

East African Medical Journal Vol. 81 No. 10 October 2004

CARRIER STATUS FOR *LISTERIA MONOCYTOGENES* AND OTHER *LISTERIA* SPECIES IN FREE RANGE FARM AND MARKET HEALTHY INDIGENOUS CHICKENS AND DUCKS

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**CARRIER STATUS FOR *LISTERIA MONOCYTOGENES* AND OTHER *LISTERIA* SPECIES IN FREE RANGE FARM AND MARKET HEALTHY INDIGENOUS CHICKENS AND DUCKS**

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**ABSTRACT**

**Background:** *Listeria* organisms are documented to be zoonotic; one of the sources of infection is the domestic fowl where it could occur as in apparent infection. The carriage of *Listeria monocytogenes* and other *Listeria* in indigenous birds has not been documented in Kenya.

**Objective:** To establish whether healthy looking indigenous chickens and ducks could be carriers of *Listeria monocytogenes* and other *Listeria* species.

**Design:** Field survey of indigenous chickens and ducks in three districts of Kenya.

**Setting:** Embakasi and Dagoreti divisions in Nairobi district; Athi river division in Machakos district; and Ngong division in Kajiado district, in Kenya.

**Subjects:** One hundred and thirty six indigenous chickens and 39 ducks reared under free range scavenging system in Nairobi, Machakos and Kajiado districts, in Kenya, were sampled.

**Methods:** In surveying the birds, the cloacal and pharyngeal swabs were taken from each bird separately using sterile cotton - tipped applicator swabs. The swabs in saline were transported in a coolbox to the laboratory for bacterial isolation and characterization.

**Interventions:** None (only compared farmed and the traded birds).

**Main outcome measures:** Isolation of *Listeria* species and pathogenicity of *Listeria* isolates.

**Results:** Two *Listeria monocytogenes* and seven other *Listeria* species were recovered from the oropharyngeal swab samples of farm and market chickens but none from respective cloacal swabs. No *Listeria* was recovered from either oropharyngeal or cloacal swabs of farmed duck and slaughter chickens. Traded chickens yielded more *Listeria* isolates as compared to farmed chickens.

**Conclusion:** This study shows that indigenous chickens in Kenya are carriers of *Listeria monocytogenes* and other *Listeria* species.

**INTRODUCTION**

*Listeria monocytogenes* bacteria are frequently overlooked as a possible causative agent of food poisoning. Six *Listeria* species are recognised, namely, *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* and *L. grayi*. Of these, *L. monocytogenes* and *L. ivanovii* are the most important pathogens(1). *Listeria monocytogenes* has been documented as a cause of food poisoning following consumption of contaminated coleslaw(2), Mexican - style cheese(3), soft ripened cheese(4,5), sausages(5-7), chickens(5,8,9), and turkey Frankfurters(5,10). Twenty percent of the 1,600-listeriosis cases seen per year in United States of America are due to consumption of uncooked hot dogs and undercooked chicken(11). Furthermore, *Listeria monocytogenes* contamination rates in minced meat products have been shown to be: turkey (20%) beef

(52%), pork (80%) and chicken (85%), respectively(5).

The natural habitats of *Listeria monocytogenes* include; soil, sewage, green plant material, decaying vegetation and silage (pH over 5.5)(12). Human foods associated with listeriosis in man include coleslaw, soft cheeses, milk and poultry meat(12). Soil contamination and ingestion of food contaminated with faeces from birds and other animals are the primary modes of transmission of *Listeria monocytogenes*(1). *Listeria monocytogenes* can grow over a wide range of temperatures (-0.4°C to 50°C) and is relatively resistant to heat(13,14). Food poisoning outbreaks occur following consumption of contaminated food products that have been inadequately cooked and heat treated or those that are consumed raw.

Since food - poisoning cases are constantly reported in Kenya, some of them due to *Listeria monocytogenes* (unpublished data), this study was undertaken to establish whether healthy looking indigenous chicken could be

carriers of *Listeria monocytogenes* thus act as silent source of food poisoning to man. So far there is no documentation on occurrence of this organism in indigenous chickens and ducks.

## MATERIALS AND METHODS

**Study area:** The study was carried out in Embakasi and Dagoreti divisions in Nairobi district; Athi river division in Machakos district; and Ngong division in Kajiado district, in Kenya. Indigenous chickens were sampled from four farms in Embakasi division and three farms from Athi river division. Indigenous ducks were sampled from two farms each in Embakasi, Dagoreti and Ngong divisions.

More village chickens were sampled at Kariokor, and Burma - Maziwa slaughter houses, Jogoo road and Kariokor open - air markets, and South and Westlands shopping centers in Nairobi districts. Swabs were taken prior to slaughter of the birds in the slaughterhouses or swabbed and returned into their cages for birds in the open-air markets.

**Birds sampled:** A total of 136 local indigenous village chickens were sampled. Of these, 55 were from peri - urban smallholder farms, 41 from the slaughterhouses, and 40 from chicken traders. All 39 ducks were from urban and peri - urban smallholder farms.

Indigenous chickens being scavengers are likely to get to hay and decaying objects and thus more opportunity of being reservoirs of *Listeria monocytogenes* compared to exotic birds. They are transported into the city in large numbers hence a likely source of food poisoning.

**Sample collection and handling:** Cloacal and oropharyngeal swabs were taken separately using sterile cotton - tipped applicator swabs. The swabs were placed in 2 ml of physiological saline and transported on ice in a cool box to the laboratory for bacterial isolation.

**Experimental mice for bacterial isolation and pathogenicity test:** Three week old, balb - C mice, free from *Listeria*, were used in this study. Both males and females were used for *Listeria* isolation and for pathogenicity testing. Mice colonies were raised and maintained within the university premises.

**Bacterial isolation:** Within one hour of sampling, each swab, was processed in two ways to increase the chances of recovering *Listeria monocytogenes*:

**Direct isolation:** The swabs were directly streaked onto 3.5 % potassium tellurite blood agar (PTBA) (BDH Chemicals Ltd. Poole, England) plate and blood agar base (CM 55: Oxoid Ltd., Basingstoke, Hampshire, England) with 5% citrated sheep blood, and then incubated at 37°C aerobically for 24 to 48 hours.

**Indirect isolation:** The content of each swab was thoroughly vortexed and 0.5 ml inoculated intraperitoneally into mice, which were observed for 48 hours. Dead mice and mice sacrificed after 5 days were post mortemed and the liver and spleen aseptically removed and macerated using sterile

wire loop. The macerated materials were streaked onto potassium tellurite blood agar plates separately, and incubated aerobically at 37°C for 48 hours.

Subcultures of isolates recovered by both methods were made onto sheep blood agar and incubated aerobically at 37°C for 48 hours, to obtain pure cultures for characterisation. The isolates were characterised following the criteria given by Bergey's manual of determinative bacteriology(15) and Cowan and Steel's Manual for the identification of medical bacteria(16).

**Characterisation of *Listeria monocytogenes*:** All black, small, pin - point colonies on PTBA that were catalase positive, oxidase negative and motile at 25°C and did not grow on sodium azide crystal violet blood agar (SACVBA) (Oxoid) were presumed to be *Listeria* species and were further identified through mice pathogenicity tests and biochemical tests (1,12,15,17,18). The reactions of the isolates were compared with those of *Listeria monocytogenes* type strains (L028 and DGH) kindly donated by professor John Olsen of the Royal Veterinary and Agriculture University, Denmark.

**Mice pathogenicity testing for *Listeria* isolates:** Five mice were inoculated with each suspect isolate or type strain of *Listeria* species. Each mouse was inoculated intraperitoneally with 0.5ml of 10<sup>9</sup> cfu /ml of *Listeria* organisms. The mice were kept in separate cages and correspondingly labeled for the respective *Listeria* isolate or type culture. The mice were observed for at least 5 days for any clinical signs and mortality. Any mouse that remained alive was euthanized. From each mouse, the liver and the spleen were thoroughly examined for necrotic foci and splenomegaly, respectively.

Once recovered and characterised, the isolates were coded as follows: LW1, LW2, LW3, LW4, LW5, LW6, LW7, LW8 and LW9.

## RESULTS

***Listeria* isolation:** Table I shows the *Listeria* organisms recovered from farms, markets, trading centres and slaughterhouses with respect to indigenous chickens and ducks.

Of the 350-oropharyngeal and cloacal swab samples from 175 indigenous birds (136 chickens and 39 ducks) only nine oropharyngeal samples from chicken yielded *Listeria* organisms. No *Listeria* organism was isolated from duck samples. Of these, one isolate was from a farm in Nairobi, while eight isolates were from the market / trading centres. No isolates were recovered from the slaughter chickens, farm ducks and all the cloacal swabs examined.

Table 2 shows the species and source of the *Listeria* isolates from various indigenous chickens. Of the nine isolates, two (22.2%) were *L. monocytogenes*, three (33.3%) *L. innocua*, two (22.2%) *L. seeligeri* and one (11.1%) each of *L. grayi* and *L. murrayi* respectively.

Table 1

*Listeria* isolations from farm, market and slaughter chickens and farm ducks

Type of birds	Source of birds		Number of birds sampled	<i>Listeria spp</i> isolation / number of samples	
	Sampling point	District		C	P
Chickens	Farm	Nairobi	26	0/26	1/26
	Farm	Machakos	29	0/29	0/29
	Total farm		55	0/55	1/55
	Market	Nairobi	40	0/40	8/40
	Slaughter house	Nairobi	41	0/41	0/41
Total chickens			136	0/136	9/136
Ducks	Farm	Nairobi	34	0/34	0/34
	Farm	Kajiado	5	0/5	0/5
Total ducks			39	0/39	0/39
Total chickens and ducks			175	0/175	9/175

C = Cloacal sample; P = Oro - pharyngeal samples

Table 2

*The species of Listeria isolated from farms and market chickens*

Source	No. of <i>Listeria</i> isolates	<i>Listeria</i> species	Laboratory code
Markets	8	<i>Listeria monocytogenes</i>	LW1
		<i>Listeria monocytogenes</i>	LW2
		<i>Listeria seeligeri</i>	LW3
		<i>Listeria innocua</i>	LW4
		<i>Listeria innocua</i>	LW5
		<i>Listeria innocua</i>	LW6
		<i>Listeria grayi</i>	LW7
		<i>Listeria murrayi</i>	LW8
Farms	1	<i>Listeria seeligeri</i>	LW9

*Mice pathogenicity test for Listeria isolates:* One *Listeria monocytogenes* and one *L. innocua* were found to be pathogenic, with *L. monocytogenes* (LW2) killing 80% of the mice within 72 hours. One isolate of *L. monocytogenes* (LW1) did not kill or cause clinical signs in mice. Interestingly *L. innocua* (LW4) did kill mice (60%) and made mice to be dull. Both reference *Listeria monocytogenes* strains were pathogenic to mice. The main clinical signs observed were, dullness and diarrhoea. The other seven *Listeria* isolates were non-pathogenic to mice.

## DISCUSSION

Systematic investigation of the occurrence of *Listeria monocytogenes* and other *Listeria* species has not previously been done on healthy appearing chickens and ducks from farms, market places and slaughterhouses in Kenya. In this study, five different *Listeria* species were isolated from village scavenging chickens. These were *Listeria monocytogenes*; *Listeria innocua*; *Listeria seeligeri*; *Listeria grayi* and *Listeria murrayi*. All these

isolates were recovered from oropharyngeal swabs and none from the cloacal. The recovery rate (5.14%) was quite low mainly because the samples were not enriched. This finding is supported by the results of Ryser *et al.*(9), who found that isolation rates for *Listeria monocytogenes* and the other *Listeria spp.* typically improve when samples are enriched in more than one primary enrichment medium. The faecal samples may have improved the recovery compared to cloacal swabs. More isolates were recovered from market birds than farmed perhaps due to stress of transportation to the market and crowding in the bird's cages at the markets. This confirms that healthy appearing village chicken are carriers of *Listeria monocytogenes* and other *Listeria* species and market birds are more likely to carry *Listeria* species compared to farm birds. The situation for ducks is not so clear as no isolation was made. This observation agrees with the work of Quinn *et al.*(12) who reported the occurrence of asymptomatic faecal carriers in man and domestic animals.

Epidemics of human listeriosis have been traced to ingestion of contaminated milk(12,13), cheese (2,3,

19,20), vegetables (21), poultry and other meat products(11). The possible presence of latent carriers in a chicken flock presents a definite public health hazard.

Felsenfeld(22) reported an outbreak of *Listeria* conjunctivitis in two poultry processing plant workers. *Listeria monocytogenes* was isolated from the spleens of five apparently healthy birds, which were being processed. It was found that these birds originated from the same area where Hurt *et al*(23) had diagnosed listeriosis four years before. This is one of the few instances where a definite mode of *Listeria* infection in man could be traced to infection in animals or birds.

The few *Listeria* isolates recovered in this study show that there is a potential risk to the farming, trading and poultry consuming communities in Kenya. The carrier birds can also transmit the *Listeria* species to other birds as reported by Cooper and Arthur(24), through environmental faecal contamination, especially the commercial breeds, causing losses of upto 40%. Primary listeriosis in birds is most commonly manifested by a septicæmia and the bacterium can be isolated from most of the viscera, particularly the liver or spleen(25,26).

Seeliger and Jones(27) have shown that *L. ivanovii* is pathogenic to sheep. Although, *L. innocua*; *L. seeligeri*; *L. grayi* and *L. welshmeri* are considered to be generally non-pathogenic, there is some evidence suggesting that some strains of both *L. seeligeri*(28, 29) and *L. innocua*(30) are occasionally pathogenic to man and animals, respectively. *Listeria innocua* has also been isolated from cases of encephalitis in ruminants, namely: Rocourt and Seeliger(30) reported one case of *L. innocua* in a deer and one in a cow while Nicolas *et al*(31) reported seven cases of encephalitis in sheep caused by *L. innocua*. The usual mode of transmission of *Listeria* organisms is through ingestion of contaminated faeces. The carrier birds with oropharyngeal infestation may also transmit the *Listeria* organisms to man and other animals through nasal and mouth discharges.

Therefore, scavenging local chicken and ducks, having been shown to be carriers of *Listeria* species, can actually play a major role in the transmission of these organisms to humans and to other livestock, especially ruminants. Mice pathogenicity results for *Listeria* isolates from chickens showed that, two isolates out of nine were pathogenic to mice. The two were identified as *Listeria monocytogenes* (LW2) and *Listeria innocua* (LW4) respectively. These, findings are similar to those of Hirsh and Zee(1), who reported *Listeria monocytogenes* and *Listeria ivanovii* as important pathogens of mice. Rocourt and Seeliger(30) also reported some pathogenic strains of *Listeria innocua*, which is in agreement with this study's findings. One of the isolates (LWI) with biochemical reactions similar to *Listeria monocytogenes* type strains was not pathogenic to mice. The production of a sulfhydryl - activated haemolysin, listeriolysin O (alpha listeriolysin) has been a factor associated with the

pathogenic potential of *L. monocytogenes*(32). Not all strains of *L. monocytogenes* are pathogenic(33). Rough variants of the organism have been shown to possess reduced virulence(34).

This study has shown that local scavenging chicken and possibly ducks are carriers of *Listeria monocytogenes* and other *Listeria* species and therefore potential reservoirs of the zoonotic infections to humans.

#### ACKNOWLEDGEMENTS

To the Chairman, Department of Veterinary Pathology and Microbiology, University of Nairobi for allowing the use of the laboratory facilities and also messrs J. Kibe, J. Matata, Z. Munene, J. Waweru, H. Kinyua and Mrs. M. Mutune and M. Wanjiru for technical assistance. The financial support for the investigation was provided through the Danida Enreca project, 'Improving the health and productivity of rural chickens in Africa', which is gratefully acknowledged.

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