

MODYFIED SONGER, KINYON and HARRIS SELECTIVE MEDIUM for PRIMARY ISOLATION of TREPONEMA HYODY-SENTERIAE

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Harris et al /2/ used TA agar supplemented 5 per cent horse blood for primary isolation of *T. hyodysenteriae*. For inoculation the specimens were filtered through millipore filters. Gas pak system provided anaerobic conditions. Songer et al /5/ used TSA medium supplemented with 5 per cent horse blood and 400 µg/ml spectinomycin for isolation of *T. hyodysenteriae*. Inoculated media were incubated in anaerobic conditions provided by Gas pak system at 42°C.

The purpose of the experiment was to prepared more selective medium for primary isolation of spirochetes from faeces associated with swine dysentery.

#### Material and Methods

Strains: 17 isolates of *Treponema hyodysenteriae*, 3 of *T. innocens*, 45 of *Bacteroides sp.* and 10 strains of aerobes were used.

Samples: 60 samples of faeces sampled from pigs suffering from swine dysentery and healthy animals were used.

Anaerobic conditions were provided by using a mixture of 20 per cent deoxygenated CO<sub>2</sub> and 80 per cent hydrogen in anaerobic jars with modified Wright's catalyst prepared in our laboratory.

Medium: basal medium TSA blood agar was used. Spectinomycin was kindly supplied by Upjohn Company. The following growth stimulating factors in TSA medium were used: 0,01% dithiotreitol DTT/4/, rumen fluid, egg yolk and yeast extract. Drug sensitivity of *T. hyodysenteriae* by means of MIC test were determined for streptomycin, chloromycin, tetracyclin, polymyxin, vancomycin and crystal violet.

#### Results

Colony forming units-CFU were the highest when basal medium was supplemented with 0,01% DTT and egg yolk or rumen fluid. Yeast extract showed a little less growth, however more stable growth stimulation of *T. hyodysenteriae* than egg yolk and rumen fluid. *T. hyodysenteriae* was resistant to vancomycin and polymyxin. Table 1 shows MIC test of vancomycin for anaerobes and aerobes.

Tab.1. Sensitivity of selected aerobes and anaerobes to vancomycin.

bacteria	number of strains	MIC µg/ml
<i>T. hyodysenteriae</i>	17	500
<i>T. innocens</i>	3	500
<i>Bacteroides sp.</i>	45	3- 250
<i>Staphylococcus aureus</i>	30	0,39-25
<i>Escherichia coli</i>	30	3,12-250
<i>Streptococcus sp</i>	10	0,09-1,56

To the spectinomycin blood TSA medium medium 250 µg/ml vancomycin was added. Table 2 shows the comparison of *T. hyodysenteriae* growth from diluted samples of faeces on 2

selective media.

Tab.2. Growth of *T. hyodysenteriae* from diluted faeces samples on spectinomycin TSA medium and spectinomycin vancomycin TSA medium

specimen	dilution	mean number of colony forming units			
		S. TSA		S. V. TSA	
		<i>T. hyodysenteriae</i>	other sp.x	<i>T. hyodysenteriae</i>	other
S9	10 <sup>4</sup>	134	154	128	0
	10 <sup>5</sup>	22	34	31	0
S10	10 <sup>4</sup>	58	18	44	1
	10 <sup>5</sup>	7	2	6	0
S11	10 <sup>3</sup>	43	83	38	0
SW	10 <sup>5</sup>	32	58	34	8
1/III	10 <sup>5</sup>	16	24	10	0

x/ usually *bacteroides sp* and cocci.

The results presented in table 2 shows that vancomycin /spectinomycin medium was more selective than Songer et al./5/ medium. The number of CFU on this medium was only 6 per cent lower than on spectinomycin TSA agar.

#### Conclusions:

Spectinomycin/vancomycin yeast extract DTT TSA medium was tested using samples of 60 faeces from animals suffering swine dysentery and healthy pigs. *T. hyodysenteriae* was not only isolated from suffering pigs, but also from carriers of this bacteria.

Selected references: 1. Binek M., Szynkiewicz Z., Rumińska A., *Medycyna Wet.* 9: 536, 1980. 2. Harris D.L., Glock R.D., Christensen C.R., Kinyon J.M., *Vet. Med. /Small Anim. Clin.* 67: 61, 1972. 3. Kinyon J.M., Harris D.L.: Symposium of Vet. Lab. Diagn. Lucerne, 1980. 4. Małuszyńska G.M., Pietraszak A.: *Acta Microbiol. Polon.* Ser. B, 3: 171, 1971. 5. Songer J.G.J., Kinyon J.M., Harris D.L., *J. Clin. Microbiol.* 4: 57, 1976.