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# SELECTIVE MEDIA FOR THE ISOLATION OF Bordetella bronchiseptica IN EXPERIMENTALY INFECTED PIGS

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*The value of different selective media for isolation of B. bronchiseptica from nasal swabs was examined.*

*The investigations were performed after experimental infection of two and three day old piglets at two week intervals using a virulent strain of B. bronchiseptica. Each swab was streaked onto 8 different nutritive media (blood agar, MacConkey agar, Bordet-Gengou agar, trypton soya agar, Endo agar, pepton agar, charcoal agar and Fc agar) which conatained different combinations of antibiotics and chemotherapeutics.*

*The selectivity of nutritive media is conditioned by the content of antibiotics and chemotherapeutics, as well as by the type of medium used. The best results were obtained using blood agar and FC agar which contained penicillin and ceporex, or pencillin and nitrofurantoin (68.0 — 69.4%). Other nutritive media with the mentioned combinations of antimicrobial substances also exhibited a satisfactory degree of selectivity. Among the media without inhibitory compounds MacConkey agar and Endo agar showed the highest degree of selectivity.*

*Key words: Bordetella, infection, pigs.*

## INTRODUCTION

Isolation of B. bronchiseptica is very important for the aetiology of atrophic rhinitis. A number of authors have pointed out the problem of isolation of B. bronchiseptica (Bertchinger and Nicod, 1970; Rutter, 1981; Smith et al., 1982). The presence of a number of accompanying bacterial flora makes isolation of B. bronchiseptica from the nose difficult and the slow growth of bordetellae adds to this difficulty. Investigations were most frequently aimed towards finding selective media by the addition of antibiotics and chemotherapeutics which would inhibit the growth of accompaying flora and which would not harm the growth of B. bronchiseptica at the same time.

Farrington (1977) used MacConkey agar for isolating bordetellae from nasal swabs. The agar contained furaltadon and nystatin.

Füzi (1975) recommanded that nitrofurantoin should be added to nutritive media for isolation of B. bronchiseptica, as 150 strains of B. bronchiseptica isolated from different animal species showed primary resistance to nitrofurantoin. Most other gram-negative bacteria showed high sensitivity. Examinations in England by Smith and Baskerville (1979) on MacConkey agar and D20 medium gave good results on both media when the number of bordetellae in the was relatively high.

Hommez et al. (1984) examined the effect of cefalexin on the selectivity of media for isolation of B. bronchiseptica. Isolation of bacteria from nasal swabs and from post mortem material proved to be successful when cefalexin was added to nutritive media. In spite of this, the authors pointed out that better results were obtained when penicillin and furaltadon were added to the media.

## MATERIALS AND METHODS

Experimental animals. Nasal swabs of piglets from three litters were used. Pregnant gilts were examined twice before the beginning of the experiment, bacteriologically and serologically. The examination was repeated at farrowing. The animals were kept under the same conditons and were given food without antibiotics. Out of 30 pigs 22 were infected artificially, while eight animals were in cohabitation. Each pig received 0.5 ml of inoculum into the nostrils during inhalation. The same procedure was repeated on the second and on the third day of age.

Inocula. Inocula of strain V of Bordetella bronchiseptica were prepared by injecting 0.1 ml. of a 24-hour broth culture into the yolk sac of 6-day old embryonating chicken eggs. The eggs were incubated at 37° C and candled each day. Embryos dying after 24 h incubation were incubated an additional 24 hours. Yolk fluids were harvested, tested for purity and stored frozen at —20° C.

Swabs were taken at farrowing and at two week intervals. The whole experiment lasted 170 days.

Culture media. Bordetella was cultivated on different solid culture media: blood agar, MacConkey agar containing 1% dextrose, Endo agar and trypton soya agar, pepton agar, Bordet-Gengou agar, charcoal agar and on a medium which we marked as FC agar.

FC agar: peptone 10 g, lactose 10 g glucose 10 g, meat extract 3 g, bile salts 1.5 g, NaCl 5 g, 0.2% phenol red 6,6 ml, distilled water 1000 ml: the final pH was set to 7.4.

Antibiotics, chemotherapeutics and antimycotics were added to these media which were thawed and brought to 45° C. The sensitivity of isolated field strains of B. bronchiseptica was examined before the addition of therapeutics.

Table 1. Sensitivity of 28 strains of B. bronchiseptica isolated from pigs

| Therapeutic agent | Degree of sensitivity of isolated strains | | | |
|---|---|---|---|---|
| | – | + | + | +++ |
| penicillin | 28 | – | – | – |
| linkomycin | 28 | – | – | – |
| nitrofurantoin | 28 | – | – | – |
| bifural | 28 | – | – | – |
| ceporeks | 28 | – | – | – |
| streptomycin | 28 | 2 | – | – |
| ampivet | 21 | 6 | 1 | – |
| specyinomycin | 27 | 9 | 2 | – |
| trimetosul | 12 | 10 | 4 | 2 |
| titan | 13 | 14 | 1 | – |
| gentamycin | 2 | – | 3 | 23 |
| flubactin | – | 4 | 4 | 20 |
| tetracycline | – | 2 | 4 | 22 |
| chloramphenicol | – | – | 3 | 25 |

Based upon these results we used the following antibiotics and chemotherapeutics: penicillin, nitrofurantoin, cefalexin and streptomycin as well as nystatin from the group of mycostatics.

The nutritive media contained antibiotics and chemotheraputics in the following combinations:

Blood agar
1. no inhibitory compounds
2. penicillin 2 mg + nitrofuratoin 10 mg
3. penicillin 2 mg + streptomycin 1 mg
4. penicillin 2 mg + ceporex 5 mg

MacConkey agar
1. no inhibitory compounds
2. penicillin 2 mg + nitrofurantoin 10 mg
3. penicillin 2 mg + ceporex 5 mg
4. nitrofurantoin 10 mg

Brodet-Gengou agar
1. no inhibitory compounds
2. nitrofurantoin 10 mg
3. penicillin 2 mg + ceporex 5 mg

Endo agar
1. no inhibitory compounds
2. nitrofurantoin 10 mg
3. penicillin 2 mg + nitrofurantoin 10 mg
4. penicillin 2 mg + streptomycin 1 mg

Trypton soya agar
1. no inhibitory compounds
2. penicillin 2 mg + streptomycin 1 mg + nitrofurantoin 10 mg
3. penicillin 2 mg + ceporex 5 mg

Pepton agar
1. no inhibitory compounds
2. penicillin 2 mg + nitrofurantoin 10 mg

Charcoal agar
1. no ihibitory compounds
2. penicillin 2 mg + nitrofurantoin 10 mg
3. penicillin 2 mg + streptomycin 1 mg

FC agar
1. no inhibitory compounds
2. ceporex 5 mg
3. penicillin 2 mg + streptomycin 1 mg
4. penicillin 2 mg + ceporex 5 mg
5. penicillin 2 mg + nitrofurantoin 10 mg

The mentioned quantities of antibiotics and chemotherapeutics were added to 100 ml of media. Beside that, nystain (500 I. U. to 10 ml of medium) was added to all media used. Media prepared in such a way were kept at 4° C. Media were used at the latest 5 days after preparation.

The selectivity of the media was checked by parallel seeding of B. bronchiseptica and of nasal swabs from pigs together with B. bronchiseptica.

Nasal swabs were streaked on solid nutritive media and incubated at 37° C for 24 to 72 hours with prolonged incubation up to 96 hours. Media were checked daily. The shape, quantity and colour of grown colonies were noted. Colonies which morphologically coresponded to B. bronchiseptica were streaked on tryptose phosphate agar in order to obtain further indentification.Colonies grown after 24 h used for biochemical exaninations and for agglutination on glass slides with antiserum which was obtained from a five times immunized rabbit.

## RESULTS

During our investigation a total of 147 nasal swabs of artifically infected piglets and piglets in cohabitation was examined. Sampling was performed at two week intervals so that 15 days after the infection 26 nasal swabs were examined and after that 22, 20, 15, 14, 13, 9, 9, 7, 6, 6 samples were also examined. Each material was streacked on 8 different media which contained different combinatins of antibiotics and chemotherapeutics. That means that every swab was streaked on 28 different media. The results of these examinations are presented in Table 2.

In our preliminary examinations we noticed that an increase in the amount of penicillin from 2 to 3 mg did not affect the growth of B. bronchiseptica or the apperance of colonies, nor did it significantly inhibit other bacterial flora. When the amount of ceporex was increased to 10 mg we noticed that colonies were smaller, rough and dried after 72 h. Gentamycin in the amount of 50 micrograms significantly inhibited growth of accompanying bacterial flora, but it also inhibited growth of B.bronchiseptica and therefore we eliminated ot from further usage.

The highest percentage of positive findings was obtained on blood agar and on FC agar when they contained penicilin and nitrofurantoin and a combination of penicillin and ceporex (68.0-69.4) (Table 2). These media also had the highest degree of isolation of B. bronchiseptica in pure culture. Other nutritive media, such as MacConkey agar, Bordet-Gengou agar, Endo agar, trypton soya agar and Charcoal agar with the mentioned combinatons of antimicrobial

substances also expressed a satisfactory degree of selectivity. The percentage of isolation of B. bronchiseptica varied from 60.5 to 63.3.

Among the media without inhibitory compounds the most selective were MacConkey agar and Endo agar. Pepton agar and Charcoal agar, with or without antibiotics ans chemotherapeutics, gave the lowest percentage of isolation of B. bronchiseptica from nasal swabs of pigs.

Table 2. The effect of diferent nutrive media on the degree of isolation of B. bronchiseptica during the whole period of examination

| Type of medium | | B.b. | B.b. other | Other bacteria | Ø | Isolation no. | Isolation % |
|---|---|---|---|---|---|---|---|
| Blood agar | 1 | – | 25 | 122 | 0 | 25 | 17,0 |
| | 2 | 80 | 22 | 30 | 15 | 102 | 69,4 |
| | 3 | 59 | 38 | 33 | 17 | 97 | 65,9 |
| | 4 | 74 | 28 | 27 | 18 | 102 | 69,4 |
| MacConkey agar | 1 | 22 | 49 | 75 | 1 | 71 | 48,3 |
| | 2 | 63 | 27 | 40 | 17 | 90 | 60,5 |
| | 3 | 63 | 30 | 35 | 19 | 93 | 63,3 |
| | 4 | 61 | 27 | 42 | 17 | 88 | 59,9 |
| Bordet-Gengou agar | 1 | – | 27 | 120 | – | 27 | 18,4 |
| | 2 | 44 | 36 | 55 | 12 | 80 | 54,4 |
| | 3 | 76 | 20 | 40 | 11 | 96 | 65,3 |
| Endo agar | 1 | 25 | 40 | 79 | 3 | 65 | 44,2 |
| | 2 | 53 | 29 | 55 | 10 | 82 | 55,8 |
| | 3 | 60 | 29 | 48 | 10 | 89 | 60,5 |
| | 4 | 61 | 26 | 49 | 11 | 87 | 59,2 |
| Trypton soya agar | 1 | – | 8 | 139 | – | 8 | 5,4 |
| | 2 | 59 | 34 | 44 | 10 | 93 | 63,3 |
| | 3 | 60 | 30 | 47 | 10 | 90 | 61,2 |
| Pepton agar | 1 | – | 26 | 118 | 3 | 26 | 17,7 |
| | 2 | 41 | 15 | 38 | 53 | 56 | 38,1 |
| Charcoal agar | 1 | – | 19 | 128 | – | 19 | 12,9 |
| | 2 | 35 | 43 | 49 | 20 | 78 | 53,1 |
| | 3 | 22 | 39 | 64 | 22 | 61 | 41,5 |
| FC agar | 1 | – | 26 | 121 | – | 26 | 17,7 |
| | 2 | 50 | 29 | 59 | 9 | 79 | 53,7 |
| | 3 | 54 | 31 | 52 | 10 | 85 | 57,8 |
| | 4 | 69 | 31 | 31 | 16 | 100 | 68,0 |
| | 5 | 69 | 32 | 30 | 16 | 101 | 68,7 |

total of 147 nasal swabs

## DISCUSSION

According to the results obtained for sensitivity of field strains of B. bronchiseptica (Table 1), the following antibiotics were added to nutritive media: penicillin, nitrofurantoin, ceporex and streptomycin. B. bronchiseptica showed a high degree of sensitivity to gentamycin, so it was not used. On the contrary, Straw et al. (1983) added gentamycin to G2OG and obtained satisfactory results. In our prelynimary work gentamycin showed marked inhibition of the accom-

panying nasal microflora, but it also inhibited growth of B. bronchiseptica at the same time. Similar to our results, Rutter 1981 and Bemis et al. (1977) also found a high sensitivity of B. bronchiseptica to gentamycin. Ceporex proved to be a statisfactory inhibitor of the accompanying nasal bacterial flora, but the quantity of 10 mg/100 ml of medium affected the apperance of the colonies. On MacConkey agar the colonies were smaller and more compact and on Endo agar the colonies were dark pink, rough and compact.

The percentage isolation of B. bronchiseptica was directly connected with the type of medium employed, as well as with the type of antibiotics and chemotherapeutics used. The highest percentage of positive findings was obtained on blood agar and on FC agar which contained penicillin and nitrofurantoin, or penicillin and ceporex, respectively. The degree of isolation of B. bronchiseptica varied from 68.0-69.4%. Other nutritive media with the same combination of antibiotics and chemotherapeutics also showed a high degree od selectivity. B. bronchiseptica was isolated in 60.5-63.3% of cases. Elias and Galgoczi (1981) also found that MacConkey agar should contain added penicilin and nitrofurantoin. The same authors also examined nasal swabs of swine and isolated B. bronchiseptica in 80% of cases. In their further examinations Elias and Szent-Iranyi (1981) found a high percentage of positive sows (36-100%) when they used the same medium. These results are very important from the epizootical point of view, having in mind that it is difficult to isolate B. bronchiseptica from nasal swabs of sows and that isolation can be successfull only from the ethmoidal sinuses after slaughtering.

The resistance of strains of B. bronchiseptica to ceporex, i. e. the sensitivity of the accompanying bacterial flora, was used for the preparation of nutritive media with the addition of ceporex. The results obtained confirm the findings of Hommez et al. (1984). Nevertheless, a greater degree of isolation of B. bronchideptica was obtained when media contained penicillin and ceporex, than with ceporex only.

It is known from the literature that MacConkey agar is the most frequantly used medium, with or without inhibitory compounds, for the isolation of B. bronchiseptica. Farrington (1977) used MacConkey agar with furaltadone, marked as G20. Smith and Bas Kerville (1979) obtained good results with the same medium when examining nasal swabs of young swine. Out of the furan preparations we used nitrofurantoin in our work. Feild strains of B. bronchiseptica were primarily resistant to nitrofurantoin. The results obtained are in ageenment with the findings of Fuzi (1975) and Elias and Galgoczi (1981).

Pepton agar with penicillin and nitrofurantoin gave isolations of bordetellae in 38% of cases. That represents the lowest percentage of isolation of B. bronchiseptica on the selective media used. Smith and Baskerville (1979) used medium G2OG, which is basically pepton agar with the addition of penicillin, furaltadon and gentamycin and isolated bordetellae in 50.01% pigs from 1-12 weeks of age. They estimated the medium as a highly selective medium.

Charchoal agar containes activated coal which neutralizes fatty acids and peroxides, the products of accompanying bacterial flora. These products distrub the growth of B. bronchiseptica which demands a slightly alkaline medium.

Charcoal agar without antibiotics and chemotherapeutics did not give the results expected, but with the addition of inhibitory compounds its selectivity increased significantly (from 12.9% to 53.14%).

We also examined a medium marked as FC agar. It consisted of pepton agar with meat extract, bile salts and phenol red. The results obtained on this agar without inhibitory compounds were similar to rhe findings on blood agar, Bordet-Gengou agar and pepton agar, also without antibiotics and chemotherapeutics. Selectivity significantly increased when FC agar was supplemented with penicillin and ceporex, or penicillin and nitrofurantoin, respectively.

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## SELEKTIVNE PODLOGE ZA IZOLACIJU Bordetella bronchiseptica KOD EKSPERIMENTALNO ZARAŽENIH SVINJA

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SADRŽAJ

Izvedena su ispitivanja vrednosti različitih selektivnih podloga za izolaciju B. brochiseptica iz nosnog brisa.

Ispitivanja su izvedena u petnestodnevnim intervalima nakon veštačke infekcije prasadi stare dva i tri dana, kulturom viruletnog soja B. bronchiseptica. Svaki bris je zasejan na 8 različitih hranjivih podloga (krvni, MacConeky, Bor-

det-Gengou, Endo, tripton soja, pepton, ugljeni i Fc agar) koje su sadržavale različite kombinacije antibiotika i homoterapeutika.

Selektivnost hranljivih podloga uslovljena je sadržajem antibiotika i hemoterapeutika, kao i vrstom korišćene podloge. Najbolji rezultati su postignuti upotrebom krvnog agara i FC agara koji su sadržavali penicilin i ceporeks, ili penicilin i nitrofurantoin (68,0—69,4%). Ostale hranljive podloge sa pomenutim kombinacijama antimikrobnih supstanci takođe su ispoljavale zadovoljavajući stepen selektivnosti. Od podloga koje nisu saržavale inhibitorne supstance najveću selektivnost su ispoljili MacConkey agar i Endo agar.