



Programme

Journey towards National Institute of One Health in India

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Background & objectives: Issues such as emerging and re-emerging infectious diseases, antimicrobial resistance, food security, biosafety and biosecurity are associated with changes in land use, population growth, urbanization, global travel and trade and climate change. As a result, a trans-disciplinary approach among human, animal and environmental health disciplines gained support. The Indian Council of Medical Research (ICMR) and Indian Council of Agricultural Research (ICAR) decided to establish a National Institute of One Health at Nagpur, Maharashtra, India. In this context, two collaborative research projects, funded by the ICAR and ICMR were initiated to conduct the epidemiological surveillance of selected zoonotic diseases in Central India.

Methods: Disease surveillance and molecular detection employing standard techniques like enzyme linked immunosorbent assay (ELISA), immuno-fluorescent assay (IFA), standard tube agglutination test (STAT), Rose Bengal plate test (RBPT) and polymerase chain reaction (PCR) were undertaken based on the disease to be screened.

Results: In animals, the seropositivities for listeriosis (7.66%) and brucellosis (11.69%) were recorded. The occurrence of tuberculosis (3.8%) and leptospirosis (6.33%) was detected by PCR. Through cross-sectional studies from suspected human population with associated risk factors for zoonotic diseases, the seropositivity of brucellosis (1.83-11%), listeriosis (1.01-10.18%), leptospirosis (8.14-12.67%) and scrub typhus (1.78-20.34%) was recorded. The investigations on scrub typhus indicated bimodal pattern during the months of pre-monsoon and post-monsoon season with a peak in post-monsoon in human cases. *Ornithonyssus bacoti* mites were identified from the rodents as a vector harbouring *Orientia tsutsugamushi*. The bovine tuberculosis was detected in 1.43 per cent human cases employing molecular assay.

Interpretation & conclusions: The data indicated the occurrence of important zoonotic diseases adversely affecting the livestock health and human wellbeing. The scientific collaboration between veterinary and medical faculties has set an example for effective implementation of One Health (OH) programme for the establishment of National Institute of OH.

Key words Brucellosis - listeriosis - One Health - scrub thypus - surveillance - zoonotic diseases

The public health challenges in today's world such as emerging and re-emerging infectious diseases, antimicrobial resistance, food security, biosafety and bio-security are associated with the changes in land use, population growth, urbanization, global travel and trade and climate change. As a result, a trans-disciplinary approach among human, animal and environmental health disciplines has gained support and visibility. On July 6, 2010 in Nagpur, India, the experts and scientists from medical and veterinary sciences in a national conference organized by the National Association of Welfare of Animals and Research in collaboration with Indian Medical Association (Nagpur Chapter) conceived the concept of establishing of a National Institute for Zoonoses (NIZ) at Nagpur. Since then, deliberations were held at the levels of the Indian Council of Medical Research (ICMR) and Indian Council of Agricultural Research (ICAR). Both the apex organizations were committed towards creating this imperative facility at Maharashtra Animal and Fishery Science University (MAFSU), Nagpur. Discussion on the setting up a dedicated facility was initiated. Subsequently, in March 2019, the ICMR declared to establish 'Centre for One Health (OH)', a satellite centre under National Institute of Virology, Pune in Nagpur. It is contemplated to be an independent institute though nomenclature may change from the NIZ to the National Institute for OH (NIOH). As a step towards activities of the institute, two research projects, funded by ICAR entitled 'Centre for Zoonoses under Niche Area of Excellence' to Nagpur Veterinary College (NVC), Nagpur, and another by ICMR to Mahatma Gandhi Institute of Medical Sciences (MGIMS), Sewagram, and Central India Institute of Medical Research (CIIMS), Nagpur, were sanctioned.

Material & Methods

The study was conducted under the projects 'Centre for Zoonoses' sanctioned by the ICAR and ICMR to NVC and CIIMS, Nagpur, and MGIMS, Sewagram, during 2015 to 2019. The zoonotic diseases namely brucellosis, listeriosis, tuberculosis, leptospirosis and scrub typhus were studied at the Centre for Zoonoses at NVC, Nagpur. the MGIMS, Sewagram, was assigned brucellosis, leptospirosis and scrub typhus, CIIMS worked on brucellosis, listeriosis and bovine tuberculosis. The research work with respect to disease surveillance and molecular detection of the pathogens was undertaken.

These studies were approved by the Institutional Ethics Committees (vide reference No. NVC/IAEC/19; MGIMS/IEC/MICR/118/2014 and 01/CIIMS/ICMR/09/14) of NVC, CIIMS and MGIMS. The samples were collected with prior consent of the animal owners and with written consent from human participants.

The team from the NVC, over a period of four years, collected the clinical samples from livestock (blood, serum, nasal swabs and vaginal swabs), other animals (blood and serum) and their human contacts (blood and serum) from different regions of Central India (Maharashtra, Madhya Pradesh, Chhattisgarh and Telangana). The samples were collected randomly. Further the number of samples included for screening for particular disease was based on the clinical symptoms. The details of clinical samples are presented in Table I.

At MGIMS, Sewagram, of the 3454 individuals, 1726 (49.97%) suspects with acute undifferentiated fever (AUF) were recruited from a tertiary care hospital. From the community, out of 10,000 population belonging to seven villages, 98 individuals were recruited by house-to-house visits over a period of one and a half year. Further, 166 individuals were selected from 8808 patients attending a rural hospital. The blood samples (2.5 ml) collected from these individuals were screened for seropositivity for brucellosis, leptospirosis and scrub typhus. The CIIMS, Nagpur, collected clinical blood samples from Central Indian population having animal-human interface. A total of 7026 blood samples were collected through cross-sectional studies from suspected populations with associated risk factors for seroprevalence against brucellosis, listeriosis and bovine tuberculosis.

The seroprevalence of listeriosis was studied by in-house standardized listeriolysin-O based indirect IgG ELISA^{1,2}. Screening for brucellosis was conducted on the basis of the detection of antibodies against *Brucella* spp. by using in-housed standardized indirect IgG ELISA against lipopolysaccharide (LPS)³. Standard tube agglutination test (STAT) and Rose Bengal plate test (RBPT) were performed according to the procedure described by the World Organization for Animal Health (OIE)⁴. The samples from animals were screened for tuberculosis and leptospirosis by targeting the *IS1160* gene⁵ and *secY* gene⁶, respectively.

Scrub typhus among various occupationally exposed patients, abattoir workers, sanitary workers

Table I. Details of the clinical samples collected

Region	Animals									Human	Total
	Cattle	Buffalo	Goat	Sheep	Pig	Dog	Rodents	Other	Total		
Nagpur	2081	928	1265	29		106	137	15	4561	2644	7205
Amravati	226	0	121	0		16	0		363	83	446
Aurangabad	2		118	71					191	72	263
Nashik	914	118							1032	53	1085
Pune	1422	104	10	30	92	15		4	1677	109	1786
Konkan	436	369	54	57		61	15		992	168	1160
Chhattisgarh	582	130				2	21		735	204	939
Madhya Pradesh	214	289	15						518	108	626
Telangana		200		94					294	29	323
Species total	5877	2138	1583	281	92	200	173	19	10,363	3470	13,833

and livestock owners was detected employing IgM-ELISA (InBios International, USA) and Immuno Fluorescent assay (IFA) (Fuller Laboratories, USA) as per the manufacturer's instructions. The samples from rodents (blood and tissue) and vectors were investigated for the 56 and 47 kDa outer membrane protein genes of *Orientia tsutsugamushi*⁷.

Results

Brucellosis: Of 9855 serum samples collected from animals, a total of 1152 (11.69%) and 898 (9.12%) samples were found to be positive for brucellosis by ELISA and by RBPT, respectively. Of the 3051 human serum samples tested, 56 (1.83%), 43 (1.40%) and 12 (0.39%) were positive by ELISA, RBPT and STAT, respectively (Table II). The antibodies for brucellosis were detected in 6.31 per cent samples collected from the human cases of AUF attending a tertiary care centre. In the samples collected from seven villages to central India, the occurrence of brucellosis was 11 per cent. Through the hospital-based observational retrospective studies, in suspected human cases with associated neurological morbidities, the presence of neurobrucellosis was found to be 8.4 per cent.

Listeriosis: In the present investigation, seropositivity for *Listeria* IgG was observed to be 7.66 per cent among animals (755/9855), whereas, of the 3346 human serum samples, 1.01 per cent were found to be positive. Of the 88 samples from women with spontaneous abortion, five (5.68%) were positive. Among the 98 samples collected from Central Indian human population, 9.3 per cent were positive for *Listeria*.

Leptospirosis: From endemic regions of leptospirosis, namely Konkan region and East Vidarbha of Maharashtra and Telangana, animal (1783) and human (1252) blood samples were tested. The *secY* gene was detected in 113 (6.33%) and 102 (8.14%) samples, respectively (Tables II and III). Among the 1726 human cases of AUF attending a tertiary care centre, 12.67 per cent (218 were positive for leptospirosis. Among the community of seven villages, the positivity for leptospirosis was seven per cent.

Tuberculosis: A total of 8363 samples from animals were screened for tuberculosis by targeting the *IS6110* gene, wherein 318 (3.8%) were found to be positive. Among the Central Indian human population who were in contact with animals; the overall occurrence of tuberculosis was 1.43 per cent and the hospital-based observational retrospective studies showed 12.3 per cent human cases of tuberculus meningitis.

Scrub typhus: A study on scrub typhus among various occupationally exposed patients, abattoir workers, sanitary workers and livestock owners was carried out. Of the, 462 serum samples, 94 (20.34%) were found to be positive by IgM-ELISA and 24 (5.14%) samples turned out to be positive by IFA (Tables II and III). The IFA could identify the infection of *O. tsutsugamushi* at strain level, viz. Karp, Gilliam, Kato and Boryong.

Blood and tissue samples of rodents trapped from different locations in Central India were processed for the detection of scrub typhus. The 56 kDa outer membrane protein gene of *O. tsutsugamushi* was detected from 10 of 59 samples, and the 47 kDa

Table II. Region wise per cent positivity to targeted diseases in humans and animals

Region	Serology						PCR					
	Brucellosis		Listeriosis		Leptospirosis		<i>Mycobacterium bovis</i>		<i>Mycobacterium tuberculosis</i>		Rickettsia	
	Animals	Human	Animals	Human	Animals	Human	Animals	Human	Animals	Human	Animals	Human
Nagpur	5.91	0.42	6.29	0.66	65.38	40.83	8.63	0.33	1.80	0.33	5.37	6.44
Amravati	5.26	0.00	0.62	0.00			0.00	0.00	0.00	0.00		
Nashik	21.71	5.66	11.43	0.00			25.28	0.00	3.93	0.00		
Pune	13.05	3.36	6.98	3.66			0.00	0.00	0.00	0.00		
Aurangabad	9.95	0.92	0.00	0.00			0.00	0.00	0.52	1.38		
Konkan	13.13	1.98	4.53	0.00	60.99	92.30	0.00	0.00	0.00	0.00	0.00	0.00
Chhattisgarh	16.36	1.14	15.03	0.98			0.00	0.00	2.24	7.35	28.57	1.785
MP	12.07	7.41	18.15	10.18			0.00	0.00	0.00	0.00		
Telangana	3.23	21.84	4.76	0.00			0.00	0.00	0.00	0.00		

Table III. Species-wise per cent positivity to targeted diseases

Species	Brucellosis	Listeriosis	Leptospirosis	Tuberculosis	Rickettsia
Human	3.64	1.01	45.86	1.04	5.92
Cattle	26.95	10.67	23.52	4.83	-
Buffalo	10.90	1.40	45.61	0.61	-
Goat	7.39	5.56	-	1.26	-
Sheep	10.32	3.56	-	0.36	-
Pig	0.00	0.00	-	0.00	-
Dog	-	-	80.00	-	-
Rodent	-	-	69.23	-	6.95
Other	0.00	0.00	0.00	0.00	1.00

protein gene was detected from four of 59 samples by nested-PCR. *Ornithonyssus bacoti* mites from the rodents were identified as a vector harbouring *O. tsutsugamushi*. The seasonal variations in the occurrence in *O. tsutsugamushi* in rodents and mites were studied by PCR detection targeting the 56 and 47 kDa genes. During pre-monsoon season, *O. tsutsugamushi* was detected in 12 and 10 per cent samples, whereas during post-monsoon season, the respective detection rates were 13.33 and 26.66 per cent predicting a bimodal pattern during the months of pre- and post-monsoon season with a peak in post-monsoon. The study conducted on the human cases having AUF attending a tertiary care centre indicated 15.79 per cent cases of scrub typhus. In the community of seven villages, four per cent positivity for scrub typhus was recorded.

Discussion

The global threats of zoonotic diseases are well known. Surveillance data help to identify areas and populations at increased risk for infection, improving ability to direct and prioritize health interventions, to monitor the effectiveness, timeliness and cost-effectiveness of prevention and control efforts and to identify gaps and new prevention strategies. In recent years, the OH approach to manage endemic and emerging/re-emerging zoonotic diseases has been promoted by international human and animal Health agencies. The presence of important zoonotic diseases, *viz.* listeriosis, brucellosis, tuberculosis, leptospirosis and scrub typhus, recorded in the present collaborative research was in accordance with Indian reports^{1,8-21}. The study on vectors revealed novel findings, especially in scrub typhus and listeriosis.

Listeria monocytogenes has potential to infect humans and animals, resulting into septicaemia, gastroenteritis, meningitis, meningio-encephalitis, still birth and abortions^{22,23}. The *Listeria* strains have been isolated from women with poor obstetric history and premature births²⁴, buffaloes with reproductive disorders^{2,25} and foods of animal and fish origin²⁶. The present study reported 7.66 and 1.01 per cent seropositivity in animals and humans for *Listeria* IgG, respectively, along with 5.6 per cent cases of spontaneous abortions in women and 9.3 per cent of *Listeria* meningitis in human patients.

The seropositivities for brucellosis in animals by ELISA and RBPT were 11.69 and 9.12 per cent, respectively. Earlier investigations recorded 6.5 per cent prevalence in organized farms²⁷; flock-wise incidence rate in sheep and goats was around 50 per cent³ and 17.8-55.5 per cent in occupationally exposed veterinarians^{28,29}. Although brucellosis in animals is involved in orchitis, arthritis, still births and late abortions, the most human cases exhibit undulant fever, headache, back pain, myalgia and arthralgia. Occupationally exposed people have more chances of getting infected during animal handling and care. Higher positivity was recorded among the individuals from the regions where livestock farming was intensified²⁹.

Tuberculosis has been a challenge to healthcare system. Our explorations indicated 1.43 and 3.80 per cent positivity of *M. bovis* in humans and farm animals, respectively. High occurrence of bovine tuberculosis was recorded in the persons living in tuberculosis-endemic region. These patients lived in poorly ventilated houses and exhibited similar symptoms to *M. tuberculosis*³⁰. Our reports in animals contradicted with earlier finding reporting 15-28 per cent infection of *M. tuberculosis* in animals³¹; however, 0.4-51.2 per cent prevalence was recorded in different studies³².

The occurrence of leptospirosis was reported from paddy areas where there are water logging conditions, soil pH is alkaline and rodent population is high³³. Our findings from animal in the endemic regions of Konkan and East Vidarbha and Telangana recorded 6.33 and 8.14 per cent occurrence in animals and humans, respectively, along with 12.67 per cent among the human cases of AUF attending a tertiary care centre. Additionally, seven per cent positivity was reported in human population of seven selected villages from

Central India. Significant reduction in positivity was reported earlier in a tertiary health care centre in north India from 26.90 per cent in 2000-2010 to 6.47 per cent in 2015-2018³⁴. Leptospiral antibodies were found in 19.7 per cent rodents and dogs from Mumbai, India of which only 5.93 per cent samples showed the presence of the pathogen as confirmed by quantitative PCR and isolation³⁵.

Rickettsiosis has been reported from almost all parts of India³⁶. We reported 20.34 per cent seropositivity of scrub typhus among various occupationally exposed patients, abattoir workers, sanitary workers and livestock owners of Central India with involvement of *O. tsutsugamushi* in 5.14 per cent cases. *O. tsutsugamushi* was detected in rodents, ticks and *O. bacoti* mites from the study area³⁷. Our study demonstrated that the *O. bacoti* mites harboured this pathogen which could be transmitted among rodent population via bites and rodents might further perpetuate the infection to human being. Earlier, flea-borne rickettsiosis was reported in human from the Western Himalayan region³⁸.

The major highlight of the study was the collaborative work conducted which provided important links towards strengthening of the One Health approach that linked human and animals for prevention and control of zoonotic infections and their drivers through holistic and interdisciplinary approaches. The infrastructure and technical expertise developed at the Centre for Zoonoses at NVC could be utilized for addressing the public health issues. The training programmes could develop skilled workforce in the various aspects of zoonoses wherein participants from veterinary, medical and other allied fields would participate as a part of capacity building programmes.

In conclusion, the present study indicated the occurrence of important zoonotic diseases that affected both animal and human health. The upcoming NIOH will be developed to serve as national leader in zoonoses and the think tank devoted to One Health involving animals, human, wild life and environment by providing inter-sectoral and inter-disciplinary approach for surveillance, response, research, capacity building and advocacy/communication for prevention and control of zoonotic diseases.

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Conflicts of Interest: None.

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