43. Omran, H. H. and El-Kholony, K. H., The influence of Nitroxynil on clinical, biochemical and hematological parameters in Egyptian buffaloes affected with liver flukes in Sharkia goveronerate. *Egyptian J. Agric. Res*., 2003, **81**, 739–751.
44. Mottelib, A. A., Haroon, E. M., Magzoob, M. and El-Basheer, E., The effect of gastrointestinal parasites on the blood picture of sheep and goats at Al-Gassim. *Assiut Vet. Med. J*., 1992, **28**, 215–223.
45. Esmaeilnejad, B., Tavassoli, M. and Asri-Rezaei, S., Investigation of hematological and biochemical parameters in small ruminants naturally infected with *Babesia ovis*. *Vet. Res. Forum*, 2012, **3**, 31–36.
46. El-Aziz, M. Z. A., Emara, S. A. and Salem, F. S., Clinicopathological studies on fascioliasis among sheep in Giza province. *Egypt. J. Veter. Sci.*, 2002, **36**, 75–86.
47. Waweru, J. G., Kanyari, P. W. N., Mwangi, D. M., Ngatia, T. A. and Nansen, P., Comparative parasitological and hematological changes in two breeds of sheep infected with *Fasciola gigantica*. *Trop. Anim. Health Prod*., 1999, **31**, 363–372.
48. Zhang, Z. W. Y., Moreau, E., Hope, J. C., Howard, C. J., Huang, W. Y. and Chauvin, A., *Fasciola hepatica* and *Fasciola gigantica*: comparison of cellular response to experimental infection in sheep. *Exp. Parasitol*., 2005, **111**, 154–159.

ACKNOWLEDGEMENT. We thank the Director CORD, University of Kashmir, Srinagar for providing the necessary facilities during this study.

Received 22 April 2016; revised accepted 18 May 2017

doi: 10.18520/cs/v113/i09/1776-1783

Isolation of *Listeria monocytogenes* from peridomestic birds and captive wild animals

U. V. Jagtap[1], R. P. Kolhe[1,*], P. D. Deshpande[1], N. V. Kurkure[2], C. V. Dhandore[1], D. M. Muglikar[3], S. N. Jadhav[4], N. K. Nighot[5] and S. B. Barbuddhe[6]

[1]Department of Veterinary Public Health,
[3]Department of Veterinary Microbiology, and
[4]Department of Veterinary Biochemistry,
Krantisinh Nana Patil College of Veterinary Sciences,
Shirwal 412 801, India
[2]Department of Veterinary Pathology, Nagpur Veterinary College,
Seminary Hills, Nagpur 440 006, India
[5]Rajiv Gandhi Zoological Park and Wildlife Research Center, Katraj,
Pune 411 037, India
[6]ICAR-National Institute of Biotic Stress Management,
Raipur 493 225, India

***Listeria monocytogenes* is an important foodborne pathogen responsible for septicaemia, meningitis and abortions. There are several animal reservoirs; however, the role of wild animals and peridomestic birds remains underestimated. We have screened 270 faecal samples of wild animals in captivity (18 species) and peridomestic birds (12 species). *Listeria* species were isolated from seven (6.66%) mammals and two (1.21%) birds. *L. monocytogenes* was isolated from barking deer, porcupine, pigeon and crow. Isolated *L. monocytogenes* were virulent strains of 4b serogroup. There is a need to explore the role of such non-conventional sources in the spread of *L. monocytogenes* in nature.**

Keywords: Antibiotic sensitivity, birds, *Listeria monocytogenes*, serotyping, wild animals.

LISTERIA monocytogenes is an emerging foodborne pathogen recognized globally. Ecology of *L. monocytogenes* is atypical and has several animal and inanimate reservoirs in nature[1]. Listeria infection is mainly acquired through a variety of contaminated non-meat and meat foods. Manifestations of listeriosis both in animals and humans include septicaemia, meningitis, abortion and still birth. Several virulence factors encoded by the *hlyA*, *plcA*, *actA* and *iap* genes play a significant role in the pathogenesis of *L. monocytogenes* infections[2].

Even though several studies have demonstrated broader distribution of *Listeria* species throughout the natural environment, the role of wild animals and birds in the ecology and as reservoirs of *L. monocytogenes* is not clearly understood[3]. Limited studies on the detection of pathogenic bacteria including *L. monocytogenes* from wild animals and birds have been documented[4–7]. Studies on the detection of *Listeria* from sources other than food-processing environments may help reveal the population genetics and natural history of *Listeria* species[8]. Due to several anthropogenic consequences, emerging trends in the epidemiology of infectious diseases have been recorded[9]. Living in close proximity and sharing the same environment virtually creates zoonotic nidus. Thus, asymptomatic healthy carriers are the cause of concern. In India, adequate studies on the occurrence of *L. monocytogenes* from foods have been conducted[5,10,11]; however, studies on wild animals and birds are largely lacking. *Listeria* species may survive for a longer period in the soil and possibly get excreted in the faeces of carrier animals without any symptoms, and may cause infection to other animals as well as personnel working in the zoo and visitors. The present study was conducted to isolate *L. monocytogenes* in peridomestic birds and wild animals in captivity.

Fresh faecal samples ($n = 270$) comprising 105 from mammals and 165 from peridomestic birds were collected. Samples were collected from the nesting sites of birds (12 species) with sterile swab without touching or disturbing their habitat. Samples of wild animals (18 mammals) were collected from the Rajiv Gandhi Zoological Park and Wild Life Research Centre, Katraj, Pune, India. The birds included pigeon ($n = 80$), sparrow

*For correspondence. (e-mail: dr_kolherahul@rediffmail.com)

(n = 26), crow (n = 10), common Indian mynah (n = 10), lovebirds (n = 4), duck (n = 5), turkey (n = 5), broiler chicken (n = 5), layer chicken (n = 5), guinea fowl (n = 5), peacock (n = 5) and owl (n = 4). Wild animals in captivity sampled included spotted deers (n = 10), barking deers (n = 5), four-horned antelope (n = 4), black buck (n = 10), blue bull (n = 15), bison (n = 3), elephant (n = 2), musk deer (n = 5), leopard (n = 8), tiger (n = 8), jackal (n = 8), jungle cat (n = 3), monkey (n = 8), bear (n = 3), hyena (n = 1) and porcupine (n = 1). The collected samples were brought to the laboratory on ice for isolation within 24 h of collection.

Listeria species were isolated by the ISO 11290/1997 method with desirable modifications[12]. The samples were enriched (two-step) followed by selective plating on PALCAM and chromogenic rapid *L. mono* differential agar (HiMedia, India). Faecal samples were first enriched in half-strength Frazer broth and then in full-strength Frazer broth at 35°C for 24 h. Following enrichment, the culture was plated on selective agar and incubated at 37°C for 24–48 h. Five typical colonies showing disperse black zone of aesculin hydrolysis on PALCAM and bluish-green colonies on chromogenic agar were subcultured for further confirmation. The isolates were characterized by Gram staining, catalase positivity and motility at 20–25°C. Further characterization was done by testing for methyle red-voges-Proskauer (MR-VP) reactions, CAMP test with *Staphylococcus aureus* and *Rhodococcus equi*, reduction of nitrate, utilization of sugars (mannitol, xylose, rhamnose and α-methyl-D-mannopyranoside) followed by hemolysis on 5% ovine blood agar. *L. monocytogenes* (MTCC1143) was used as known positive culture during the study.

Detection of *Listeria* species and *L. monocytogenes* was done using multiplex PCR assay[13]. Table 1 provides details on the oligonucleotides used. DNA was extracted by snap chilling method. Briefly, 0.5 ml pure bacterial culture was pelleted at 10,000 rpm for 5 min. The pellets obtained were resuspended in 100 µl nuclease-free water and heated for 10 min in a water bath. The tubes were removed and snap-chilled immediately. Subsequently, PCR reactions were performed employing the supernatant as DNA template.

Multiplex PCR assay was performed using 25 µl reaction mixture containing 10× PCR buffer, 1 mM dNTPs, 25 mM $MgCl_2$, 50 µM primer set, 3U *Taq* DNA polymerase, DNA template (50 ng) and adjusted to 25 µl by nuclease-free water. PCR cycling conditions were set as initial denaturation at 95°C for 5 min followed by denaturation at 95°C for 30 sec, annealing at 56°C for 1 min, extension at 72°C for 2 min and one cycle of final extension at 72°C for 8 min (40 cycles). Resultant products were resolved in 1% agarose gel with 10 µg/ml ethidium bromide (EtBr) visualized under gel documentation system.

The *Listeria* isolates were serotyped using multiplex PCR[14]. The PCR reaction mixture contained 2.5 µl of PCR buffer (10×), 2 mM dNTPs, 3 mM $MgCl_2$, 0.3 µM each of primers, 2U *Taq* DNA polymerase, DNA template (50 ng) and finally adjusted to 25 µl volume by nuclease-free water. The cycling conditions for PCR were initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 40 sec, 53°C for 75 sec, and 72°C for 75 sec, and one final extension cycle at 72°C for 7 min. The resultant products were separated on 1.5% agarose gel followed by staining with EtBr (10 µg/ml).

The virulence-associated genes of *L. monocytogenes* were detected using multiplex PCR assay[15–18]. The assay was performed in 50 µl volume with 5.0 µl of 10× PCR buffer, 1 µl of 10 mM dNTPs, 4 µl 25 mM $MgCl_2$, 100 nM of each primer, 1U *Taq* DNA polymerase, and DNA template (50 ng) and adjusted to 50 µl using nuclease-free water. Multiplex PCR was performed with initial denaturation at 95°C for 2 min with further 35 cycles of denaturation at 95°C for 15 sec, annealed at 60°C for 30 sec and extended at 72°C for 90 sec followed by a final extension at 72°C for 10 min. After amplification, the products were electrophoresed in 1.5% agarose gel and visualized with EtBr (0.5 µg/ml) staining in gel documentation system.

Disc diffusion method employing Mueller Hinton agar was used for antimicrobial susceptibility testing[19]. Agar plates were inoculated with overnight-grown pure bacterial culture in BHI broth, antibiotic discs were placed on plates and incubated at 37°C for 24 h. Results were interpreted as resistant, intermediate and sensitive according to the manufacture's instructions (HiMedia Laboratories, Mumbai). The following antibiotics were used: amikacin (30 µg), ampicillin-sulbactum (10/10 µg), cefriaxone (5 µg), chloramphenicol (30 µg), erythromycin (10 µg), gentamicin (5 µg), kanamycin (30 µg), neomycin (30 µg), norfloxacin (10 µg), oxytetracycline (30 µg), penicillin-G (10 iu) and tetracycline (10 µg).

Detection of *tetA*, *tetB* and *tetC* genes was done using PCR assay[20]. The assay was carried out in 25 µl volume containing PCR master mix (12.5 µl; Thermo Scientific), 1 µl of each primer, DNA template (3 µl) and nuclease-free water to make up the volume. Primers used were: *tetA* (F-5′-GGCGGTCTTCTTCATCATGC-3′ and R-5′-CGGCAGGCAGAGCAAGTAGA-3′), *tetB* (F-5′-CATTAATAGGCGCATCGCTG-3′ and R-5′-TGAAGGTCATCGATAGCAG-3′) and *tecC* (F-5′-GCTGTAGGCA-TAGGCTTGGT-3′ and R-5′-GCCGGAAGCGAGAAGAATCA-3′). Amplification of DNA was performed as follows: 95°C for 15 min of initial denaturation; 34 cycles of 94°C for 30 sec, 64°C for 90 sec, 72°C for 90 sec and final extension at 72°C for 15 min. Amplicons were electrophoresed in 1.5% agarose gel with EtBr (0.5 µg/ml) and visualized under gel documentation system.

Out of 270 faecal samples examined, *Listeria* species were isolated from 7 (6.66%) mammals and 2 (1.21%) bird species. Among wild animals in captivity, *Listeria* species were detected in seven animals: spotted deer

Table 1. Multiplex PCR assays performed

Target genes	Sequence (5′–3′)	Product size (bp)	Reference
Detection of genus *Listeria* and *Listeria monocytogenes*			
prs	F-5′-AGCTGAAGAGATTGCGAAAGA-3′	844	13
	R-5′-TTCACCAAGAAGAGCTGCAA-3′		
isp	F-5′-TGCAGCGAATGCTCTTAGTG-3′	713	
	R-5′-AGCCAAGCACGGCTACTTTA-3′		
L. monocytogenes serotyping			
Lmo0737	F-5′-AGGGCTTCAAGGACTTACCC-3′	691	14
(*1/2a, 1/2c, 3a, 3c*)	R-5′-ACGATTTCTGCTTGCCATTC-3′		
ORF2819	F-5′-AGCAAAATGCCAAAACTCGT-3′	471	
(*1/2b, 3b, 4b, 4d, 4e*)	R-5′-CATCACTAAAGCCTCCCATTG-3′		
ORF2110	F-5′-AGTGGACAATTGATTGGTGAA-3′	597	
(*4b*, *4d*, and *4e*)	R-5′-CATCCATCCCTTACTTTGGAC-3′		
prs	F-5′-GCTGAAGAGATTGCGAAAGAAG-3′	370	
(all *Listeria* spp.)	R-5′-CAAAGAAACCTTGGATTTGCGG-3′		
Detection of virulence genes of *L. monocytogenes*			
plcA	F-5′-CTGCTTGAGCGTTCATGTCTCATCCCCC-3′	1484	15
	F-5′-CATGGGTTTCACTCTCCTTCTAC-3′		
hlyA	F-5′-GCAGTTGCAAGCGCTTGGAGTGAA-3′	456	16
	R-5′-GCAACGTATCCTCCAGAGTGATCG-3′		
actA	F-5′-CGCCGCGGAAATTAAAAAAAGA-3′	839	17
	R-5′-ACGAAGGAACCGGGCTGCTAG-3′		
iapA	F-5′-ACAAGCTGCACCTGTTGCAG-3′	131	18
	R-5′-TGACAGCGTGTGTAGTAGCA-3′		

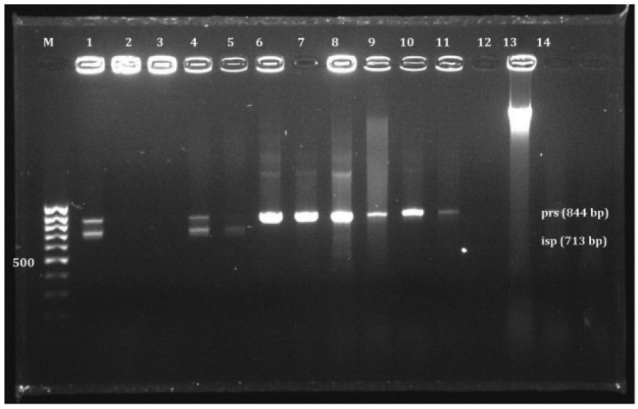

Figure 1. Detection of genus *Listeria* and *Listeria monocytogenes* by multiplex PCR. M, 100 bp DNA ladder; lanes 1–4, *prs* & *isp* genes; lanes 6–11, *prs* gene.

(2/10), black buck (1/10), bison (1/3), jackal (1/8), barking deer (1/5) and porcupine (1/1). Further biochemical characterization confirmed *L. monocytogenes* from samples collected from barking deer and porcupine. Similarly, examination of peridomestic birds yielded *L. monocytogenes* from the droppings of pigeon and crow (Figure 1). Faecal samples screened from other species of mammals and birds were negative for *L. monocytogenes*.

Interestingly, all the four *L. monocytogenes* strains, two from mammals and two from birds were of serogroup 4b as revealed by multiplex serotyping PCR (Figure 2). The virulence genes *plcA*, *hlyA*, *actA* and *iap* were found to be present in all the *L. monocytogenes* strains (Figure 3).

Isolates were sensitive to all the 12 antimicrobials tested by disc diffusion method, except one *L. monocytogenes* isolate from porcupine that was resistant to neomycin. *L. monocytogenes* strains were also screened for the presence of genotypic resistance to tetracycline; however, none of the isolates was positive for *tetA*, *tetB* and *tetC* genes.

L. monocytogenes serogroup 1/2a, 1/2b and 4b strains are largely implicated in human foodborne listeriosis outbreaks. *Listeria* species are saprophytes having environmental reservoirs and they may enter into the food chain through various routes. Infection of *L. monocytogenes* is mainly acquired through contaminated food; several studies have highlighted the incidence of this pathogen in foods and food-processing environment[21,22]. However, not may studies have been conducted on non-conventional sources of *L. monocytogenes* like the role of wild animals and birds. It is also a poorly studied pathogen in the Indian context[10]. Therefore the aim of the present study was to examine different captive wild animals and bird species for the detection of pathogenic serovars of *L. monocytogenes*. Faecal samples of all the mammalian species reared in the Rajiv Gandhi Zoological Park and Wildlife Research Centre, Pune were screened for *L. monocytogenes*. Simultaneously, faecal droppings of peridomestic birds were also screened. The occurrence of *Listeria* species (6.66%) and *L. monocytogenes* (1.90%)

was found to be low. The detection of virulent strains of 4b serogroup in barking deer (*Muntiacus muntjak*), porcupine (*Hystrix indica*), pigeon (*Columba livia*) and crow (*Corvus splendens*) is a cause of concern. Prevalence of *L. monocytogenes* in captive wild animals[4,23,24] and bird species[4,6,25] has been studied earlier worldwide. Recently, the red deer has been reported to excrete *L. monocytogenes* and other zoonotic pathogens in the environment in Europe[26]. In India, *Listeria* species have been detected in wild animals in captivity and birds[5,7,27]. Faecal samples of 623 wild mammals were screened in Japan and were found to harbour *Listeria* species and *L. monocytogenes* in 6.1% and 1% samples respectively[4]. *L. monocytogenes* was detected in Japanese monkey (1.25%), red fox (1.33%), raccoon dog (0.96%), Japanese marten (0.79%) and Sika deer (1.05%). In contrast, comparatively higher prevalence of *L. monocytogenes* (18.6%) in zoo animals of Taronga Zoological Garden, Australia was recorded[23]. High detection rates of *Listeria* species (21%) and *L. monocytogenes* (7%) were recorded in faeces of healthy animals from Antwerp Zoo, Belgium[24]. Fairly high prevalence of *L. monocytogenes* (10.57–18%) in zoo animals and birds was reported from India[5,27]. Yadav *et al.*[7] recovered *Listeria* species (5.36%) and *L. monocytogenes* (1.79%) from faecal samples of healthy wild animals and birds from Gujarat, India.

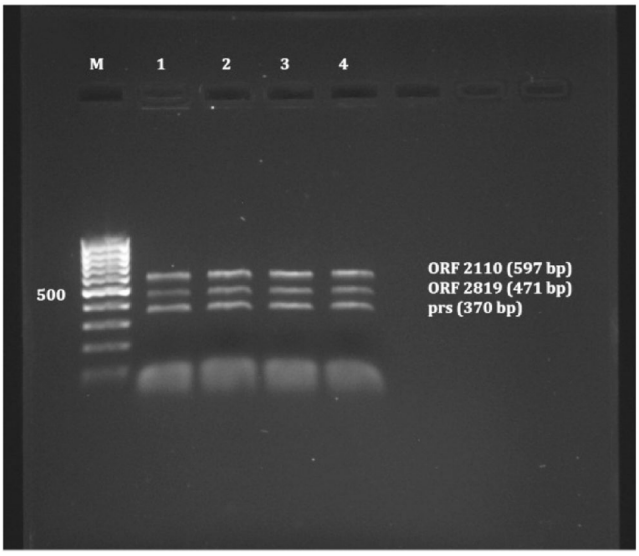

Figure 2. Multiplex PCR-based serotyping of *L. monocytogenes*. M, 100 bp DNA ladder; lanes 1–4, ORF2110, ORF2819, and *prs* genes.

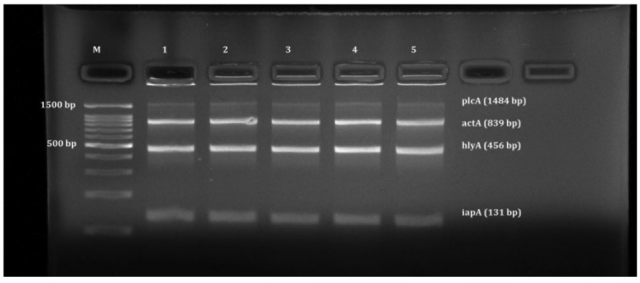

Figure 3. Detection of *L. monocytogenes* virulence genes. M, DNA ladder; lane 1, *L. monocytogenes* MTCC1143; lanes 2–5, *L. monocytogenes* isolates studied.

Screening of the 165 faecal samples from 12 birds species revealed 2 *Listeria* species, both being virulent *L. monocytogenes* serogroup 4b strains. Droppings of Indian pigeon and crow contained *L. monocytogenes.* This was detected in 13.4% birds from Japan earlier[4]. In the present study, we could not detect *Listeria* species and *L. monocytogenes* in broiler-layer chickens, sparrows, Indian mynah, duck, turkey, guinea fowl and lovebirds. Earlier, *L. monocytogenes* was isolated from 36% faecal samples from wild birds of Helsinki region[6] and the most common serotypes detected were 1/2a, followed by 4b and 1/2b. Healthy wild birds were found to carry *L. monocytogenes* in their intestinal contents with higher prevalence of *L. monocytogenes* at a landfill site than in urban areas[6].

Variations in the prevalence rates can be attributed to several factors like difference in feeding habitat and living environment. Detection of *Listeria* species, including virulent *L. monocytogenes* strains in captive wild animals and peridomestic birds is indicative of their role as reservoirs or carriers of this pathogen. Undoubtedly, *L. monocytogenes* is a soil-borne pathogen; how it has been detected in different animal reservoirs is debatable. Antibiotic resistance was not detected in any of the isolates, except one strain showing resistance against neomycin. *Listeria* species sensitive to ciprofloxacin, levofloxacin, amoxicillin, azithromycin and enrofloxacin, and resistant to gentamicin, oxytetracycline, penicillin-G, cephadroxil, and nalidixic acid have been observed earlier[25]. In this study, isolates were sensitive to amikacin, ampicillin-sulbactum, cefriaxone, chloramphenicol, erythromycin, gentamicin, kanamycin, norfloxacin, oxytetracycline, penicillin-G and tetracycline. Tetracycline resistance has been extensively studied and shown to be widespread in bacterial populations. It has been widely used in animals as a therapeutic agent and animal growth promoter in the past. Thus we wanted to understand the diversity of tetracycline genes in *L. monocytogenes*. However, *tetA*, *tetB* and *tetC* genes could not be detected.

Although ubiquitous, the ecology of *L. monocytogenes* in outside environments is poorly understood[3]. Studies on *L. monocytogenes* in faeces of zoo animals and birds from India are scanty and there is great dearth of research on understanding the molecular epidemiology and ecological niche of *L. monocytogenes* in diverse environments. The present study showed the presence of virulent *L. monocytogenes* in captive wild animals and peridomestic birds harbouring similar serotype as that reported earlier to be predominant in the Indian subcontinent[28]. Further studies are warranted to understand the sources and diversity of *Listeria* species in newer niches.

1. Szymczak, B., Szymczak, M., Sawicki, W. and Dabrowski, W., Anthropogenic impact on the presence of *L. monocytogenes* in soil, fruits and vegetables. *Folia Microbiol*., 2014, **59**, 23–29.
2. Rawool, D. B., Malik, S. V. S., Barbuddhe, S. B., Shakuntala, I. and Aurora, R. A., Multiplex PCR for detection of virulence associated genes in *L. monocytogenes. Int. J. Food Saf.*, 2007, **9**, 56–62.
3. Vivant, A. L., Garmyn, D. and Piveteau, P., *Listeria monocytogenes,* a down-to-earth pathogen. *Front. Cell. Infect. Microbiol*., 2013, **3**, 1–10.
4. Yoshida, T., Sugimoto, T., Sato, M. and Hirai, K., Incidence of *Listeria monocytogenes* in wild animals in Japan. *J. Vet. Med. Sci.*, 2000, **62**, 673–675.
5. Kalorey, D. R., Kurkure, N. V., Warke, S. R., Rawool, D. B., Malik, S. V. S. and Barbuddhe, S. B., Isolation of pathogenic *Listeria monocytogenes* in faeces of wild animals in captivity. *Comp. Immunol. Microbiol. Infect. Dis*., 2006, **29**, 295–300.
6. Hellstrom, S., Kiviniemi, K., Autio, T. and Korkeala, H., *Listeria monocytogenes* is common in wild birds in Helsinki region and genotypes are frequently similar with those found along the food chain. *J. Appl. Microbiol*., 2008, **104**, 883–888.
7. Yadav, M. M., Roy, A., Bhanderi, B. and Jani, R. G., Prevalence of Listeria species including *L. monocytogenes* from apparently healthy animals at Baroda Zoo, Gujarat State, India. *J. Threat. Taxa*, 2011, **3**, 1929–1935.
8. Saunders, D. B., Overdevest, J., Fortes, E., Windham, K., Schukken, Y., Lembo, A. and Wiedmanna, M., Diversity of *Listeria* species in urban and natural environments. *Appl. Environ. Microbiol.*, 2012, **78**, 4420–4433.
9. Morse, S. S., Factors in the emergence of infectious diseases. *Emerg. Infect. Dis*., 1995, **1**, 7–15.
10. Barbuddhe, S. B., Malik, S. V. S., Kumar, J. A., Kalorey, D. R. and Chakraborty, T., Epidemiology and risk management of listeriosis in India. *Int. J. Food Microbiol*., 2012, **154**, 113–118.
11. Doijad, S. *et al.*, Incidence and genetic variability of *Listeria* species from three milk processing plants. *Food Control*, 2011, **22**, 1900–1904.
12. Anon., ISO 11290 Part 1:1997 – horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection, International Organization for Standardization, Geneva, 1997.
13. Rawool, D. B. *et al.*, A multiplex PCR for detection of *Listeria monocytogenes* and its lineages. *J. Microbiol. Methods*, 2016, **130**, 144–147.
14. Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C. and Martin, P., Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol*., 2004, **42**, 3819–3822.
15. Notermans, S. H. W., Dufrenne, J., Leimeister-Wachter, M., Domann, E. and Chakraborty, T., Phosphatidylinositol specific phospholipase C activity as a marker to distinguish between pathogenic and non-pathogenic *Listeria* species. *Appl. Environ. Microbiol*., 1991, **57**, 2666–2670.
16. Paziak-Domanska, B. *et al.*, Evaluation of the API test, phosphatidylinositol-specific phospholipase C activity and PCR method in identification of *Listeria monocytogenes* in meat foods. *Fed. Eur. Microbiol. Soc. Microbiol. Lett*., 1999, **171**, 209–214.
17. Suarez, M. and Vazquez-Boland, J. A., The bacterial actin nucleter protein ActA is involved in epithelial cell invasion by *Listeria monocytogenes*. PUBMED, 2001 (accession no. AF103807).
18. Furrer, B., Candrian, U., Hoefelein, C. and Luethy, J., Detection and identification of *Listeria monocytogenes* in cooked sausage products and in milk by *in-vitro* amplification of haemolysin gene fragments. *J. Appl. Bacteriol*., 1991, **70**, 372–379.
19. Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M., Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol*., 1966, **45**, 493–496.
20. Lanz, R., Kuhnert, P. and Boerlin, P., Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. *Vet. Microbiol*., 2003, **91**, 73–84.
21. Chasseignaux, E., Gerault, P., Toquin, M. T., Salvat, G., Colin, P. and Ermel, G., Ecology of *Listeria monocytogenes* in the environment of raw poultry meat and raw pork meat processing plants. *FEMS Microbiol. Lett*., 2002, **210**, 271–275.
22. Ho, A. J., Lappi, V. R. and Wiedmann, M., Longitudinal monitoring of *Listeria monocytogenes* contamination patterns in a farmstead dairy processing facility. *J. Dairy Sci*., 2007, **90**, 2517–2524.
23. Arumugaswamy, R. and Gibson, I., *Listeria* in zoo animals and rivers. *Aust. Vet. J*., 1999, **77**, 819–820.
24. Bauwens, L., Vercammmen, F. and Hertsens, A., Detection of pathogenic *Listeria* spp. in zoo animal faeces: use of chromogenic separation and a chromogenic isolation medium. *Vet. Microbiol*., 2003, **91**, 115–123.
25. Fenlon, D. R., Wild birds and silage as reservoirs of *Listeria* in the agricultural environment. *J. Appl. Bacteriol.*, 1985, **59**, 537–543.
26. Gnat, S. *et al.*, Experimental studies of microbial populations and incidence of zoonotic pathogens in the faeces of red deer (Cervus elaphus). *Lett. Appl. Microbiol*., 2015, **61**, 446–452.
27. Sarangi, L. N. and Panda, H. K., Occurrence of *Listeria* species in different captive wild animals of Nandankanan Zoo, Baranga, Odisha, India. *J. Threat. Taxa*, 2013, **5**, 3542–3547.
28. Barbuddhe, S. B. *et al.*, Presence of a widely disseminated *Listeria monocytogenes* serotype 4b clone in India. *Emerg. Microbes Infect*., 2016, **5**, e55.

ACKNOWLEDGEMENTS. We thank the Associate Dean, KNP College of Veterinary Science, Satara, for support and In Charge, Central Instrumentation Facility for providing the necessary laboratory facilities. The research was supported by grants from the Department of Biotechnology, Government of India to R.P.K. and S.B.B.

Received 12 March 2017; revised accepted 17 May 2017

doi: 10.18520/cs/v113/i09/1783-1787