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Amandus P Muhairwa

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Occurrence of *Pasteurella multocida* and related species in village free ranging chickens and their animal contacts in Tanzania

A.P. Muhairwa[a,b,*], M.M.A. Mtambo[a], J.P. Christensen[b], M. Bisgaard[b]

[a]*Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania*

[b]*Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University, Stigbøjlen 4, DK-1870 Frederiksberg C, Copenhagen, Denmark*

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Abstract

Investigation was done to determine the presence of *Pasteurella multocida* and related species in free ranging chickens and ducks, dogs, cats and pigs in three climatic zones (cool, warm and hot) of rural Morogoro, Tanzania. A total of 153 isolates of *P. multocida* ssp. *multocida* and related species were obtained by direct culture on blood agar, selective medium and mouse inoculation. *P. multocida* ssp. *multocida* was isolated from 0.7% of chickens and 7% of ducks. In dogs and cats, *P. multocida* ssp. *multocida* was isolated from 1 and 68%, respectively. One isolate of *Pasteurella gallinarum* was isolated from a duck. Other species obtained were; *P. multocida* ssp. *septica*, *Pasteurella stomatis* and taxon 16 from dogs and cats, while *Pasteurella dagmatis* and *Pasteurella canis* were found in dogs only. Prevalence of *P. multocida* ssp. *multocida* was significantly higher ($P < 0.01$) in ducks of the warm zone (22%) than in ducks of other zones (0%). No significant difference was observed between the prevalence of *P. multocida* ssp. *multocida* in chickens of the warm zone (2%) and chickens of the cool and hot zones (0%). Extended phenotypic characterization revealed phenotypic similarities between two isolates from chickens and the duck strains. Mouse inoculation appeared to be more sensitive in detecting *P. multocida* ssp. *multocida* than blood agar and selective medium. Direct culture on blood agar recovered most of the isolates from dogs. This study has demonstrated for the first time the presence of *P. multocida* and related species in the village free ranging chickens, ducks, dogs and cats in Tanzania. Other non-classified *Pasteurella* spp. were also observed in the study, but further characterization is required before the final classification can be made. This paper reports for the first time the isolation of unclassified *Pasteurella* from dogs and cats in Africa. The results implies that fowl cholera might be occurring

* Corresponding author. Tel.: +45-35-28-27-49; fax: +45-35-28-27-57.
E-mail address: apm@kvl.dk (A.P. Muhairwa).

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in free ranging poultry, and dogs and cats kept in contact might serve as sources of *P. multocida* to chickens and ducks. Subsequent applications of molecular techniques to analyse the epidemiological relatedness of clones isolated from different host species is indicated.

Keywords: *Pasteurella multocida*; *Pasteurellaceae*; Carriers; Chicken-bacteria; Ducks; Dogs; Cats

1. Introduction

Lack of reliable animal diseases statistics in Tanzania as in most developing countries, limits the information of disease prevalence and significance in free ranging village chickens. Also the availability of dead birds from the villages for post-mortem examination is difficult. Most dead birds are either eaten by predators, fed to dogs and cats, or are thrown away so that most cases go unreported (Melewas, 1989). In other incidences sick birds may be slaughtered for human consumption. This means that information on the prevalence and significance of disease agents can only be obtained through indirect studies on the carrier rate of the causal agents in "healthy" birds or serology.

The prevalence of *Pasteurella multocida* and its disease significance in poultry, cattle, small ruminants, pigs, rabbits and other animals has been studied extensively in the past century (Barnum, 1990). Fowl cholera has been reputed a leading killer of domestic and wild birds in some developed countries (Rimler and Glisson, 1997). The few available reports from the developing world show the presence of fowl cholera in village free ranging chickens in Asia (Thitisak et al., 1989; Aini, 1990) and Zimbabwe (Kelly et al., 1994).

The potential of domestic cats as natural carriers of *Pasteurella* in causing Pasteurellosis in feral birds has been indicated by Korbel et al. (1992). Subsequently, van Sambeek et al. (1995) demonstrated pathogenic serotypes of *P. multocida* in the buccal cavities of cats kept as mousers in poultry farms. In wild mammals and birds, Snipes et al. (1988) identified somatic serotypes of *P. multocida* isolates from two out of seven premises examined to be the same as somatic serotypes of infected turkeys in the same location. Recently, Christensen et al. (1998) showed that a single clone of *P. multocida* ssp. *multocida* affected several avian species in an outbreak involving different geographic regions. The same clone was subsequently demonstrated in semi-confined commercial poultry (Christensen et al., 1999). Although village free ranging poultry are always in contact with other domestic animals, no documented report is available on the role of this contact in disease occurrence. In addition, no reliable knowledge about the occurrence of fowl cholera in Tanzania or other developing countries is available.

Reclassification of the genus *Pasteurella* sensu stricto by Mutters et al. (1985) has resulted into 11 recognised species within the genus. The reorganisation has lead into addition of new species and removal of organisms previously classified under the genus *Pasteurella.* This has made it difficult to identify with precision organisms reported in previous studies. Thus, the epidemiology of these organisms is little known, and reinvestigation of the epidemiology of the organisms is needed (Christensen and Bisgaard, 1997).

It was the objective of the present work to investigate the occurrence of *P. multocida* and other related species in chickens, ducks, dogs, cats, and pigs kept in the same environment. Since haemorrhagic septicaemia in ruminants seems to be associated with certain geographic areas (Carter and De Alwis, 1989) this study also aimed at observing the effect of climate on the presence of *P. multocida* in domestic animals in the villages. Moreover, high and low environmental temperatures have been shown to have different effects on the pathogenesis of fowl cholera in turkeys (Simmensen et al., 1980). This paper presents the first report in Tanzania and Africa about the carrier rate of *P. multocida* and related species after reclassification of the genus *Pasteurella* by Mutters et al. (1985).

2. Materials and methods

2.1. Study area

The study was conducted in Morogoro Region, Tanzania. The sampling area was divided into three zones; cool, warm and hot zones. In all three zones, heavy rains due to the El-Niño effect dominated throughout the sampling period. In each zone two villages were selected as follows; Langali and Nyandira from the cool highland area (Mgeta), Kiroka and Mkuyuni from the warm zone (Mkuyuni) and Kipera and Kongavikenge from the hot area (Mlali). Differences in the temperatures, altitude and animal population in the three zones are shown in Table 1. Migration of warthogs, hedgehogs, squirrels, buffaloes, monkeys and baboons during the dry season is common in all three zones, and a wide variety of wild birds are found in all three locations.

2.2. Sample size

The study was conducted between November 1997 and June 1998. To raise the number of chickens per unit of population, three households were regarded as a unit of sampling. The required sample size was 110 per location based on the formula $n = 4\ \mathrm{pq/l}^2$. Since the prevalence (P) of the infection was not known, a prevalence of 50% was estimated (Martin et al., 1987), the prevalence could then be established to be within 10% of the true prevalence 90% of the time. Ducks, dogs, cats and pigs, kept by the selected owners and their neighbours were screened as a part of animals kept in contact (Table 2).

Table 1
Climate and estimated population size in the zones investigated

Zone	Location	Temperature (°C)	Altitude (m)	Chickens	Ducks	Dogs	Cats	Pigs
Hot	Mlali	23–32	760	6674	1800	100	25	–
Warm	Mkuyuni	18–25	1200	9500	1300	88	8	–
Cool	Mgeta	12–18	1500	3440	1200	65	14	200

Table 2
Prevalence of *P. multocida* ssp. *multocida* and *P. multocida* ssp. *septica* in the chickens, ducks, dogs, and cats investigated[a]

Host	Zone	*P. multocida* ssp. *multocida*				*P. multocida* ssp. *septica*			
		BA	SM	MI	*n*/*N*	BA	SM	MI	*n*/*N*
Chickens	Cool	–	–	–	0/110	–	–	–	0/110
	Warm	1 (1%)	2 (2%)	2 (2%)	2/110 (2%)	–	–	–	0/110
	Hot	–	–		0/110	–	–	–	0/110
	Subtotal	1 (0.3%)	2 (0.7%)	2 (0.7%)	2/330	–	–	–	0/330
Ducks[b]	Cool	–	–	–	0/50	–	–	–	0/50
	Warm	1 (2%)	–	10 (20%)	11/50 (22%)***	–	–	–	0/50
	Hot	–	–	–	0/52	–	–	–	0/52
	Subtotal	1 (0.7%)	–	10 (7%)	11/152	–	–	–	0/152
Dogs	Cool	–	–	1 (4%)	1/25 (4%)	–	–	–	0/25
	Warm	1 (4%)	–	–	1/25 (4%)	–	–	–	0/25
	Hot	–	–	1 (3%)	1/30 (3%)	–	2 (7%)	–	2/30
	Subtotal	1 (1%)	–	2 (3%)	3/80	–	2 (3%)	–	2/80
Cats	Cool	5 (100%)	5 (100%)	5 (100%)	5/5 (100%)*	–	–	–	0/25
	Warm	3 (43%)	2 (29%)	3 (43%)	3/7 (43%)[c]	–	1 (14%)	1 (14%)	1/7 (14%)**
	Hot	3 (12%)	7 (28%)	11 (44%)	11/25 (44%)[d]	7 (28%)	8 (32%)	5 (20%)	10/25 (40%)**
	Subtotal	11 (30%)	14 (38%)	19 (51%)	19/37	7 (19%)	9 (24%)	6 (16%)	11/37

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$. Significantly different compared with two other zones.
[a] BA: blood agar; SM: selective medium; MI: mouse inoculation; *n*/*N*: number of positive animals/number animals sampled. The higher prevalence was used where identification failed to match (see text).
[b] One isolate of *P. gallinarum* obtained in the warm zone from blood agar is not listed in the table.
[c] Prevalence by mice inoculation findings.
[d] Prevalence by findings on blood agar and selective medium plates.

2.3. Isolation media

Sheep blood agar (5%) (Columbia; ADSA^{MICRO}) was used for initial culture and propagation. The modified 8HPG (Smith and Baskerville, 1983) was used as a selective medium, which was made by adding bacitracin (5000 U/l), polymixin B (200 μg/l), and gentamycin (30 μg/l) to a 5% sheep blood agar. *Pasteurella*-free Swiss strain mice (21-days old) were used for intraperitoneal passage. Subsequent isolates from 8HPG and mice were propagated on 5% sheep blood agar.

2.4. Collection of samples, bacterial culture and mouse inoculation

Laryngotracheal and cloacal swabs from a total of 330 chickens and 152 ducks from the three locations were cultured and screened for *Pasteurella* species. A total of 80 dogs and 37 cats were swabbed around the canine tooth-gingival junction, while nasal swabs were taken from 30 pigs. Each swab was streaked on two plates of 5% sheep blood agar to obtain single colonies and one plate of selective medium. The swabs were then placed in 3 ml of sterile physiological saline. Blood agar plates were incubated under aerobic conditions for 24 h at 37°C, whereas selective medium was incubated for 48 h under the same conditions. Up to four subcultures were made from the blood agar plates and from each selective medium plate.

A physiological saline tube with an infected swab was shaken thoroughly, then 0.5 ml of the contents were injected into Swiss strain mice by intraperitoneal route. The inoculated mice were observed for 48 h and any mouse that remained alive was euthanised. Spleen samples were aseptically removed and macerated under sterile conditions following dissection of the mice. Using a sterile loop, spleen material was inoculated on blood agar and incubated overnight under aerobic conditions at 37°C. Blood agar and selective medium plates were marked for each animal sampled. However, because of the inefficiency of the method of marking mice not all mice isolates could be matched with respective animals marked on the plates.

2.5. Identification of Pasteurella species

Identification of *Pasteurella* species was performed according to the methods described by Bisgaard and Mutters (1986) and Bisgaard et al. (1991). Colonies suspected to be *P. multocida* from blood agar, selective medium and mouse passage were subcultured on blood agar plates. Pure isolates were propagated in tryptophan broth (ADSA^{MICRO}) and were incubated at 37°C for 8 h. Each isolate in broth culture was inoculated into glucose broth (with Durham tubes to detect gas formation), ornithine decarboxylase, decarboxylase control medium, sucrose, maltose, mannitol, dulcitol, sorbitol, urea broth and urea broth control medium. An overnight tryptophan broth culture was tested for indole production by using dimethylaminobenzaldehyde (Kovac's reagent). Each isolate was again subcultured on blood agar to confirm purity of the culture. All sugar broth cultures were incubated at 37°C for 48 h. Pure colonies from blood agar were used for motility test, catalase test, oxidase test, Hugh and Leifson's oxidation-fermentation test, and Gram staining. Each strain was tested three times to assess repeatability of the results.

All the isolates that were preliminary identified as *Pasteurella* or *Pasteurella*-like were subsequently characterized by extended phenotypic methods described by Bisgaard et al. (1991).

2.6. Statistical analysis

A χ^2-square test was used to compare the differences on prevalence of *Pasteurella* species between the zones, host species, and the media of isolation (Martin et al., 1987).

3. Results

3.1. Overall results

A total of 153 isolates of *P. multocida* and related species were obtained from the 629 apparently healthy animals investigated. Each of the positive chickens and ducks carried a single species, whereas in dogs and cats few carriers of two and three different species were also found, and no isolate came from pigs. Because of inefficiency of mice identification the combination of all three methods in the interpretation of results could not be considered for every animal investigated (Tables 2 and 3). Out of 330 chickens and 152 ducks examined in the three zones, a total of 16 laryngotracheal isolates from the warm zone were confirmed to be *P. multocida* ssp. *multocida* and one isolate from ducks was *Pasteurella gallinarum* (Table 2). *Pasteurella* species were not obtained from the cloaca. Overall prevalence of *P. multocida* ssp. *multocida* in chickens was 0.7% (2/330), significantly ($P < 0.05$) lower than that of ducks (7%). Five isolates were obtained from two chickens. From one chicken, all three methods of isolation demonstrated the *P. multocida* ssp. *multocida*, while the second bird yielded *P. multocida* ssp. *multocida* from the selective medium and from mouse inoculation. From the 11 positive ducks, one bird yielded *P. multocida* ssp. *multocida* through blood agar inoculation, while the remaining 10 isolations were done through mice (Table 2).

Extended phenotypic characterization confirmed all five isolates from chickens to be *P. multocida* ssp. *multocida.* Two isolates from one chicken were identical, but they appeared different from three identical isolates from the second chicken. The first chicken clone (two isolates) was positive for L (+) arabinose and negative for D (+) xylose, trehalose, D (−) arabinose, L (−) fucose and PNPG, the second clone (three isolates) was negative for L (+) arabinose and positive for the other tests. A total of 11 strains from ducks shared all 80 phenotypic features, and appeared similar to the L (+) arabinose positive clone from chickens. Ducks and chickens carrying *P. multocida* ssp. *multocida* were in the same vicinity having boundless contact, despite being kept by different villagers. Apart from *P. multocida* ssp. *multocida* and *P. gallinarum,* no other species of *Pasteurella* were recovered from poultry. None of the methods used detected *Pasteurella* spp. in the 30 pigs investigated.

P. multocida ssp. *multocida* was demonstrated in 4% (3/80) of dogs and 51% (19/37) of cats investigated (Table 2). Thus, a carrier rate of *P. multocida* ssp. *multocida* in cats was statistically significantly higher ($P < 0.001$) than those of dogs, chickens, and ducks.

Table 3
Occurrence of *Pasteurella* species other than *P. multocida* in dogs and cats in the three zones[a]

Zone	Dogs				Cats			
	BA	SM	MI	*n*/*N*	BA	SM	MI	*n*/*N*
P. canis								
Cool	2 (8%)	2 (8%)	–	3/25 (12%)	–	–	–	0/5
Warm	1 (4%)	1 (4%)	3 (12%)	3/25 (12%)	–	–	–	0/7
Hot	4 (13%)	–	–	4/30 (13%)	–	–	–	0/25
Total	7 (10%)	3 (4%)	3 (4%)	10/80	–	–	–	0/37
P. dagmatis								
Cool	2 (8%)	–	–	2/25 (8%)	–	–	–	0/5
Warm	–	–	1 (4%)	1/25 (4%)	–	–	–	0/7
Hot	2 (3%)	–	–	2/30 (7%)	–	–	–	0/25
Total	4 (4%)	–	1 (4%)	5/80	–	–	–	0/37
P. stomatis								
Cool	–	–	–	0/25	–	–	–	0/5
Warm	–	1 (4%)	–	1/25 (4%)	–	1 (14%)	–	1/7 (14%)***
Hot	–	–	1 (3%)	1/30 (3%)	–	–	–	0/25
Total	–	1 (1%)	1 (1%)	2/80	–	1 (3%)	–	1/37
Taxon 16								
Cool	5 (20%)	2 (8%)	–	7/25 (32%)	–	–	–	0/5
Warm	2 (8%)	2 (8%)	–	4/25 (16%)**	1 (14%)	1 (14%)	2 (28%)	2/7 (28%)**
Hot	9 (30%)	4 (13%)	–	10/30 (33%)	2 (8%)	–	–	2/25 (8%)
Total	16 (20%)	8 (10%)	–	21/80	3 (8%)	1 (3%)	2 (5%)	4/37

Table 3 (*Continued*)

Zone	Dogs				Cats			
	BA	SM	MI	*n*/*N*	BA	SM	MI	*n*/*N*
Unclassified								
Cool	1 (4%)	1 (4%)	–	2/25 (8%)*	–	–	–	0/5
Warm	3 (12%)	–	–	3/25 (12%)	1 (14%)	–	–	1/7 (14%)
Hot	6 (20%)	–	–	6/30 (20%)	2 (8%)	–	–	2/25 (8%)
Total	10 (8%)	1 (1%)	–	11/80	3 (3%)	–	–	3/37

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$. Significantly different compared with two other zones.
[a] BA: blood agar; SM: selective medium; MI: mouse inoculation; *n*/*N*: number of positive animals/number of animals sampled.

P. multocida ssp. *septica* was also statistically significantly more prevalent ($P < 0.001$) in cats 30% (11/37) compared with dogs 3% (2/80).

Other species of *Pasteurella* identified included *Pasteurella canis, Pasteurella dagmatis, Pasteurella stomatis,* taxon 16, and unclassified *Pasteurella* (Table 3). The carrier rate of *P. canis* in dogs was 14% (11/80), which was statistically significantly ($P > 0.05$) higher than in cats where no carrier was detected. *P. dagmatis* was demonstrated in five out of 80 dogs (6%). The prevalence of *P. dagmatis* in dogs was statistically significantly ($P < 0.05$) higher in dogs compared with cats where *P. dagmatis* was not detected. *P. stomatis* was found in two dogs (3%), a similar prevalence to that of cats 3% (1/37).

Taxon 16 was found in 26% (21/80) of dogs investigated, which was statistically significantly ($P < 0.01$) higher than that of cats 11% (4/37). A variety of unclassified *Pasteurella*-like strains were found in 14% (11/80) of dogs which is not statistically significant higher ($P < 0.05$) than that of cats 8% (3/37).

The presence of more than one taxon in one animal was shown in 9 out of 80 dogs (11%) and 5 of 37 (13%) cats investigated (data not shown in the tables). The difference in prevalence of multiple carriers between dogs and cats was not statistically significant ($P < 0.05$). In dogs carriers of more than one taxon were found to host the following bacteria; *P. multocida* ssp. *multocida* and *P. stomatis* (one dog), *P. multocida* ssp. *septica*, *P. canis* and unclassified *Pasteurella* (one dog), *P. multocida* ssp. *septica* and taxon 16 (one dog), *P. canis* and taxon 16 (one dog), *P. canis* and unclassified *Pasteurella* (one dog), *P. dagmatis* and unclassified *Pasteurella* (one dog), taxon 16 and unclassified (three dogs).

The five cats found to carry more than one species were shown to have *P. multocida* ssp. *multocida* and *P. multocida* ssp. *septica* (one cat), *P. multocida* ssp. *multocida*, *P. stomatis* and taxon 16 (one cat), *P. multocida* ssp. *multocida* and unclassified *Pasteurella* (one cat), *P. multocida* ssp. *septica* and unclassified *Pasteurella* (one cat) and *P. multocida* ssp. *septica* and taxon 16 (one cat).

All carriers of more than one species, were detected by either blood agar, selective medium or a combination of both methods. Out of nine multiple carrier dogs, two were detected by blood agar alone and seven by selective medium and blood agar. All cats that carried more than one species were detected by combination of results from blood agar and selective medium.

3.2. Occurrence of Pasteurella spp. in the three zones

The prevalence of *P. multocida* ssp. *multocida* in chickens of the warm zone 2% (2/110) was not statistically significantly different ($P > 0.05$) from that of chickens from the other zones (Table 2). However, prevalence of *P. multocida* ssp. *multocida* in ducks of the warm zone 22% (11/50) was statistically significantly ($P < 0.001$) higher than that of ducks in the cool and hot zones (0%). Level of differences in prevalence of isolates obtained are indicated in the Tables 2 and 3.

3.3. Efficacy of the methods of isolation

Mouse inoculation was more efficient than the other methods for isolating *P. multocida* ssp. *multocida* (Table 4). A total of 33 (52%) isolates were obtained by mouse inoculation

Table 4
Efficacy of media used (%) for isolation of *Pasteurella* spp. from chickens and their animal contacts

	Taxon (*n*)	BA	SM	MI
P. multocida ssp. *multocida*	Chickens (5)	20	40**	40**
	Ducks (11)	9	–	91***
	Dogs (3)	33	–	66***
	Cats (44)	25	32	43**,a
P. multocida ssp. *septica*	Dogs (2)	–	100	–
	Cats (22)	32	41*,b	27
P. canis	Dogs (13)	54***	23	23
P. dagmatis	Dogs (5)	80***	–	20
P. stomatis	Dogs (2)	–	50**	50***
Taxon 16	Dogs (24)	67***	33	–
	Cats (6)	50***	17	33***,c
Unclassified *Pasteurella*	Dogs (11)	91***	9	–
	Cats (3)	100***	–	–

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$
[a] Significantly higher than BA only.
[b] Significantly higher than MI only.
[c] Significantly higher than selective medium only.

which was statistically significantly ($P < 0.001$) higher than blood agar and selective medium which recovered 22% (14) and 25% (16) of the isolates, respectively. Out of 24 isolates of *P. multocida* ssp. *septica* 46% (11) were isolated by selective medium which was statistically significantly higher ($P < 0.05$) than that of blood agar (29%) and mouse inoculation (25%). Direct culture on blood agar was generally more efficient that the other methods used in detecting the remaining species of *Pasteurella*. Efficacy of the three techniques in isolating *P. multocida* ssp. *multocida* and other species in different host animals is shown in Table 4.

4. Discussion

Most of the works published before reclassification of the genus *Pasteurella sensu stricto* (Mutters et al., 1985) do not safely allow the present species and subspecies identification within the genus *Pasteurella*. This is the first systematic investigation of *P. multocida* and related species in free ranging chickens and their animal contacts. Contrary to previous investigations in commercial flocks both poultry and contact animals are kept free under village management system which allow optimum interaction between them. According to the recent classification (Mutters et al., 1985) our study demonstrated two subspecies of *P. multocida*, four species of *Pasteurella* and at least one taxon of uncertain affiliation (taxon 16) among the animals investigated. We could not accurately compare the organisms described in most previous reports (Saphir and Carter, 1976; Arnbjerg, 1978; Curtis and Ollerhead, 1981; Carpenter et al., 1989) with our findings because of limited number of tests employed in those studies.

Isolation of *P. multocida* from apparently healthy poultry has been associated with previous outbreaks of fowl cholera in the flock (Curtis and Ollerhead, 1981; Carpenter et al., 1989). In the present investigation, *P. multocida* ssp. *multocida* was detected in laryngotracheal swabs in 2% of chickens and 22% of ducks in the warm climatic zone of the studied areas. Extended phenotypic characterization showed that one chicken clone was similar in all 80 phenotypic features with 12 duck strains kept in the same area. This suggests that the strains might be related and there can be exchange of *P. multocida* ssp. *multocida* between chickens and ducks, confirming the recent observations of exchange of clones of *P. multocida* between commercial poultry and the avifauna by Christensen et al. (1999). This exchange of *P. multocida* ssp. *multocida* might also have implications on cats and dogs kept in the same area. Three out of seven cats and one of 25 dogs from this zone carried *P. multocida* ssp. *multocida.* However, all five cats from the cool zone and 44% of the cats from the hot zone carried *P. multocida* ssp. *multocida* while chickens and ducks from these zones sampled negative for *P. multocida* ssp. *multocida.* Consequently, exchange of clones of *P. multocida* ssp. *multocida* between animal species might not be the same in all zones for reasons which remains to be investigated. Reliable data about the extent to which cats and dogs had access to dead poultry in the different zones were not available. Finally, the relevance of cats and dogs in the epidemiology of *P. multocida* in birds in the present study remains to be further analysed by molecular characterization of the strains to understand the epidemiological relationship between the clones from different host species under investigation.

The prevalence data of cats in our study support the previous findings that *P. multocida* ssp. *multocida* is part of normal flora of oral mucosa in majority of cats. Investigations done in recent years have demonstrated that the prevalence of *P. multocida* ssp. *multocida* in cats varies from 21 to 38% (Baldrias et al., 1988; Ganiere et al., 1993; Mohan et al., 1997). High isolation rates in cats of the cool zone may be influenced by the small sample size. *P. multocida* ssp. *multocida* has been reported to be less prevalent in dogs compared with cats (Baldrias et al., 1988; Ganiere et al., 1993; Mohan et al., 1997). Agreement of our results with these findings may suggest that dogs seems to represent a less likely source of *P. multocida* ssp. *multocida* to poultry compared with cats.

Although *P. multocida* ssp. *septica* is scarcely reported in poultry, as also seen in the current study, it has been associated with different outbreaks of fowl cholera in both domestic and wild birds (Hirsh et al., 1990; Blackall et al., 1995). Studies by Snipes et al. (1990) and Fegan et al. (1995) found *P. multocida* ssp. *septica* in less than 1% of all isolates of *P. multocida* from outbreaks of fowl cholera. Characterization of more isolates from birds in Tanzania and other countries will provide more knowledge about the distribution of *P. multocida* ssp. *septica* in birds.

Several reports about the presence of *P. multocida* ssp. *septica* in cats and dogs are available (Baldrias et al., 1988; Hirsh et al., 1990; Ganiere et al., 1993). An equal proportion of *P. multocida* ssp. *septica* to *P. multocida* ssp. *multocida* carriers in cats has been documented (Baldrias et al., 1988; Ganiere et al., 1993). A 1:2 ratio of *P. multocida* ssp. *septica* to *P. multocida* ssp. *multocida* was observed in our investigations. However, differences in the sample size and precision of isolation techniques may contribute to the variation in the results between our study and others. Cats in the warm zone were not found to harbour *P. multocida* ssp. *septica,* but since *P. multocida* ssp. *multocida* was isolated in cats, ducks and chickens in this zone, it may be too early to speculate that climate is the factor guiding the prevalence here. Subculture of more isolates per plate might have documented the existence of more species than four subcultures per plate in the current study.

Previous reports of carriers of *P. multocida* in poultry have been associated with previous occurrence of disease in the flocks (Curtis and Ollerhead, 1981; Carpenter et al., 1989). Thus, our results may be considered an indication of previous fowl cholera in village free ranging poultry, although the clinical form of the disease was not seen during the period of study. A report on carriers of *P. multocida* which were not associated with previous occurrence of disease in the flocks has been published recently (Muhairwa et al., 2000). *P. multocida* ssp. *multocida* from cats has been reported to cause disease in feral birds (Korbel et al., 1992). Since free contact between dogs and cats and free ranging poultry might favour exchange of pathogenic strains leading into clinical disease, *P. multocida* ssp. *multocida* found in cats and dogs in the present study might have significant impact on village free ranging chickens.

For the first time this study has shown the presence of *P. gallinarum* in an apparently healthy duck. Although this finding does not rule the possible presence of convalescent carriers or the beginning of infection, it demonstrate that ducks can also be infected by *P. gallinarum*. Previous reports associated the bacterium with pathological lesions in chickens (Hall et al., 1955; Mraz et al., 1980; Droual et al., 1992). Further studies in healthy and diseased ducks will be necessary to confirm the importance of *P. gallinarum* in ducks.

P. canis, P. dagmatis, P. stomatis, and taxon 16 have never been reported in chickens and ducks (Mutters et al., 1985; Bisgaard, 1993). *P. canis* was predominantly isolated from dogs and in equal proportions in the three zones. The present results show that *P. canis* is more common than *P. multocida* in dogs. This agrees with the results of Ganiere et al. (1993). Low occurrence of *P. dagmatis* and *P. stomatis* in dogs confirms previous observations by Ganiere et al. (1993), who found 11% *P. dagmatis,* and Mohan et al. (1997) who found less than 1% *P. stomatis* in dogs. The organism tentatively designated taxon 16 by Bisgaard and Mutters, (1986) was found in twenty-one dogs and in four cats in this study. Ganiere et al. (1993) recovered an organism from dogs with features related to taxon 16 which they named atypical *P. stomatis.* Seemingly a prevalent taxon in dogs, taxon 16 could represent misclassified *P. gallinarum* and unidentified *Pasteurella* reported in dogs (Baldrias et al., 1988).

Unclassified *Pasteurella* described represent organisms the biochemical patterns of which failed to match with any of species in the new classification of Mutters et al. (1985). Similar organisms have also been reported in dogs and cats in Europe (Bisgaard and Mutters, 1986; Ganiere et al., 1993) and Australia (Baldrias et al., 1988). To the author's knowledge this is the first report on the occurrence of unclassified *Pasteurella* in dogs and cats of Africa. It is possible that some of these organisms might later form new taxa or classify in the known species of *Pasteurella*. Thus, subsequent characterization by extended phenotypic characterization and molecular techniques is required to decide the taxonomic position of these organisms.

Simultaneous isolation of *P. dagmatis* and *P. multocida* from human wounds related to cat bites was reported by Zbinden et al. (1988). Subsequently, Ganiere et al. (1993) demonstrated multiple carriers of *Pasteurella* species/ subspecies in 19% of dogs and 30% of cats examined. The present results show that, 11% of dogs and 13% of cats are double carriers and therefore confirms the previous findings. However, it is presumed that swabbing of multiple sites in the oral cavity, and subculturing on more plates and several colonies per plate could significantly improve the results.

The use of different isolation methods has been reported to increase the combined isolation rate of *Pasteurella* species (Baldrias et al., 1988). A total of 33 out of 63 strains of *P. multocida* ssp. *multocida* in the present investigation were collected by mouse inoculation. Comparison of our results with those of Baldrias et al. (1988) may suggest that mouse inoculation is superior to other methods we used for isolating *P. multocida* ssp. *multocida*. However, the possibility that different clones of *P. multocida* ssp. *multocida* could vary in their pathogenicity to mice needs to be addressed. Consequently, the genetic relatedness of clones obtained by different methods of isolation should be investigated to reach a firm conclusion.

The present findings indicate that all three methods of isolation applied can isolate *P. multocida* ssp. *septica* from cats. Although the selective medium isolated more strains than direct culture on blood agar and mouse inoculation, the number of strains examined is too low reach a safe conclusion. In addition, the virulence of these organisms for mice needs to be addressed. Generally, the present results show that blood agar is more sensitive for the isolation of *Pasteurella* other than *P. multocida.*

Baldrias et al. (1988) isolated a total of 21 strains of *P. canis* from dogs by using blood agar only. In the same study, 20 out of 27 strains of *P. canis* from cats came from blood agar, only seven strains came from selective medium and mouse inoculation. These findings correlate with our results where we have obtained 8 of 14 strains of *P. canis* from dogs by direct culture on blood agar and three isolates each, from selective medium and mouse inoculation. Lack of pathogenicity to mice and susceptibility to drugs in the selective medium may be the reasons for inefficiency of mouse inoculation and selective medium in isolating *P. canis*.

In conclusion, isolation of *P. multocida* ssp. *multocida* from village free ranging chickens and ducks suggest that fowl cholera might be common in the village poultry of Tanzania. More efforts should be directed in understanding the impact of the *P. multocida* and other species in village birds. The presence of *P. multocida* in dogs and cats has never been observed to influence the epidemiology of fowl cholera in free ranging chickens and ducks. However, their potential as a source of disease is still there. Studies using molecular techniques are needed to clarify the significance of dogs and cats in the epidemiology of fowl cholera in free ranging poultry.

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