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A cross-sectional study on swine brucellosis sero-survey in Nagaland state of North Eastern India

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ABSTRACT

Swine brucellosis caused by the bacterium *Brucella suis*, is an important zoonotic disease characterized by abortion, birth of dead or weak piglets, orchitis in males, lameness and sometimes paralysis. Brucellosis is endemic to many Indian states but disease status is unknown in the pig-rearing state of Nagaland in North-Eastern India. The present study aimed to survey presence of *anti-Brucella* antibodies in pigs to understand the serological status of disease. A cross-sectional study design was adopted resulting in collection of 1550 serum samples from nine out of eleven districts, 43 out of 74 blocks (58.10% of blocks) and 59 out of 1428 epiunits called villages (4.13% of epiunits) and serum samples were tested by in-house developed indirect ELISA. In the study, more males (n-932) were tested compared to female pigs (n-618) which is approximately in the ratio of 3:2. Overall, 6.84% (5.69-8.2) apparent prevalence for brucellosis (AP) was noted with the highest sero-prevalence of 53.77% in Wokha district followed by Zunhebot, Tuensang and Kiphire districts as 6.82, 5.22 and 4.14%, respectively and least sero-positivity (2%) in Dimapur (the largest district) and Kohima district (the capital of Nagaland). At epiunit level, 100% sero-prevalence was recorded in two epiunits, >15% in three epiunits and >10% prevalence in four epiunits. Non-significantly higher sero-prevalence was recorded in males (7.40%) alongwith significantly higher prevalence in 24-month-old pigs (17.85%). The study identified swine brucellosis endemic regions in the state of Nagaland, which will be helpful for planners in implementing intervention measures in the absence of a vaccination policy for pigs in the country.

Key words: iELISA, India, Nagaland, Swine brucellosis, Sero-epidemiology

Brucellosis is recognized as one of the seven neglected, under-detected and under-reported zoonoses affecting multiple livestock species, wild animals, some marine mammals and humans (McDermott *et al.* 2013, Olsen and Palmer 2014, OIE 2018). Swine brucellosis is caused by the bacterium *Brucella suis*, ans is a contagious and emerging zoonoses characterized by late abortion, infertility, orchitis, inflammatory lesions in the joints, paralysis of posterior limbs and lameness (Olsen *et al.* 2012, Olsen and Palmer 2014). India ranks fifth in the world share of pig population with 09.06 million pigs, of which majority is concentrated in North-Eastern (3.95 million) and Eastern (2.8 million) Indian states (DAHD 2019). Pig farming is an important component of the Indian livestock sector and diseases affecting production can affect livelihood of the pig farmers and growth of piggery sector in the country.

Although there are reports of brucellosis in Mithun, an indigenous livestock species (Bos frontalis) but swine brucellosis has not yet been reported in the Nagaland state (Nath *et al.* 2009, Rajkhowa *et al.* 2005). The effective regulatory mechanisms such as whole-herd depopulation is recommended but it is difficult to implement in India (Olsen and Tatum 2017). In the absence of control policy for swine brucellosis, periodical brucellosis sero-prevalence studies are needed to understand the disease burden in the pig rearing state -Nagaland, India.

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MATERIALS AND METHODS

Ethics statement: The study approved by Institutional Animal Ethics Committee, Indian Council for Agricultural Research-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR- NIVEDI), Bengaluru, India and permission has been taken from farm owners to publish the data.

Study plan and sampling design:India is broadly divided into five regions (Northern, Central, Western, Southern and North-Eastern) and the study aimed to survey the presence of *anti-Brucella* antibodies in pigs from Nagaland during the year 2018-2019. Nagaland state is one among the 29

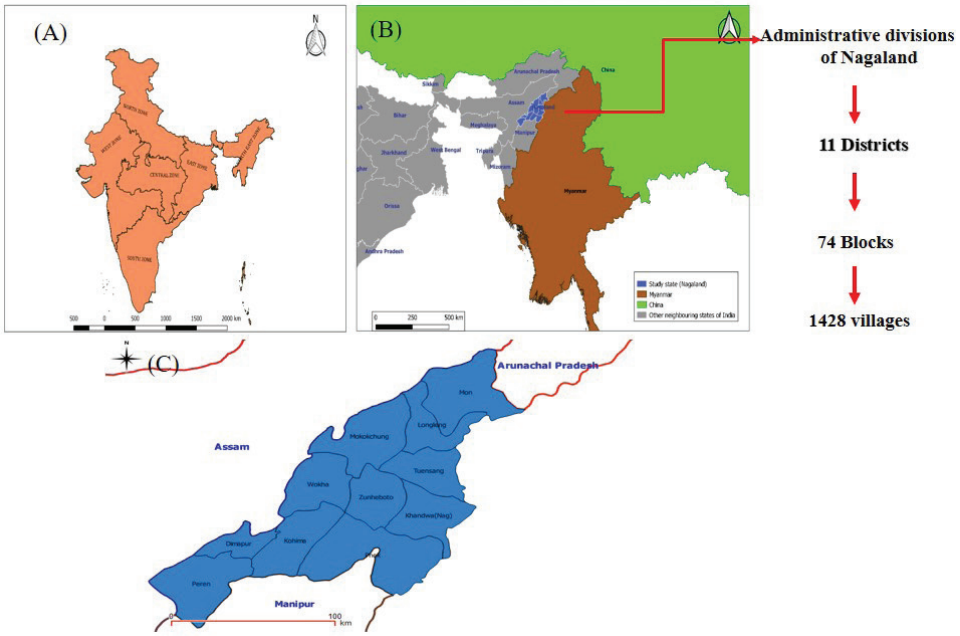

Fig. 1 (A) Zonal map of India. (B) Administrative divisions of Nagaland, India. (C) Different districts within Nagaland state.

states located in the North-Eastern region of the country (Fig. 1 A,B,C) and bounded by the Indian states of Arunachal Pradesh to the northeast, Manipur to the south, Assam to the west and northwest and Myanmar (Burma) to the east.There are 11 districts, 74 blocks and 1428 epiunits (villages) with a population of two million pigs. Pork is the staple diet with a per capita consumption of around 51 kg/ year (AH and VS 2016, Patra *et al.* 2016).

A questionnaire was designed for the study with complete animal description (sex and age) and demographic details such as the name of epiunit, block and district within the state. In the first strata, districts in the state, blocks within the district and epiunits within the blocks were selected by means of a simple random approach and animals were selected based on probability proportional to swine population. In the second strata, the households were randomly selected to collect the designated samples from animals based on sample size calculations with 10-30% prevalence, 0.05 precision, 95% confidence level (https://nivedi.res.in/Nadres_v2/Epical/herd_level_sample_size.php).

Sample collection, serological screening and statistical analysis:Approximately 2-3 ml of blood was collected and serum was separated from the ear vein of pigs in vaccutainers without anticoagulant (Becton Dickson, Oxford, UK) Serum samples were transported to ICAR-NIVEDI, Bengaluru, India by maintaining cold chain and tested by iELISA using smooth lipopolysaccharide (sLPS) antigen extracted from *B. abortus* S99 and rabbit anti-pig IgG-HRP conjugate (Sigma-Aldrich, St. Louis, MO, USA). The optical density (OD) values thus obtained were converted to percent positivity (PP) values relative to OD of positive control and samples with greater than 50 PP was considered positive (Shome *et al.* 2016, 2022). Apparent prevalence(AP) and true prevalences (TP) were calculated with 95% confidence interval as per the sensitivity (94%) and specificity (92%) (Rogan and Rogan, 1978, Thrushfield 2005). The chi-square values and odds ratio was calculated to interpret significant differences between the different age groups and sex as per the VassarStats website (http://vassarstats.net) and $P<0.05$ was considered significant. All the maps were prepared using QGIS software version 3.16.

RESULTS AND DISCUSSION

A total of 1550 serum samples were sourced from nine out of eleven districts, 43 out of 74 blocks (58.10% of total blocks) and 59 out of 1428 epiunits (4.13% of total epiunits). The study analysed stratified samples representing majority of the districts in the state unlike isolated studies of a farm or outbreak investigations. Overall, 6.84% (CI: 5.69-8.2) brucellosis AP was recorded by Ielisa. Till date the disease has not been reported from the study region for comparison. However, swine brucellosis reports reviewed from 1969 to 2000 in the country, revealed a prevalence of 16.17% in slaughtered and adult pigs of Punjab state (Boral *et al.* 2009, Jindal *et al.* 2017, Kaur *et al.* 2020) and 41.04% in pig farms with history of abortion from Karnataka (Shome *et al.* 2019). Similarly, in Tamil Nadu, AP of 10.1% was recorded in pig farms using iELISA (Preena *et al.* 2024). A pan-India level swine brucellosis was reported based on stratified random serum samples revealed apparent prevalence of 4.33% for samples collected during 2018–2019 (Shome *et al.* 2022). Distinct difference were observed between sampling methodology and sero-prevalence reports except a for study conducted during 2018-2019. These location specific studies clearly indicated swine brucellosis sero-prevalence across the country and the present study is a region-specific reporting

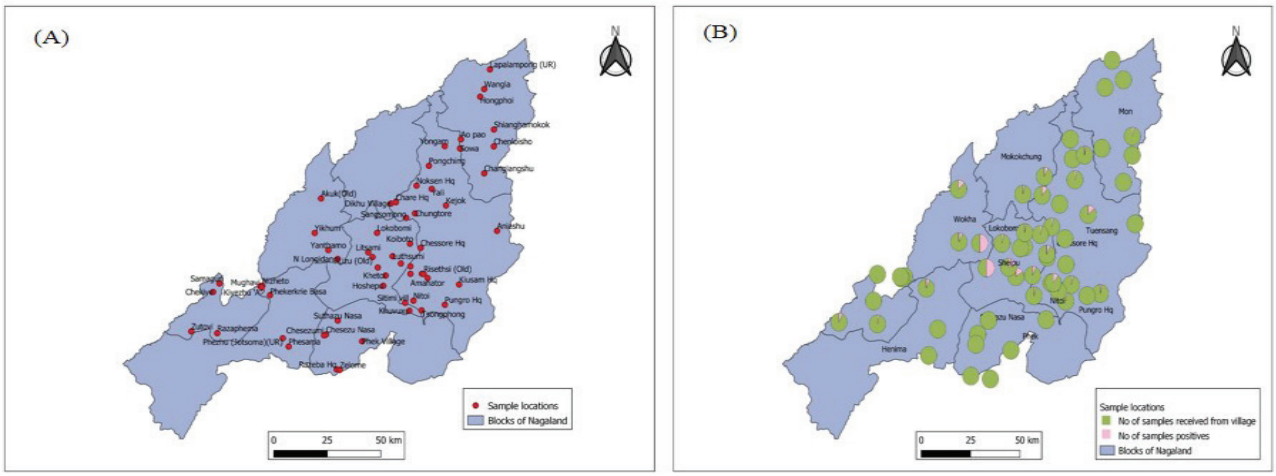

Fig. 2 (A) Sampling sites (B) map depicting swine brucellosis sero-prevalence in Nagaland state

of disease important to Nagaland state .

Among the districts, highest sero-prevalence (53.77%, CI: 43.4-62.07) was recorded in Wokha district followed by Zunhebot, Tuensang and Kiphire districts with 6.82% (CI:4.36-10.52), 5.22% (CI:3.01-8.9) and 4.14% (CI:2.32-7.25), respectively and least sero-positivity (2%, CI: 0.69-8.66) in Dimapur (the largest district) and Kohima district (the capital of Nagaland). Absence of anti-Brucella antibodies was noted in serum samples from two districts (Longleng and Phek), in 18 blocks and 25 epiunits surveyed in the study (Fig. 2 A and B, Tables 1 and 2). Brucellosis risk is perceived to be in the larger herds (greater than 100 animals) or densely populated regions or overcrowding of the animals in the farms (Mai 2012). In contrary to this, The piggery production system in the region is mostly unorganized with large pig farms (Patra *et al.* 2016) and increased disease prevalence noticed in few regions might not be due to pig population or herd size or overcrowding.

Table 1. Swine brucellosis sero-prevalence in different districts of Nagaland State

Sl. No	District name	No. of blocks	Pig population	No. of samples received	No. of positives	Apparent prevalence (95% CI)	True prevalence (95% CI)*
1	Wokha	12	48591	106	57	53.77 (43.4-62.07)	52.13 (41.16-62.87)
2	Zunheboto	13	59691	264	18	6.82 (4.36-10.52)	-
3	Tuensang	15	57567	230	12	5.22 (3.01-8.9)	3.24 (-5.8-1.04)
4	Kiphire	8	43836	266	11	4.14 (2.32-7.25)	-
5	Kohima	8	53928	80	2	2.5 (0.69-8.66)	1.69 (-8.5-0.77)
6	Dimapur	8	69561	183	4	2.19 (0.85-5.48)	-
7	Longleng	5	10891	52	0	0 (0-6.88)	-
8	Mon	14	47155	210	2	0.95 (0.26-3.41)	-
9	Phek	14	45315	159	0	0 (0-2.36)	-
10	Peren	7	16232	Not sampled	-	-	-
11	Mokokchung	9	50920	Not sampled	-	-	-
	Total	113	503687	1550	106	6.84 (5.69-8.2)	-1.35 (-2.69-0.24)

*CI: Confidence interval

Table 2. Swine brucellosis sero-prevalence in different epiunits of Nagaland state

Sl.No	District name	Block name	Epiunit name	No. of samples received	No. of positives	Apparent prevalence (95% CI)
1	Dimapur	Aquqhnaqua	Mughavi	26	0	0 (0-12.87)
			Nizheto (UR)	25	0	0 (0-13.32)
		Chumukedima	Chekiye	28	0	0 (0-12.06)
			Samaguri	25	0	0 (0-13.32)
		Dhansiripar	Zutovi	26	2	7.69 (2.14-24.1)
		Medziphema	Razaphema	27	1	3.70 (0.66-18.8)
		Niuland	Kiyezhu 'A'	26	1	3.84 (0.6-18.8)
	District level total			183	4	2.19 (0.85-5.48)
2	Kiphire	Amahator	AmahatorVill	27	1	3.70 (0.66-18.8)
			Risethsi (New)	27	0	0 (0-12.46)
			Risethsi (Old)	26	0	0 (0-12.87)
		KiphireSadar	Anatongre	27	3	11.11 (3.85-28.06)
		Kiusam	KiusamHq	26	1	3.84 (0.6-18.8)
		Longmatra	Tsongphong	27	1	3.70 (0.66-18.8)
		Pungro	PungroHq	28	0	0 (0-12.06)
		Seyochung	Phisami	26	3	11.54 (4.0-28.98)
		Sitimi	Nitoi	26	1	3.84 (0.6-18.8)
			SitimiVill	26	1	3.84 (0.6-18.8)
	District level total			266	11	4.14 (2.32-7.25)
3	Kohima	Chiephobozou	PhekerkrieBasa	26	2	7.69 (2.14-24.1)
		Jakhama	Phesama	27	0	0(0-12.46)
		Sechu-Zubza	Phezhu (Jotsoma) (UR)	27	0	0 (0-12.46)
	District level total			80	2	2.5 (0.69-8.66)
4	Longleng	Longleng	Pongching	26	0	0 (0-12.87)
		Yongnyah	Yongam	26	0	0 (0-12.87)
	District level total			52	0	0 (0-6.88)
5	Mon	Aboi	Aopao	26	0	0 (0-12.87)
		Angjangyang	Sowa	26	1	3.84 (0.6-18.8)
		Chen	Chenloisho	27	0	0 (0-12.46)
		Longshen	Shianghamokok	26	1	3.84 (0.6-18.8)
		Mon Sadar	Hongphoi	26	0	0 (0-12.87)
			Wangla	26	0	0 (0-12.87)
		Monyakshu	Changlangshu	27	0	0(0-12.46)
		Tizit	Lapalampong (UR)	26	0	0 (0-12.87)
	District level total			210	2	0.95 (0.26-3.41)
6	Phek	Chetheba	ChesezuNasa	26	0	0 (0-12.87)
			Chesezumi	27	0	0 (0-12.46)
		PhekSadar	Phek epiunit	27	0	0 (0-12.46)
		Razieba	RazebaHq	26	0	0 (0-12.87)
			Zelome	27	0	0 (0-12.46)
		Sekruzu	SuthazuNasa	26	0	0 (0-12.87)
	District level total			159	0	0 (0-2.36)

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Sl.No	District name	Block name	Epiunit name	No. of samples received	No. of positives	Apparent prevalence (95% CI)
		Chare	ChareHq	24	3	12.5 (4.34-31)
			Dikhu epiunit	25	1	4 (0.71-19.54)
		Longkhim	Chungtore	26	0	0 (0-12.87)
			Sangsomong	27	1	3.70 (0.66-18.8)
7	Tuensang	Ngoungchung	Yali	26	1	3.84 (0.6-18.8)
		Noksen	NoksenHq	25	2	8 (2.22-24.97)
		Sangsangnyu	Kejok	26	4	15.38 (6.15-33.53)
		Chessore	ChessoreHq	25	0	0(0-13.32)
		Nokhu	Aniashu	26	0	0 (0-12.87)
	District level total			230	12	5.22 (3.01-8.9)
		Aitepyong	Akuk (Old)	29	4	13.79 (5.5-30.56)
		Englan	Yikhum	26	2	7.69 (2.14-24.1)
8	Wokha	WokhaSadar	N Longidang	27	27	100 (87.54-100)
			Yanthamo	24	24	100 (86.2-100)
	District level total			106	57	53.77 (43.4-62.07)
		Aghunato	AghunatoHq	27	2	7.41 (2.06-23.37)
			Hoshepu	27	5	18.52 (8.18-36.7)
			Khetoi	27	2	7.41 (2.06-23.37)
		Asuto	Luthsumi	27	0	0 (0-12.46)
9	Zunheboto		Koiboto	26	1	3.84 (0.6-18.8)
		Atoizu	Litsami	26	1	3.84 (0.6-18.8)
			Lokobomi	26	1	3.84 (0.6-18.8)
		Satoi	Khuvuxu	26	0	0 (0-12.87)
		Zunheboto Sad	Lizu (Old)	26	0	0 (0-12.87)
			Sheipu	26	6	23.08 (11.03-42.05)
	District level total			264	18	6.82 (4.36-10.52)
State level Total		43	59	1550	106	6.84 (5.69-8.2)

Within Wokha district, Wokha Sadar block showed 100% sero-prevalence followed by 13.79 and 7.69% in Aitepyong and Englan blocks, respectively (Table 2). At epiunit level, 100% sero-prevalence was recorded in two epiunits, >15% in three epiunits and >10% prevalence in four epiunits. Each epiunit is called an epidemiological unit practicing same management habits such as breeding and feeding and in such situations, many risk factors are presumed to augment spread of brucellosis (Jonsson 2013). Due to remoteness, mountainous terrain and inaccessibility to veterinary health care, farmers have evolved a self-sustainable production systems and hence conserved disease pattern was observed in few epiunit regions.

Frequent movement of animals from neighboring states through road transportation is presumed to be an important risk for disease transmission (Jonsson 2013) and few epiunits which showed 100% brucellosis sero-prevalence are located adjoining to Assam state. Brucellosis prevalence as high as 87.10% in a pig breeding farm with a history of abortion from Assam (Nath *et al.* 2009) and 5% from another bordering state, Manipur (Shome *et al.* 2016) earlier have been reported and it is most likely that the infected pigs are transported for breeding or meat purpose which is probably responsible for ingression of disease into the regions.

In the study, more males (n-932) were tested compared to female pigs (n-618) which is approximately in the ratio of 3:2. Prevalence estimation within the gender disclosed higher sero-prevalence in males (7.40%) compared to female pigs (5.98%), however there was no significant difference in sex-wise prevalence as shown by odds ratio [1.255 (0.830-1.897)] with P-value of 0.362. In three districts, odds for male pigs >1.0 was observed (Table 3). High proportions of male pigs were found positive compared to female pigs though statistically insignificant, and this justifies the fact that male pigs are exposed to females during natural breeding which increases chances of infection in males (Olufemi *et al.* 2018). In addition, boars excrete Brucella in the semen and act as potential spreaders of disease (Thirlwall *et al.* 2008). Higher prevalence in the older pigs (24 months) could be attributed to retention of sows and boars for breeding purpose in the infected farm

Table 3. Sex-wise brucellosis sero-prevalence in swine population of Nagaland state

Sl. No	District name	Pig population	Male		Female		χ^2 value	P-value	Odds ratio
			Number of animals tested	Number of positive animals	Number of animals tested	Number of positive animals			
1	Phek	45315	122	0 (0)*	37	0 (0)	-	-	-
2	Longleng	10891	17	0 (0)	35	0 (0)	-	-	-
3	Mon	47155	145	2 (1.37)	65	0 (0)	0.03	0.862	-
4	Dimapur	69561	118	2 (1.69)	65	2 (3.07)	0.1	0.920	0.543 [0.07-3.948]#
5	Kohima	53928	46	1 (2.17)	34	1 (2.94)	0.26	0.610	0.733 [0.044-12.156]
6	Kiphire	43836	144	8 (5.55)	122	3 (2.45)	0.82	0.365	2.33 [0.641-7.495]
7	Tuensang	57567	112	8 (7.14)	118	4 (3.38)	0.84	0.359	2.192 [0.641-7.495]
8	Zunhebot	59691	161	13 (8.07)	103	5 (4.85)	0.49	0.483	1.721 [0.595-4.981]
9	Wokha	48591	67	35 (52.2)	39	22 (56.4)	0	1.000	0.845 [0.382-1.869]
	Total	436535	932	69 (7.40)	618	37 (5.98)	0.96	0.327	1.255 [0.830-1.897]

*Values within round brackets indicate the percentage positivity, # values within square bracket indicate 95% CI.

environment.

With respect to age, large number of swine samples were from 7-12 months age group [56% (n-873)], 31.87% samples (n-494) belonged to 1-6 months, 10% of the samples came from 13-24 months (n-155) and only 28 samples received from older pigs of >24 months (1.81%). Brucellosis within the age groups disclosed significantly higher sero-prevalence in (0.0094) >24 month old pigs (17.85%) followed by 13-24 month pigs (12.25%) and comparatively less sero- prevalence in the pigs of 12 months (Table 4).

There is no formal infrastructure for slaughtering and pork marketing. The pork meat is sold in gunny bags or banana leaves (*Musa paradisiaca*) or in polythene sheets during regular or weekly markets. Very high risk of zoonotic infection with *B. suis* associated with the handling of infected meat is reported (Deqiu *et al.* 2002) and lack of human brucellosis reports necessitates screening of pig handlers for zoonotic diseases including brucellosis. Pig alone accounts for 53.77% of the total livestock population of Nagaland and still a wide gap exists between the demand and availability of pork mainly due to the traditional production system and poor health care (Patra *et al.* 2016). Brucellosis prevalence of 6.84% in the Nagaland state with highest sero-prevalence of 52.83% in a district and 100% in a epiunit represents a significant disease threat to pig population in the region. In the absence of vaccination policy in India, risk-based prevention measures such as continuous surveillance, test-and-slaughter of infected animals, and creating awareness among farmers are important measures. Regular disease surveys to alert governments for implementing targeted intervention measures in hyper-endemic regions are unoptional to minimize operational cost for the control of diseases and human exposure.

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Table 4. Age-wise brucellosis sero-prevalence in pig population of Nagaland state

Sl.no	Intervals of month	Number of samples	Number of positives	χ^2 value	P-value
1	1-6	494	30 (6.07)	11.48	0.0094*
2	7-12	873	52 (5.95)		
3	13-24	155	19 (12.25)		
4	>24	28	5 (17.85)		
	Total	1550	106 (6.83)		

Values within the parenthesis indicate % positivity; *indicate the P<0.05.

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