

A selective medium for *Pasteurella multocida* and its use with animal and human specimens

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SUMMARY A selective medium (CGT medium), containing clindamycin, gentamicin, potassium tellurite and amphotericin B in 5% horse-blood agar, allowed unimpaired growth of almost all strains of *Pasteurella multocida*, and *P pneumotropica*, while inhibiting other bacteria that might be encountered in upper respiratory tract secretions. With its use, *P multocida* was readily detected in oral swabs from four of 23 dogs, and 10 of 25 cats, but not detected in oral swabs from 47 human subjects. One of 500 sputum specimens yielded *P pneumotropica*.

Pasteurella multocida is a common commensal of the upper respiratory tracts of domestic and other animals, and also causes disease in many species.¹⁻⁴ It is encountered clinically in man mainly as a cause of acute inflammation in bites and scratches from dogs, cats and other animals,^{5,6} and it is also sometimes found as the predominant micro-organism in acute chest infections.⁷ Many of these cases have no known animal association⁸ and it has been suggested that there may be long-term carriage by humans. Isolation of *P multocida* from human respiratory tract secretions may be difficult because of overgrowth by other bacteria, and because the prevalent *Haemophilus* strains may readily be confused with pasteurellae. In this investigation, we set out to devise and assess a selective medium which would be effective for such specimens.

Material and methods

Antibiotics and chemicals that showed differential activity against *P multocida* strains and other bacteria commonly encountered in respiratory specimens were used alone and in combination in a base of heart infusion agar (Difco) with and without 5% defibrinated horse blood. The media were first tested by streaked inocula of the *Pasteurella* strains from the National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London, which are listed in the Table, and with clinical isolates of *P multocida* (identified by the methods of Cowan¹⁰) and clinical isolates of commensal bacteria. The medium finally chosen was

further tested by spot inoculation of 0.02 ml of ten-fold dilutions of overnight broth cultures of the bacteria, with, after overnight incubation at 37°C in air, observation of the highest dilution showing growth on blood agar and on the selective medium.

Oral specimens were taken with wool swabs (Medical Wire and Equipment Co) applied to the anterior surface of the gums and the interior of the cheek, from 48 animals (23 dogs and 25 cats) without evidence of infection, and from 47 volunteers, including children, members of an animal-loving charitable society, and kennel workers. Five hundred routine sputum specimens were homogenised with N-acetyl cysteine (Sputasol, Oxoid). All the specimens were plated on the selective medium and on blood agar; the growths obtained after overnight incubation in air were compared and the growth on the selective medium, if any, investigated for the presence of *P multocida*.

Results

The optimal composition finally established was: heart infusion agar with 5% defibrinated horse blood, containing clindamycin phosphate (Upjohn) 5.0 mg/l, gentamicin sulphate (Roussel Laboratories) 0.75 mg/l, potassium tellurite (BDH) 2.5 mg/l, and amphotericin B (Sigma) 5.0 mg/l. Quantitative comparison of the growth of selected cultures on this medium and on blood agar is shown in the Table. The selective medium permitted undiminished growth of 21/22 *P multocida* clinical isolates and of NCTC 8489 (*P multocida*) and NCTC 8141 (*P pneumotropica*). One clinical isolate of *P multocida* and NCTC 3195 (*P multocida*) grew poorly, as did NCTC 10365 (*P haemolytica*) and

Growth of selected cultures on selective medium and blood agar: highest dilution of culture at which growth detectable, overnight incubation, 37°C.

Bacterial strain	Highest tenfold dilution of overnight broth culture at which growth detectable	
	Blood agar	Selective medium
NCTC 8489 <i>Pasteurella multocida</i>	- 9	- 9
NCTC 3195 <i>P multocida</i>	- 7	- 4
NCTC 10365 <i>P haemolytica</i>	- 6	- 3
NCTC 10219 <i>P haemolytica</i> var <i>ureae</i>	- 9	- 1
Clinical isolates:		
<i>P multocida</i> 1	- 7	- 6
<i>P multocida</i> 2	- 8	- 7
<i>P multocida</i> 3	- 8	- 8
<i>P multocida</i> 4	- 7	- 8
<i>Pseudomonas aeruginosa</i>	- 7	- 7
<i>Proteus mirabilis</i>	- 7	- 2

Other commensal bacteria were completely inhibited by selective medium.

NCTC 10219 (*P haemolytica* var *ureae*). Strains of other bacteria were completely inhibited, with the exception of *Ps aeruginosa* and *Proteus* spp. Only one of seven *Proteus* isolates grew well on the medium; the others were prevented from swarming and grew poorly. The medium was more effective than that of Morris¹¹ in the suppression of haemophili. The medium retained its selective properties during storage at 4°C for four weeks, despite lysis of the blood component.

In animal specimens the presence of *P multocida* was easily recognised on half plates of the selective medium, whereas on blood agar it was usually obscured by other bacteria (Fig. 1a and b). Of 23 dogs, 4 (17%) yielded *P multocida* from gum swabs; as did 10 (40%) of 25 cats. Four cats were sampled in

an identical manner at weekly intervals for five weeks, with very careful spreading of the inoculum and examination of the blood agar cultures for pasteurellae. *P multocida* was detected on both selective medium and blood agar in three instances, on selective medium alone in 10 instances, and never on blood agar alone. None of the human oral specimens yielded *P multocida*, but commensal mouth bacteria were effectively suppressed by the selective medium (Fig. 2a and b). Of 500 sputum specimens examined, only one yielded a pasteurella, *P pneumotropica*. This specimen was obtained from a 42-year-old woman, owner of a pet dog, who had suffered an acute exacerbation of chronic bronchitis. *P pneumotropica* was plentiful in this specimen and detected readily on non-selective media, as well as

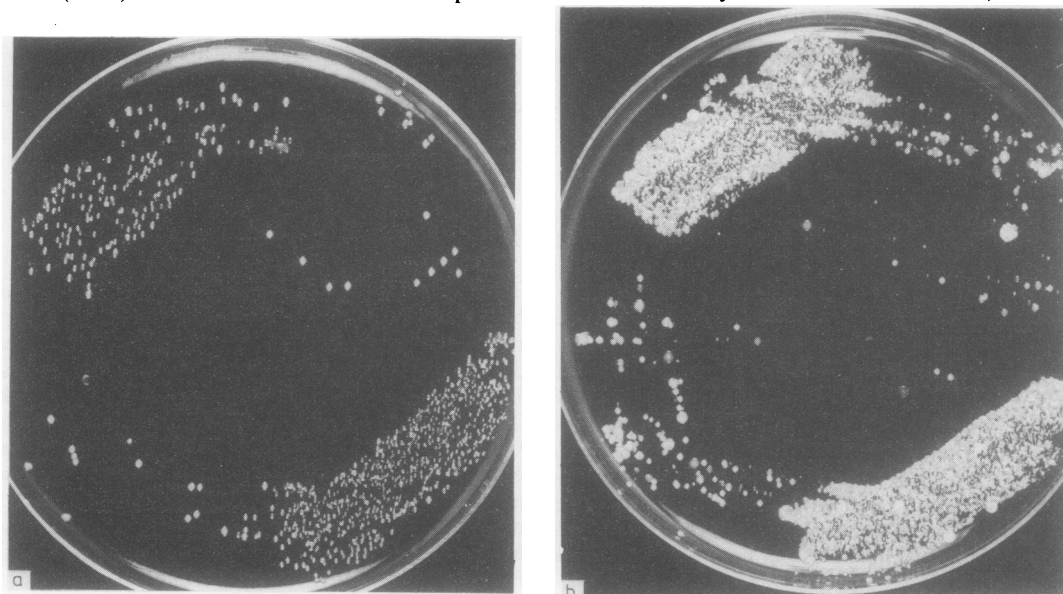


Fig. 1 (a) Oral swabs from two dogs plated on selective medium, showing a pure growth of *Pasteurella multocida*; (b) the same swabs plated on blood agar, showing a mixed growth of commensal bacteria. Both overnight incubation, 37°C, in air.

selective medium. *P. multocida* in smaller amounts was consistently detectable on selective medium from sputum artificially inoculated with *P. multocida* and processed in a similar manner.

Discussion

In this investigation, the rate of isolation of *P. multocida* from animal specimens was of the same order as that obtained by others. Hawkins¹² found 14% of gum and tonsil swabs from 50 dogs to be positive, and 52% of similar specimens from 50 cats. Woodgyer¹ found 42% of gum swabs from 199 cats to yield *P. multocida*. Smith¹³ obtained *P. multocida* from 54% of tonsil swabs from 111 dogs, and from 10% of nose swabs, but he was sampling dead animals, and the greater ease and efficiency of specimen taking may have increased the yield.

P. multocida has occasionally been isolated from the upper respiratory tract of healthy humans. Smith⁹ obtained it from the throats of 2/71 veterinary students; in one it persisted for at least four months, and in the other *P. multocida* was detected by mouse inoculation only for a few days. Hubbert and Rosen⁸ reported five positive isolations from normal upper respiratory tracts. Henriksen and Jysum¹⁴ detected 10 *Pasteurella* strains in two years in nose and throat cultures from patients with respiratory complaints but in whom the pasteurellae could not be definitely adjudged the responsible pathogen. Six were *P. multocida* and four were *P. haemolytica* var *ureae*; the authors estimated the positivity rate in such specimens as 2–4 per thousand. Jones,¹⁵ in describing 15 isolations of *P. haemolytica* var *ureae* from 1% of sputum specimens received during eight months, reported only two isolations of *P. multocida* during this period. Our results in the short series of volunteers, including many who had close association with cats and dogs, again suggest that carriage of *P. multocida* is uncommon, unless there is an underlying pathological process.

Although the selectivity of the medium described here is not perfect, its practical usefulness in the culture of specimens from the upper respiratory tracts of animals has clearly been demonstrated, and it should prove valuable in extending the observations on human carriage.

We are indebted to the pet owners and volunteers, including members of the Patients Animal Welfare Society, for providing specimens; to Dr GR Smith and Dr TE Curtis for information and advice; and to Dr LR Hill of the National Collection of Type Cultures for confirming the identification of an atypical *P. multocida* isolate.

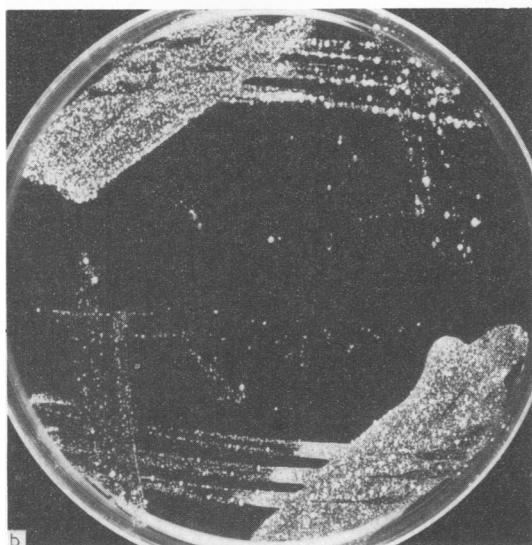
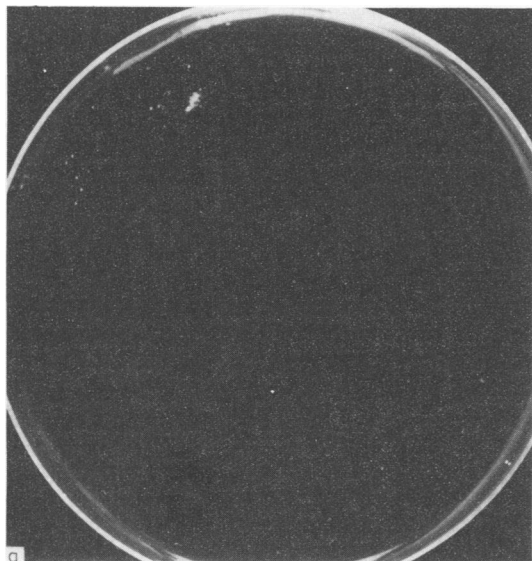


Fig. 2 (a) Oral swabs from two human subjects plated on selective medium, showing almost complete inhibition of commensal flora; (b) the same swabs plated on blood agar. Both overnight incubation, 37°C, in air.

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