The role of H_2S in the recovery of *Salmonella* spp. from animals

G. Evangelopoulou (DVM, PhD Student)[1], A.R. Burriel (DVM, MSc, MSc, PhD)[1]

[1]Laboratory of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Thessaly, Greece

Introduction

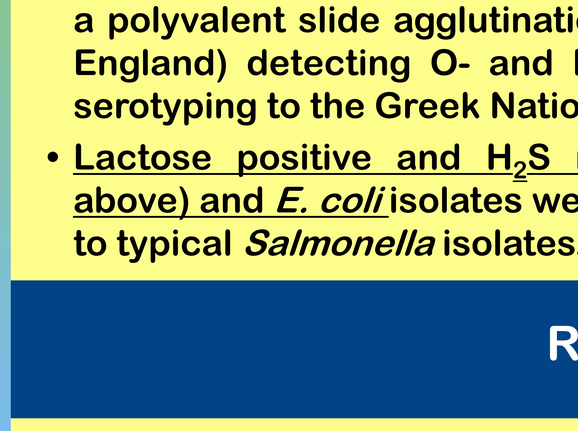
- The ability of a microorganism to produce H_2S is a detrimental taxonomic characteristic, with *Citrobacter, Proteus* and *Salmonella* being the major H_2S-producing genera of the Enterobacteriaceae. H_2S is a highly toxic compound to mammalian cells contributing, perhaps, to their ability to colonize tissues, playing a specific role in gastroenteritis and in the pathogenesis of ulcerative colitis. However, loss of H_2S production may occur in environmental strains of *Salmonella* spp. due to mutations or it can be "masked" by acid production during sugar fermentation on typical diagnostic media.

Materials and Methods

- Samples: 615 samples, from a variety of pig carcass sites were examined using ISO 6579:2002, Annex D (ISO 2002).
- Culture media: XLD agar (XLD Oxoid – England) and Salmonella Shigella agar (SS Merck - Germany) were used for isolating suspect colonies from MSRV (Biokar -France). Colonies suspected of being *Salmonella* spp. were subcultured on Columbia blood agar (CBA, Oxoid, England) for further examination with Gram stain, oxidase production test and utilization of Triple Sugar Iron Agar (TSI, Merck- Germany).
- Identification: Suspect isolates were assigned to species using the API 20E (Biomerieux, France) and the MicrogenTM GnA+B-ID (Microgen Bioproducts Ltd, UK) Systems.
- Serotyping: Recognized *Salmonella* spp. isolates were tested with a polyvalent slide agglutination test (Remel Europe Ltd; Dartford, England) detecting O- and H- antigens and mailed for specific serotyping to the Greek National Reference Laboratory (GNRL).
- Lactose positive and H_2S negative salmonellae (identified as above) and *E. coli* isolates were cultured on SS and TSI media next to typical *Salmonella* isolates.

Results

- Of the 59 serotyped *Salmonella* isolates five (5) were lactose positive and H_2S negative when cultured on XLD, SS and TSI media.
- They were assigned to serovars: S.I.6,14,25:-:1,2 (1), *S. enterica* subsp. houtenae 40:g,t:- (1), *S. enterica* subsp. *salamae* (1) and S.I. 6,7:k:- (2).
- The above five (5)*Salmonella* spp. isolates and three (3) *E. coli* produced H_2S when co-cultured with typical strong H_2S - producing *Salmonella* isolates, after prolonged incubation (30h to 48h).



Negative control (Strain 69): *E.coli* H_2S(-) isolate on TSI (a), SS (b) and XLD (c) media.

Positive control (Strain 79): Lactose negative -H_2S(+) *Salmonella* isolate on XLD (a), SS (b) and TSI (c) media.

Strain 17: Lactose positive-H_2S(-) *Salmonella* isolate on SS (a) and TSI (b) media.

Typical *Salmonella* isolate (strain 79/ red arrows) on SS (a) and TSI (b) media co-cultured with: i) *Salmonella* H_2S (-) (strains 17, 50/ yellow arrows) and ii) *E.coli* (strains 30,113 /green arrows, negative control /blue arrows) after 36h incubation.

Conclusions

- The observations indicate an increasing number of false negative results (present investigation ~ 8.5%), decreasing the recovery of *Salmonella* spp., if ISO recommendations are strictly followed.
- The presence of H_2S-producing isolates in the gut may synergically reactivate the "masked" ability of H_2S production of non- H_2S producing *Salmonella* spp. and that of the abundant in the gut *E. coli.*
- This may result in an increased exposure of the colonic mucosa to H_2S, causing, perhaps, local tissue damage, if cecal mucosa fails to detoxify it.

References

- Barrett EL and Clark MA. 1987. Tetrathionate Reduction and Production of Hydrogen Sulfide from Thiosulfate. Microbiological Reviews, 51: 192-205.
- Bulmash, J.M., Fulton, M., Jiran, J. 1965. Lactose and sulfide reactions of an aberrant Salmonella strain. J. Bacteriol. 89:259.
- Levitt MD, Furne J, Springfield J, Suarez F, DeMaster E. 1999. Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. J. Clin.Invest. 104:1107–1114.
- Sakano C, Kuroda M, Sekizuka T, Ishioka T, Morita Y, Ryo A, Tsukagoshi H, Kawai Y, Inoue N, Takada H, Ogaswara Y, Nishina A, Shimoda MA, Kozawa K, Oishi K, Kimura H. 2013. Genetic analysis of non-hydrogen sulfide-producing Salmonella enterica serovar Typhimurium and S. enterica serovar Infantis isolates in Japan. J. Clin. Microbiol. 51:328–330.