevalences of *Salmonella* bacteria.