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Zoonoses and Food Hygiene News, published four times a year, provides a medium for disseminating technical information on matters related to zoonoses and food hygiene generated in the world, particularly in Nepal. The editors welcome submissions on these topics with appropriate illustrations and references. The views and opinions expressed in the News are those of the authors.

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## Population Structure of South Asian Indigenous Pigs (*Sus scrofa*) Determined by Microsatellite Markers

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## INTRODUCTION

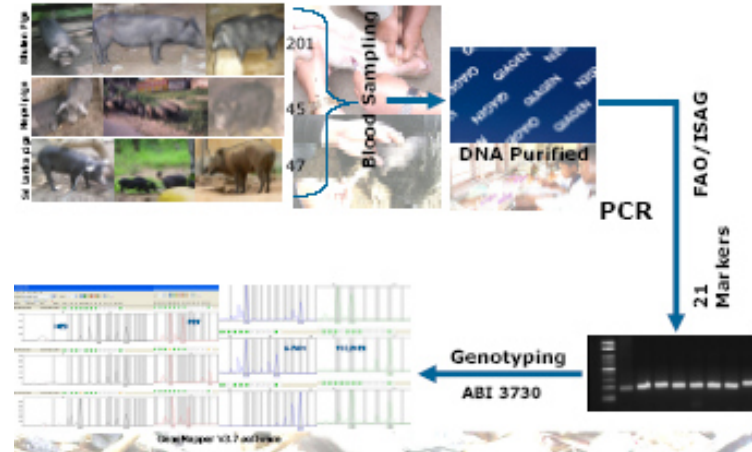
- Indigenous pigs have socio-economic, cultural and traditional, and biodiversity importance in the lives of many people around the world including Bhutan, Nepal, and Sri Lanka (South Asia).
- Improvement of pig industry in South Asia is mainly oriented towards exotic germplasm.
- Current knowledge and genetic information on indigenous pigs from the region are very limited.
- The number of indigenous domestic pigs in South Asia is declining.

## Objective

- Determine genetic diversity and structure of indigenous pigs in South Asia.

- Generate pre-requisite genetic data to devise relevant strategies for conservation of biodiversity and sustainable use of genetic resources in the region.

## MATERIAL AND METHOD



## Data Analysis

Genotypes analyzed using: Structure, GenAIEx 6.1, POPGENE, GenePop, Mega 4, and Population 1.2.30

## RESULT AND DISCUSSION

- Preliminary analysis of genetic structure reveals five populations of indigenous pigs in Bhutan, two in Nepal, and two clearly segregated populations of village and wild pigs in Sri Lanka.
- Deviation ( $P < 0.05$ ) from HWE at most loci.
- Mean expected heterozygosity ranges from 0.70 to 0.81 (SE = 0.01) for Bhutanese pigs, 0.71 and 0.74 (SE = 0.01) for Nepalese pigs, 0.66 and 0.76 (SE = 0.03) for Sri Lankan pigs, and 0.67 (SE = 0.03) for out-group.
- Phylogenetic analysis clusters all populations into three major clades according to the country of origins (Figure 1).

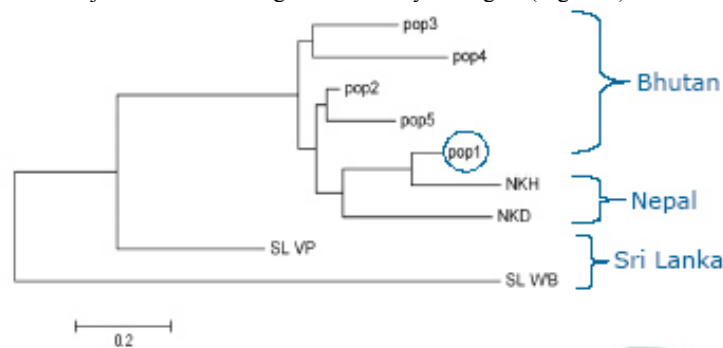


Figure 1: Neighbour-joining (Saitou & Nei, 1987) dendrogram of South Asian pigs.

- Himalayan pigs are more closely related when compared with Sri Lankan pigs.
- Bhutanese POP 1 clusters with Nepalese pigs. This is consistent with live-pig trade activity as Nepal export piglets to north-east India via which piglets are smuggled into Bhutan across Bhutan-India border.

## Prevalence of *Taenia Solium* Cysticercosis in Swine in Kathmandu Valley

Ajit Kumar Karna and Durga Datt Joshi, NZFHRC

### CONCLUSION

- Confirms rich biodiversity in domestic pig populations in south Asia and the need to manage and conserve these populations.
- Findings would be useful for conservation and sustainable utilization of porcine genetic resources in the region.

### Further Study

Mitochondrial DNA sequences are being generated from 200 of the above pigs to further examine population structure and relationships.

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### Abstract

With an increase in pig and consumption by small holder, there have been problems with zoonotic parasitic diseases especially porcine cysticercosis. A study was conducted among 200 pigs from nine different slaughter slabs from Kathmandu valley during June to August 2009. The objectives of this study were to determine prevalence of *Taenia solium* cysticercosis in swine by carcass, lingual and ELISA examination. The prevalence rate of cysticercosis by lingual examination, carcass examination and ELISA was found out to be 0.0%, 0.005% and 35.5% respectively. The collected cysts were confirmed as *Taenia solium* cyst by the histopathology and microscopic examination. Neurocysticercosis (NCC) patients were found at an overall rate of 9.8% (179) of 1839 epilepsy patients from the survey of five hospitals of Kathmandu valley viz. TUTH, Bir, Patan, Norvic and NMC. The overall age wise distributions of NCC patients were 55.63%, 24.02% and 22.35% for 15-35yrs, 0-14 yrs and above 35 yrs respectively. Sex wise distribution of NCC in male and female was 66.5% and 33.5% respectively.

**Keywords:** *Taenia solium*, Cysticercosis, Pigs, ELISA, NCC/ Epilepsy

### Introduction

It is reported that Human and Porcine taeniasis/cysticercosis is one of the major zoonotic diseases in Nepal. Estimation made by WHO, that 50 million persons primarily from budding countries are infected with taeniasis, and 50 thousands people die every year due to this disease. Some of the ethnic groups, up to 25% of the total population of Nepal, are pig farmers and pork consumers having very low sanitation and hygienic practices, and have no power over pig husbandry and slaughtering (Joshi et al., 2007). It is now emerging as a major public health problem of worldwide dimensions (Sciutto et al, 2000).

Human infection caused by adult tapeworm, belonging to the class Cestoda (Soulsby, 1982), *Taenia solium* and *Taenia saginata* (Joshi et al, 2007) and also by *Taenia asiatica* (Shulman, 1982) is referred to as taeniasis and similarly the infection of larval stage of these parasites is known as cysticercosis (Garcia et al., 2003; Joshi et al, 2007 and Ngowi et al, 2008). The infective stage of *T.solium* is *Cysticercus cellulosae* that develops in pig and adult form is an obligatory intestinal parasite for man as reported by Joshi et al, 2007.

Agrawal, (2006) reported 66 cases of Neurocysticercosis (NCC) at Neurology service T.U. Teaching Hospital. Individuals of 16-25 years of age were mostly affected. 77.2% presented with seizures of one or other type, 40.9% had weakness of the limbs and 18% presented with headache alone and 9% had signs of increased intracranial pressure. 63.6% (42 out of 66 cases) showed single ring enhancing lesion and 36.3% (24) showed multiple ring enhancing lesions. And most of the lesions were seen in parietal region (63.6%) followed by frontal (13%), temporal and occipital (9% each).

Dorny et al. (2004) in a study of 868 slaughtered pigs at Lusaka (Zambia) found the sensitivity and specificity of lingual examination, meat inspection, Ab-ELISA and Ag-ELISA as 0.210, 0.221, 0.358, 0.867 and 1.000, 1.000, 0.917 and 0.947 respectively. Souza and Hafez, (1999) reported serodiagnosis as the major reliable technique than meat inspection. ELISA detected the highest percentage of porcine cysticercosis.

Joshi et al., (2006) conducted a survey of porcine cysticercosis where by 200 pigs were subjected to lingual examination, antibody detection by ELISA and Post-mortem carcass examination with 10.5,

22.5 and 20.5% found positive, suggesting lingual examination method for detecting porcine cysticercosis is easy, inexpensive and could be utilized as a surveillance tool in developing countries like Nepal where technical resources and technological capacity are very limited.

#### OBJECTIVES

- To determine prevalence of *T. solium* cysticercosis in swine by carcass examination, lingual examination and ELISA.
- To compare the result of lingual examination, carcass examination and ELISA

#### MATERIALS AND METHODOLOGY:

##### Study Area

This study was conducted in Kathmandu valley. The Kathmandu valley consists of three districts as Kathmandu, Lalitpur and Bhaktapur, which share its boundaries with Kavrepalanchok district on east, Dhading on west, Nuwakot and Sindhupalchok districts on the north and Makwanpur on the south. This valley is located at an altitude of 1300 m from the sea level and extends about 25 km east to west and 20 km from north to south. The temperature in summer (May, June, July) ranges from 19.5°C to 28.1°C and in winter (October, Jan., February) ranges from 3°C to 19.3°C (NTB, 2009).

##### Lingual examination

The tongues of pigs after slaughtering were palpated and the tongues were sliced by a knife and the cut surfaces were examined thoroughly for the detection of any cyst if present.

##### Carcass examination

The head, carcass and viscera were thoroughly examined visually as per the OIE guideline. Meat inspection was done by visual inspection of the carcass and its cut surfaces for the detection of cyst. The muscles of diaphragm, heart, shoulder, thigh and abdomen were thoroughly examined visually; similarly the masseters and the pterygoid muscles were examined on incisions.

##### Sampling schedule

Sample was collected during the period of June to July 2009. The individual pig was the sampling unit, which was selected randomly at the time of slaughter. Total 200 pigs were examined for cysticercosis from all nine slaughter slabs.

##### Collection of Blood samples for serology

About 10 ml blood sample was collected in a sterile blood-collecting vial without EDTA directly from the Jugular vein after the slaughtering of pigs and from the heart at the places where stabbing or hammering was done. The vial was numbered appropriately and the detail record was kept in a notebook. Then the blood was centrifuged at 5000 rpm for 10 minutes and the serum was separated in a serum-collecting vial (ependorf tube) for serology with the help of a micropipette.

##### Serological study (ELISA)

Enzyme-Linked Immunosorbent Assay for the detection of circulating antigen (Ag-ELISA) was conducted in National Zoonoses and Food Hygiene Research Centre (NZFHRC), Kathmandu. For this study, the sandwich antigen-ELISA as described by Dorny *et al.*, (2000) and adapted by Dorny *et al.* (2004) was used. This test makes use of the IgG type monoclonal antibodies developed for the diagnosis of *Taenia saginata* cysticercosis (Van Kerckhoven *et al.*, 1998), but cross-reaction makes it possible to use these antibodies for the diagnosis of *T. solium* cysticercosis as well (Brandt *et al.*, 1992).

##### Microscopic examination of cyst

The microscopic examination of the cyst was done at NZFHRC. Thawing of frozen cyst was done. The cyst was kept in 10% HCl for

2 minutes to dissolve the outer layer. Then the cyst was kept in between the two slides and pressed so that the scolex was separated. Then the slide was examined under the microscope at 100x magnification (Eyepiece 10X, Objectives 10X). The Scolex was searched and the hooks were counted. The Size of hooks was measured with the help of an ocular micrometer, which was fitted in the Eye Piece.

#### RESULTS

##### Results of Laboratory analysis (ELISA)

Out of 200 serum samples of pigs, 72 were found positive by ELISA reader. The prevalence rate found out by simple mathematics is 35.5% ie. Prevalence rate=  $72/200 = 35.5\%$

The OD (Optical Density) value for five set of ELISA was 0.061-9.885 (Set I), 0.046-9.911 (Set II), 0.052-9.929 (Set III), 0.525-9.796 (Set IV) and 3.015-83.33 (Set V).

**Table 1: Age wise distribution of cysticercosis among pigs**

Age (months)	Positive	Negative	Total
0-3	0	0	0
3-5	1	0	1
5-7	33	51	84
7-9	29	58	87
9-11	9	16	25
>12	0	3	3
Total	72	128	200

Age was taken as the guess by the farmers and abattoir workers

**Table 2: Sex-wise distribution of cysticercosis among pigs**

Sex of the pigs	Positive	Negative
Male	66	118
Female	06	10
Total	72	128

##### Results of Lingual and Carcass examination

Out of 200 lingual examinations, none of the tongues of pig was positive. So the prevalence by lingual examination was found to be 0.0%.

Similarly out of 200 carcass examination, only 1 was found to be positive to cysticercosis (Cysts of *Taenia solium* in muscle). So the prevalence by carcass examination was found to be 0.005%

**Table 3: Results of Hospital Survey From 2002-2008 in Kathmandu valley.**

Name o Hospitals	Total Epileptic patients	Total NCC patients
TUTH	890	36
Bir	68	35
Patan	380	31
Norvic	321	55
NMC	180	22

##### Age and sex wise distribution of NCC patients

The age and sex wise distribution of total 179 NCC patients from the five hospitals of Kathmandu valley has been shown below. The age wise distribution of NCC cases shows the highest in the age of 15-35 years having 96 out of 179 (53.63%) followed by 43 out of 179 (24.02%) in 0-14years and 40 out of 179 (22.35%) in above 35 years age group. Out of total 179 NCC cases, the proportion among male is 119 (66.5%) and among female is 60 (33.5%).

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**NEWS:**

**World Association for the Advancement of Veterinary Parasitology (WAAVP):**

Dr. Durga Datt Joshi, Executive Chairman participated in 22<sup>nd</sup> Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP) August 9-13, 2009 Calgary, Alberto, Canada. Three following technical papers were presented at WAAVP an oral and poster presentation.

- Survey on porcine trichinellosis in Nepal diagnosed by ELISA and Pepsin digestion Methods.
- Prevalence of Visceral Leishmaniasis in Human Population of Siraha District in Nepal.
- Assessment of Helminthological Contamination in Drinking Water Sources of Kathmandu Valley, Nepal.

**The 3<sup>rd</sup> International Symposium of Integrative Zoology (ISZS):**

The 3<sup>rd</sup> International Symposium of Integrative Zoology held at the Olympic Village Science Park in Beijing from 8 – 10 July 2009. This symposium was organized by the ISZS, the Institute of Zoology, Chinese Academy of Sciences and China Zoological Society with support from the Bureau of International Cooperation, Chinese Academy of Sciences and the Division of International Affairs, China Association of Science and Technology.

Dr. Durga Datt Joshi, Executive Chairman participated in ISZS. Three following technical papers were presented at ISZS an oral and poster presentation.

- "Epidemiological Surveillance Outbreak Record of Visceral Leishmaniasis/Kala-azar in Nepal during the Year 2005-2008"
- Epidemiological Surveillance Study of Snakes and Snakebite Human Cases in Nepal.

**World Rabies Day 2009 Celebrated, Nepal:**

In Nepal World Rabies Day September 28, 2009 was organized by NZFHRC. On the occasion of WRD, free dog rabies vaccination was carried out at Ward no. 13, Ward no. 15, Ward no. 19 and Ward no. 20 of KMC. Total 163 dogs were vaccinated against rabies.

**K.D.M.A. Research Award:**

*Please kindly submit your research work paper on allergy for trust award consideration by the end of December 2009 to KDMART office Chagal, G.P.O. Box 1885, Kathmandu, Nepal, Phone: 4270667 and Fax 4272694. This award was established by Dr. D.D. Joshi in 2049 B.S. on the memory of his wife, the late Mrs. Kaushilya Devi Joshi. The award includes a grant of NCRs. 10,001 with certificate.*

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