PASTEURELLOSIS AND *PASTEURELLAE* IN ZIMBABWE: AN UPDATE

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Abstract

Pasteurellosis broadly refers to any of the disease conditions caused by species of the genus *Pasteurella*. These conditions appear to be prevalent in Zimbabwe, and they range from slow latent infections to rapid fatal septicaemias. Apart from known classical disease syndromes like bovine haemorrhagic septicaemia (HS), snuffles in rabbits and fowl cholera, other conditions which range from polyarthritis, surface wound infections, pneumonia, sinusitis, rhinitis and genito-urinary tract infections have been reported. A wide spectra of hosts affected by species of the genus *Pasteurella* include cattle, rabbits, chickens, sheep, pigs, goats, crocodiles, dogs and cats. In cattle, the condition has predominantly been the pneumonic form. In pigs, pneumonia has commonly been encountered. Classical progressive atrophic rhinitis was recorded in two farms. Sporadic cases of pneumonia have also been observed in sheep and goats. Besides fowl cholera, keratoconjunctivitis due to *Pasteurella haemolytica*, sinusitis and arthritis due to *P. gallinarum* have been reported in chickens. In dogs and cats, *Pasteurella* species were isolated from bite wounds, rhinitis, pyothorax, bronchitis, uterine and vaginal infections. *Pasteurella stomatis*, *P. dagmatis* and *P. multocida* were isolated from a single dog with chronic bronchitis. We also reported the first isolation of Communicable Diseases Centre (CDC) group EF-4 bacteria from dogs in Southern Africa. Of all identified species, *P. multocida* was found to be predominant, and serological typing revealed that serogroup A was most prevalent, with serogroups E and F absent. A single outbreak of bovine HS was found to be associated with serogroup B as opposed to an earlier belief that serogroup E was the causative agent in Southern Africa. The significance and implications of observations made on selected disease syndromes are discusssed in detail.

Introduction

Of the several species of the genus *Pasteurella*, only two are regarded as of immense veterinary significance – *P. multocida* and *P. haemolytica*. The two species are easily differentiated in the laboratory using biochemical or physiological characters (Cowan and Steel, 1993). *P. multocida* infects a wider range of hosts than *P. haemolytica*.

The taxonomy of *Pasteurella* (*sensu stricto*) has recently been reviewed by Christensen and Bisgaard (1997). Although a wide range of disease manifestations

have been attributed to either *P. multocida* and/or *P. haemolytica*, the pathogeneses of these infections are not well-understood. Detailed descriptions of virulence factors of *P. multocida* have been reviewed by Christensen and Bisgaard (1997) and Dziva (1997). Virulence factors found in all serotypes include; the capsule, endotoxin, outer membrane proteins and neuraminidase. In addition, hyaluronic acid has been demonstrated in type A strains (Rush, 1989), hyaluronidase and chondroitinase activity in type B strains (Carter and Chengappa, 1980; Rimler and Rhoades, 1994), osteolytic toxin in type A and D strains (Rutter and Luther, 1984; Nakai *et al*., 1984), IgA proteases in type D strains (Pouedras *et al*., 1992) and transferrin binding activity in *P. haemolytica* and *P. multocida* (Ogunnariwo and Schryvers, 1991; 1992; Veken *et al*., 1994).

There is little information on the molecular determinants of virulence apart from the cloned and expressed osteolytic toxin gene, designated *tox*A (Petersen and Foged, 1989). Various other genes have been cloned and identified (Ruffolo and Adler, 1996; Kasten *et al*., 1995; Dziva, 1997), but their role in pathogenicity has not been defined. On the other hand, the pathogeneses of *P. haemolytica* infections are known to be mediated primarily by the leukotoxin, which destroys the ruminant neutrophils and macrophages (Berggren *et al*., 1981; Markham and Wilke, 1980). The gene encoding for the leukotoxin has been cloned and expressed in *E. coli* (Lo *et al*., 1985). However, there appears to be specific biotypes associated with a particular disease in a specific host. For example, biotype A is associated with pneumonia in cattle, whereas biotype T is associated with septicaemia in feeder lambs. Virulence factors, including molecular aspects of the organism have been reviewed by Confer *et al*. (1990). Generally, *Pasteurellae* are susceptible to most commonly available antibiotics, hence their infections are easy to treat when detected early.

Laboratory isolation and identification

Clinical specimen were received from our teaching veterinary hospital and private veterinarians from around the country. Specimen were processed within 1–2 hrs of collection. Post-mortem samples were in the form of organs (liver, spleen, lung tissue), aspirates, exudates or heart blood. Specimen from live animals were in the form of swabs, exudates, aspirates and trans-tracheal washes. Isolation and identification were done following standard procedures described by Cowan and Steel (1993). In our laboratory, primary isolation on sheep blood agar (Oxoid) proved very efficient. The isolates were then kept at –20°C in brain heart infusion broth or lyophilised until required for further characterisation. Disease categories and types of specimen from which pasteurellae organisms were isolated are shown in Table 1.

Phenotypic characterisation

Phenotypic characterisation (Mohan *et al*., 1994; 1997) revealed that Zimbabwean isolates of *P. multocida* could be classified into different taxa and

Table 1: Examples of diseases categories and type of specimen from which *Pasteurellae* organisms were recovered

Disease	*Specimen*
Pneumonia	i) Postmortem samples; lung, pleural exudate or swabs from exudates. ii) Bronchial washes
Septicaemia	Postmortem samples; heart blood, liver, spleen, etc.
Upper respiratory infections	Swabs of nasal discharge, pharyngeal region, etc.
Urogenital infections	Swabs from vaginal discharge, urine samples, etc.
Surface wounds, ulcers, bite wounds	Swabs from affected areas.
Arthritis	Joint aspirate

subspecies as described by Mutters *et al.* (1985) and Bisgaard *et al.* (1991). The important sugars used for the classification into species and subspecies were; trehalose, xylose, mannitol, sorbitol, salicin, maltose, lactose, inositol, sucrose, arabinose and dulcitol. Consistent results were obtained in the tests for inositol, salicin and sucrose. No relationship between the phenotype and the reclassified taxa based on DNA homology studies (Mutters *et al.*, 1985) could be found in some strains. We therefore proposed that the utilisation of dulcitol and sorbitol should be treated as variable characters of *P. multocida*, rather than used to split the taxon into subspecies (Mohan *et al.* 1994). The species that have been reported in Zimbabwe and their approximate proportions are shown in Table 2.

Serological typing

Serotyping of *P. multocida* (the predominant species) using an indirect haemagglutination test (Sawada *et al.*, 1982; Rhoades and Rimler, 1987) revealed that capsular serogroup A was widespread among various animal hosts and the most prevalent serotype in Zimbabwe. However, capsular serogroup E and F were not detected, but a relatively high incidence of nontypable isolates was encountered (Dziva *et al.*, 1999a). Details of the common serogroups of this organism are shown in Table 3.

Diseases syndromes observed in Zimbabwe

A wide variety of infections in different hosts due to *Pasteurella* species have been recorded. Hosts included nearly all domestic animals found in Zimbabwe,

Table 2: Relative proportions of each of the *Pasteurella* species observed during the last ten years in Zimbabwe based on our laboratory findings

Species	*Relative proportion expressed as percentage of all Pasteurellae organisms*
P. multocida	75%
P. haemolytica	18%
P. canis	1%
P. gallinarum	3%
P. stomatitis	1%
P. dagmatis	1%
Pasteurella-like (e.g. group EF-4) organisms	1%

Table 3: Common capsular serogroups of *Pasteurella multocida* and their relative frequencies observed in Zimbabwe

Capsular serogroup	*Relative frequency*
A	59%
B	5%
D	14%
E	0%
F	0%
Non-typable	22%

and also some wildlife species – crocodiles. The prevalence of the infections in wildlife species is yet to be determined. Table 4 summarises the hosts, disease conditions and predominant *Pasteurella* species encountered in our laboratory in the last ten years. A single outbreak of bovine haemorrhagic septicaemia has been reported (Lane *et al*., 1992), and this was controlled using broth bacterins prepared from the field isolate. Two isolates from cases of navell-ill and arthritis in neonatal calves due to *P. multocida* have also been recorded. In addition, an outbreak of arthritis in broilers due to *P. gallinarum* has been reported by Mohan and Dziva (1999). Pneumonia in various animals (calves, sheep, goats, pigs, rabbits and cats), due to either *P. multocida* or *P. haemolytica* has been commonly encountered (Mohan and Dziva, unpublished data). Two cases of meningitis in pigs associated with *P. multocida* were also recorded (Mohan and Dziva, unpublished data). A single case of suspected keratitis in a horse was also recorded in our laboratory. However, the classical progressive atrophic rhinitis (AR) has been reported (DVS Report, 1993), but not confirmed with toxinogenicity

assays. In a preliminary study (Mohan and Muzira, unpublished data), pigs were observed to harbour *P. multocida* in their tonsils, but these strains were found to be nontoxinogenic when studied by polymerase chain reaction using primers for the osteolytic toxin gene and by a commercial ELISA kit (Dziva *et al*., unpublished data). Dogs and cats appeared to be highly susceptible to pasteurellosis, mainly in the form of upper respiratory tract infections, pneumonia and bite wound

Table 4: Disease conditions in different hosts caused by species of the genus *Pasteurella* recorded in Zimbabwe in the last ten years

Host	*Disease condition*	*Predominant species*
Cattle	Calf pneumonia	*P. multocida*
	Haemorrhagic septicaemia	*P. multocida*
	Navel-ill and polyarthritis	*P. multocida*
	Nephritis	*P. multocida*
Chickens	Fowl cholera	*P. multocida*
	Arthritis	*P. gallinarum*
	Sinusitis	*P. gallinarum*
	Keratoconjunctivitis	*P. haemolytica*
Pigs	Pneumonia	*P. multocida*
	Meningitis	*P. multocida*
	Carrier status	*P. multocida*
Dogs	Bronchitis	*P. multocida*
	Stomatitis	*P. dagmatis*
	Septic arthritis	*P. canis*
	Utero-vaginal infections	*P. stomatitis*
	Rhinitis	Group EF-4 organisms
	Wound infections (bite, surgical)	Bisgaard taxon 16
Cats	Pyothorax	*P. multocida*
	Bronchopneumonia	*P. multocida*
	Rhinitis	*P. stomatitis*
	Wound infections (bite, scratch, surgical, etc)	Group EF-4 organisms
Rabbits	Pneumonia	*P. multocida*
	Septicaemia	*P. multocida*
Sheep	Pneumonia	*P. multocida*
	Lymphadenitis	*P. haemolytica*
Goats	Bronchopneumonia	*P. multocida*
		P. haemolytica
Horses	Suspected keratitis	*P. multocida*
Crocodiles	Septicaemia	*P. multocida*

(Source: Mohan and Dziva, unpublished data)

infections. *Pasteurella multocida* was the predominant isolate, except in two isolated cases from which CDC group EF-4 organisms were recovered (Mohan *et al.*, 1997).

Discussion

Pasteurellosis is undoubtedly one of the commonly encountered infections of animals in Zimbabwe. The data presented (Table 1) shows that a wide host spectrum exists. The pathogeneses of the infections caused by these organisms are poorly understood. Species of the genus *Pasteurella* are believed to inhabit the upper respiratory tracts of a wide variety of animals (Carter *et al.*, 1995) and oral mucosae of dogs and cats (Baldrias *et al.*, 1988), from which they can be isolated in apparently healthy animals. Consequently, most *Pasteurellae* diseases are thought to be either secondary or opportunistic infections. However, there are diseases like haemorrhagic septicaemia, fowl cholera and snuffles in rabbits, which are still referred to as primary pasteurelloses.

The relatively increased proportion of untypable isolates of *P. multocida* has been discussed elsewhere (Dziva *et al.*, 1999a). A single outbreak of HS was reported, and the isolate was characterized as serogroup B (Mohan *et al.*, 1994, Dziva *et al.*, 1999a). The earlier epidemiology of HS suggested that serogroup E was the causative agent in Africa, whereas serogroup B was for the Asian form of the disease (De Alwis, 1987). Although serogroup B has been reported in Africa, particularly North Africa (Hassan and Mustafa, 1985), this had not reached the southern region, where serogroup E was only being encountered (Francis *et al.*, 1980; Bastianello and Jonker, 1981). Recent reports in the Southern Africa region associated HS with serogroup B *P. multocida* (Lane *et al.*, 1992, Martrenchar and Njanpop, 1994; Voigts *et al.*, 1997). This certainly changes the epidemiology of the disease. We wonder whether serogroup E is still in existence in its pathogenic form. In a recent serotyping study of *P. multocida* isolates (Dziva *et al.*, 1999a), we could not find any serogroup E isolate, suggesting its probable absence from Zimbabwe. There has been some speculation that serogroup E could have evolved from serogroup B (Smith, J.E., pers. comm.). Since cattle in southern Africa are of Asian origin, there is a possibility of serogroup E having changed antigenicity with change of geographical region (continent). The decline in the serogroup, could suggest some form of adaptation, with reversion to the original form. This remains as a hypothesis which needs to be proven.

Classical atrophic rhinitis was reported in two separate farms (DVS Report, 1993). Confirmation of the disease was done by isolation of the *P. multocida* from clinically affected animals (Makaya, pers. comm.). Isolation of *P. multocida* from clinically affected animals without subsequent assays for toxin production is generally regarded as being insufficient, as apparently healthy pigs have been shown to harbour nontoxinogenic strains in their tonsils (Farmer, 1996; Mohan and Muzira, unpublished data). Evidence indicates that only toxin-producing strains of *P. multocida* are responsible for clinical manifestations of atrophic rhinitis

(Farmer, 1996; Goodwin *et al*., 1990). It would, therefore, be ideal to confirm the presence of the disease by performing capsular serotyping and toxinogenicity assays, since the all-important osteolytic toxin is produced only by some serogroups of A and D strains. Ruziwa *et al*. (1999) observed turbinate atrophy in an indigenous pig characteristic of atrophic rhinitis, but reported it as an anatomical defect as bacteriological isolation and toxinogenicity assays were not performed. This further suggests that the disease may be prevalent in the pig industry.

Biochemical characterization remains one of the fundamental procedures of classifying bacterial isolates and most adaptable to developing countries. Modern technology has tackled characterization of bacteria at genotypic level, and this has taken the form of DNA fingerprinting, ribotyping, random amplification of polymorphic DNA and 16S rRNA sequencing. Though these have provided salient differentiation among isolates, there has been no close correlation between genotype and phenotypic expression. Our recent findings indicated that random amplification of polymorphic DNA using arbitrary primers did not correlate with capsular typing (Dziva *et al*., 1999b).

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