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Research Article

PREVALENCE OF SALMONELLA IN EQUIDS DETERMINED BY MICROBIOLOGICAL CULTURE, STANDARD TUBE AGGLUTINATION TEST AND PCR

N. BABU[1], B. R. SINGH[2], HARISHANKAR, R. K. AGRAWAL, MUDIT CHANDRA[3], T. V. VIJO[4], S. K. SRIVASTAVA and M. P. YADAV[5]
National Salmonella Centre (Vet.), Division of Bacteriology and Mycology
Indian Veterinary Research Institute, Izatnagar-243 122

ABSTRACT

The *Salmonella enterica* strains were isolated from 35 faecal samples (14.2%) of 245 equines examined using four selective enrichment broths simultaneously. Eight animals were excreting *Salmonella* strains of two serovar each. Of the 43 isolates of *Salmonella*, 42 belonged to 10 serovars of *Salmonella enterica* subspecies *enterica* (*S.*) namely, *S. I.* 4,5,12,27: r,i: 1,5 (13), *S.* Drogana (9), *S.* Lagos (6), *S.* Kottbus (4), *S.* Bovismorbificans (4), *S.* Dumfries (3), *S.* Tshiongwe (1), *S. I.* 3, 10, 15: r:- (1) and *S. I.* 6,7: y: 1,z_{28} (1), and one isolate to *S. enterica ssp salamae* 6,7: g t: z_{42}. All the 43 isolates from 35 faecal samples were obtained using selective enrichment in tetrathionate broth (TTB) while only five samples were positive using Rappaport-Vassiliadis R10 (RV) medium. No sample was detected positive for *Salmonella* using either selenite cystine (SC) or *Salmonella* selective enrichment broths (SEB). With PCR, 66 faecal samples yielded a *Salmonella* specific amplicon of 496 bp using *hisJ* gene primers. All the 35 faecal samples positive for *Salmonella* with conventional bacteriological culture method were also positive with PCR. Although, using *Salmonella* spiked (10 cfu of *S.* Abortusequi-E158 g^{-1} of faeces) faecal samples of 10 *Salmonella* free horses, PCR had 100% sensitivity (Se) and 100% specificity (Sp). On field samples of equine faeces Se and Sp of PCR was 1.00 and 0.85, respectively. Standard tube-agglutination test on serum samples of equines using 'O' 4,12 antigen (STAT-O) had Sp and Se of 0.87 and 0.61, respectively while using 'H' e,n,x antigen (STAT-H), Sp and Se was 0.87 and 0.61, respectively. Corrected prevalence (P_{RG}) of salmonellosis with conventional bacterial culture, STAT-O, STAT-H and PCR in equines was 14%, 5%, 0% and 14%, respectively. Prevalence was significantly higher in mules and female donkeys (~70%).

Key words: *Salmonella*, equines, PCR, STAT, tetrathionate broth, prevalence

Salmonellosis, an important infectious disease of equids, caused by different serovars of *Salmonella enterica* subspecies *enterica* is often associated with fatal septicaemia and severe diarrhoea in foals and colitis/typhilitis in equids of all age groups (Singh *et al.,* 2007). Many of the infected animals become chronic asymptomatic carriers of the pathogen serving as a potential source of infection to susceptible hosts (Smith *et al.*, 1978). Isolation of *Salmonella* organisms from faecal samples of subclinically infected carriers or diseased equids may be difficult due to limitations of sensitivity of the technique, excretion of injured or non-viable pathogen and due to intermittent shedding (Carter *et al.*, 1986). Therefore, standard tube agglutination test (STAT) is considered valuable in detecting of intermittent *Salmonella* shedding carriers (House *et al.*, 1993). The identification of *Salmonella* spp. from faeces through conventional culture technique is a labour intensive, time consuming process, hence, for rapid and reliable diagnosis, polymerase chain reaction (PCR) based tests are now being widely used (Gentry-Weeks *et al.*, 2002). However, false negative results due to a variety of inhibitory substances such as bilirubin and chelating agents in clinical samples (Widjojoatmodjo *et al.*, 1992) could limit the use of PCR. Enrichment broth, incubation temperature, incubation time and

[1]Captain, Equine Breeding Stud, Babugarh, Ghaziabad, Uttar Pradesh
[2]Corresponding author & Present address Principal Scientist, ICAR Research Complex for NEH Region, Nagaland Centre, Jharnapani, Medziphema-797 106, India
[3]GADVASU, Ludhiana, Punjab
[4]The Donkey Sanctuary, Gurgaon, Haryana-122 001
[5]Vice-Chancellor, SVBP University of Agriculture and Technology, Meerut

enrichment technique (single enrichment or double enrichment) might alter sensitivity of PCR and culture techniques used for detection of *Salmonella* in faecal samples of different animal species (Carli *et al.*, 2001). This study reveals the prevalence of *Salmonella* infection in equids through bacteriological culture, standard tube-agglutination test (STAT) and PCR.

MATERIALS AND METHODS

***Salmonella* strain:** Reference strain of *Salmonella* Abortusequi (E-158) used for preparation of 'O' 4, 12 and 'H' e, n, x antigens and for spiking the control faecal samples was revived from the glycerol stocks maintained at the National *Salmonella* Centre (Vet.), Indian Veterinary Research Institute, Izatnagar.

Faecal samples: About 10 g faecal sample from each equid was collected directly from the rectum using pre-sterilized disposable full sleeves gloves (Bareilly Biologicals, India). Samples were immediately transferred to the labelled sterile faecal sampler (Tarson, India Ltd. Kolkata) and brought to the laboratory on ice. Equine faecal samples (245) were collected from equids under unorganized sector with permission from the owners in villages of different parts of India (Tamilnadu, 91, Rajasthan, 68, Uttar Pradesh, 30 and Haryana, 56) over a period of 6 months from November, 2002 to June, 2003. Consent of owner for sampling was the only criteria for sampling the animals.

Besides, faecal samples, from 10 known *Salmonella* negative horse mares maintained in the Institute, were spiked with 10 cfu of *S. enterica* ssp *enterica* serovar Abortusequi (*S.* Abortusequi E-158) g^{-1} of faeces. The spiked and non-spiked samples were processed in the similar way as the test samples as positive and negative controls, respectively.

Serum samples: About five ml blood from each animal was collected using labeled sterile vacutainer (Becton Dickinson, USA) from all the equids sampled for faeces. Serum was separated after clotting and transported to laboratory on ice packs. All the serum samples were stored at -20°C until tested.

***Salmonella* isolation:** All of the 245 faecal samples were extended in nine volumes of buffered peptone water (BPW) and incubated at 37° C for 18 h to resuscitate the organisms under stress. After pre-enrichment, faecal samples were selectively enriched at 37°C for 24 h using four different media namely tetrathionate (Difco) broth (TTB), selenite-cystine (Difco) broth (SCB), *Salmonella* selective enrichment broth (Agrawal, 2003) and Rappaport-Vassiliadis R10 (Difco) medium (RV-10). Thereafter, a loopful of inoculum from the enrichment broth was streaked on to hektoen enteric agar (HEA, Hi-Media, Mumbai) and brilliant green agar (BGA, Hi-Media) plates. Plates were incubated at 37°C for 24 h. Presumptive *Salmonella* colonies showing smooth, transparent, with or without black centre with greenish periphery on HEA and transparent reddish colonies with pink periphery on BGA were picked up and confirmed (Edwards and Ewing, 1972) before submitting for serotyping at NSC (Vet), Izatnagar.

Standard tube-agglutination test: Somatic 'O' 4, 12 and flagellar 'H' e, n, x antigens were prepared with reference strain of *S.* Abortusequi E-158 (Edwards and Ewing, 1972). The STAT was performed by making serial two-fold dilutions of test sera in NSS and adding equal volume (0.5 ml) of antigen in each tube (Hudson and Hay, 1991). Negative and positive controls, having NSS and factor specific rabbit antiserum (titer 1:80), respectively were included. Highest dilution of serum yielding complete agglutination by mat formation at bottom with clear fluid at top was taken as agglutination titre. Agglutinin titres of $\geq$80 and $\geq$320 against 'O' and 'H' antigens, respectively were considered positive.

Polymerase chain reaction: To determine *Salmonella* in faeces, 496 bp fragment of a conserved sequence of *his*J gene was amplified (Cohen *et al.*, 1994) by PCR. For PCR, primer1 (5' ACT GGC GTT ATC CCT TTC TCT GGT G) and 2 (5' ATC TTG TCC TGC CCC TGG TAA GAG A) custom synthesized by Genei (Bangalore) and template DNA from 1 ml aliquot after selective enrichment in TTB (Babu, 2003) were used. A 25 µl of reaction mix consisting of 5µl of the template, 2.5 µl of 10x Taq polymerase reaction buffer, 1.5 mM $MgCl_2$, 300 µM dNTP

mix, 37.5 pM of each of the two primers and 1 U of Taq DNA polymerase was used. For amplification in Eppendorf Thermocycler (Corning, USA), an initial denaturation (5 min at 94°C) was followed by 30 cylces of denaturation (1 min at 94°C), annealing (1 min at 55°C) and extension (90 s at 72°C) and a step of final extension for 10 minutes at 72°C. The amplicon of 496 bp was visualized under uv illumination after agarose gel electrophoresis (5volts/cm) using 1.5% agarose gel made in 0.5*X* Tris-borate buffer (TBE) containing ethidium bromide ($0.5 \mu g\ ml^{-1}$).

Statistical Analysis: Prevalence of salmonellosis, determined by different tests, was adjusted using Rogan and Gladen (1978) method. Sensitivity, specificity, and predictive value (negative and positive) of different tests were also calculated (Thrusfield, 1995) using *Salmonella* isolation method as gold standard. To determine the effect of different sex and equine species on prevalence Chi-square test statistics was used (Thrusfield, 1995).

RESULTS AND DISCUSSION

Evaluation of different enrichment media: Forty three *Salmonella* were isolated from 35 (14.3%) of the 245 equine faecal samples using selective enrichment in TTB while no *Salmonella* could be isolated following selective enrichment in SCB and *Salmonella* selective enrichment broths (SEB). Only 5 samples were positive for *Salmonella* using RV-10 medium for selective enrichment. All five faecal samples positive for *Salmonella* using RV-10 were also positive with TTB enrichment. Thus, TTB was the best selective enrichment medium for isolating *Salmonella* from equine faecal samples similar to earlier reports (Carli *et al.*, 2001).

Isolaion of *Salmonella* serovars: *Salmonella* isolates were serotyped into 10 serovars of *Salmonella enterica* subspecies *enterica* (*S.*) namely, *S.* 4,5,12,27: r,i: 1,5 (13), *S.* Drogana 1,4,12,27: r,i: e,n,z_{15}; (9), *S.* Lagos 1,4,5,12: i: 1,5 (6), *S.* Kottbus 6,8: e,h: 1,5 (4), *S.* Bovismorbificans 6,8,20: r,i: 1,5 (4), *S.* Dumfries 3,10: r,i: 1,6 (3), *S.* Tshiongwe 6,8: e,h: e,n,z_{15} (1), *S. I.* 3, 10, 15: r:- (1) and *S. I.* 6,7: y: l,z_{28} (1), and one isolate to *S. enterica ssp salamae* 6,7: g t: z_{42}. Isolation of *Salmonella* from 14.3% apparently healthy equids confirmed the endemic status of *Salmonella* infection in equids in India (Gupta and Verma, 1993). Excretion of strains of zoonotic *Salmonella* serovars by apparently healthy animals might be a potential danger to equine caretakers. Eight (22.6%) of the 35 faecal samples were positive for *Salmonella*e isolates belonging to more than one serovars proving existence of mixed infection of *Salmonella* serovars in equids. Moreover, frequent isolation of the strains of *S.* Drogana, *S.* Dumfries, *S.* Tshiongwe, *S.* Kottbus, *S. I.* 4, 5, 12, 27: r,i: 1, 5 and *S. I.* 6,7: y: lz_{28} serovars rarely reported in India either from human or other animals (Gupta and Verma, 1993) indicated that epidemiology of salmonellosis in equids in India is more complicated than in other animals probably due to easy international movement of equids for races and sports and close contact with their owners and caretakers being a companion animal.

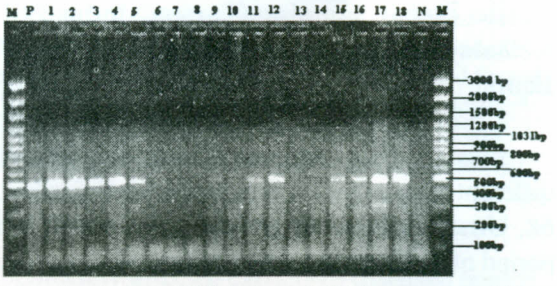

Fig 1. Amplicons (496 bp) on 1.5 % agarose gel from PCR assays on equines faecal samples using primer 1 and 2 for *hisJ* gene of *Salmonella* genus. M, molecular weight markers (100bp ladder); N, negative control; P, positive control; 1 to 18, different faecal samples of equines.

Detection of *Salmonella* by PCR: Using control spiked faecal samples from 10 mares PCR had 100% sensitivity and specificity. Out of 245 faecal samples screened with PCR for specific amplicon of 496 bp (Fig 1), 66 (26.9%) faecal samples were positive for *Salmonella*. All the 35 samples positive for *Salmonella* spp. by culture method were also detected positive by PCR yielding a 1.00 and 0.85 in-field Se and Sp, respectively (Table 1) similar to earlier reports (Gentry-Weeks *et al.*, 2002, Babu, 2003). Presence of similar amplicon (496 bp) in culture-

Table 1

Prevalence of salmonellosis in equids using different tests for diagnosis of *Salmonella* infection

Animal type (n)	Prevalence estimate	Prevalence estimate of salmonellosis in equids using			
		Isolation	STAT-O	STAT-H	PCR
Mules (40)	P_A	0.33	0.35	0.28	0.68
	P_{RG}	0.33	0.25	0.00	0.62
Male horses (14)	P_A	0.07	0.00	0.29	0.07
	P_{RG}	0.07	0.00	0.00	0.00
Female horses (118)	P_A	0.03	0.02	0.25	0.05
	P_{RG}	0.03	0.00	0.00	0.00
Male donkeys (33)	P_A	0.09	0.39	0.49	0.09
	P_{RG}	0.09	0.30	0.27	0.00
Female donkeys (40)	P_A	0.38	0.33	0.23	0.75
	P_{RG}	0.38	0.22	0.00	0.71
Total (245)	P_A	0.14	0.17	0.28	0.27
	P_{RG}	0.14	0.05	0.00	0.14

negative faecal samples might be attributed either to the higher sensitivity of PCR than culture or the ability of PCR assay to detect even non-viable *Salmonella* (Cohen *et al.*, 1996) or false detection due to mispriming with related bacteria. In a few studies, PCR had low sensitivity (Carli *et al.*, 2001, Feder *et al.*, 2001) which might be due failure of enrichment of the pathogen as observed with three enrichment broths which were proved inferior to TTB as earlier (Gouws *et al.*, 1998, Soumet *et al.*, 1999) or due to inhibitory activity of TTB (Stone *et al.*, 1994) which could be removed by washing of the TTB cultures. In this washing of cell pellet after enrichment, particularly TTB enrichment might be responsible for higher sensitivity of PCR, while use of unwashed cultures, in earlier studies (Stone *et al.*, 1994) might be responsible for reduced sensitivity because of inhibitory activity of TTB due to its high calcium contents. The choice of primer set may be the other crucial factor for variation in the sensitivity of PCR assay (Gentry-Weeks *et al.*, 2002). The primers used in this study have been reported to have no cross reaction with other bacteria present faeces (Cohen *et al*, 1994). Although PCR protocol used in this study took about 36 h to detect *Salmonella* in faeces in contrast to 24 h in earlier studies (Feder *et al.*, 2001, Carli *et al.*, 2001), the higher sensitivity (1.00) of this protocol justified the use. With the use of TTB enrichment protocol, all 35 Salmonella positive and control spiked samples yielded positive results in PCR, which could not be achieved earlier (Feder *et al.*, 2001, Carli *et al.*, 2001).

STATs for anti 'O' 4, 12 and anti 'H' e, n, x antibodies: Antibodies against serogroup B *Salmonella* ('O' 4, 12) and 'H' e, n, x, present in more than 20 serovars prevalent in India (Singh, 2005) were detected in ~17% and 28% equids respectively (Table 1). Absence of correlation between high *Salmonella* agglutinin titers and isolation of different *Salmonella* serovars from the faecal samples of equids yielded low Se and Sp for the STATs which are in concurrence with similar findings in cattle and pigs (Veling *et al.*, 2002, LoFo-Wong *et al.*, 2003). In this study ~65% and 33% *Salmonella* isolated from equids possessed antigens inducing antibody generation detectable with STAT-O (4, 12) and STAT-H (e, n, x), respectively. Thus to improve the sensitivity of STATs one should use more antigens for STATs particularly 'O' 6, 7 (group C) and 3, 10 (group E). Presence of high antibody titres but no isolation of the pathogen might be due to the persistence of antibodies in convalescent animals or after a transient infection (Veling *et al.*, 2000) or excretion by latent carriers (House *et al.*, 1993). Thus, the use of bacteriological examination and PCR for the detection of *Salmonella* in clinical samples from latent carriers and intermittent excretors is consequently limited.

Se and Sp of different tests: Although isolation of *Salmonella* has many limitations (House *et al.*, 1993), there is no other gold standard. Hence calculation of Se and Sp of serological tests with *Salmonella* isolation might not reflect the real

Table 2

Chi-square statistics to estimate effect of sex and species in equids on prevalence of salmonellosis by different tests

Compared groups	STAT-O	STAT-H	Isolation	PCR
Three different kinds of equids	0.00	0.49	0.00	0.00
Mules versus horses	0.00	0.78	0.00	0.00
Mules versus donkeys	0.96	0.54	0.45	0.12
Horses versus donkeys	0.00	0.23	0.00	0.00
Male horses versus female horses	0.63	0.78	0.35	0.63
Male donkeys versus female donkeys	0.62	0.06	0.01	0.00

situation. The PCR assay had the higher Se (1.00) and Sp (0.85) than STATs because both *Salmonella* isolation and PCR assay depends on the presence of the pathogen in the sample while STATs depends on presence of *Salmonella* agglutinins (Gentry-Weeks *et al.*, 2002).

Effect of species, sex, and place on prevalence of *Salmonella* in equids: The prevalence of *Salmonella* (Table 1) in equids indicated that mules and female donkeys were more common excretors of *Salmonella* than horses and male donkeys. Chi-square analysis (Table 2) further proved that female donkeys were more susceptible to *Salmonella* colonization and excretion than males and mules were equally susceptible as their grannies. More prevalence of *Salmonella* in female donkeys than in males might be due to the effect of sex on prevalence of *Salmonella* as reported earlier in human beings and other animals (Singh and Verma, 2003, Chandra *et al.*, 2007) due to modulation of immune response by sex hormones (Singh and Verma, 2003). Place of sampling had no significant effect on prevalence of *Salmonella* infection in equids however; *S.* Tshiongwe and *S.* Lagos were isolated exclusively from equids of Tamilnadu and Rajasthan, respectively.

It can be concluded that PCR is the most sensitive and specific test for detection of *Salmonella* excretion in faeces of equids but have the limitation of not indicating about the serovar of the infecting *Salmonella* which might be an important factor in understanding epidemiology of the disease. For isolation of *Salmonella* from faecal samples, TTB was found to be more suitable selective enrichment. Although STATs for *Salmonella* had no correlation with the detection of *Salmonella* with PCR or isolation technique and had low Se and Sp, but it can be used as global sero-surveillance test to detect latent and convalescent carriers being simple and informative about the infecting serovar.

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REFERENCES

Agrawal, R.K. (2003). *Salmonella* surveillance in foods. Annual Report, IVRI, Izatnagar, pp. 14.

Babu, N. (2003). Epidemiological studies on *Salmonella* infection in equines. M. V. Sc. thesis, IVRI, Izatnagar, India.

Carli, T.K., Unal, B.C., Caner, V. and Eyigor, A. (2001). Detection of *Salmonellae* in Chicken. faeces by a combination tetrathionate broth enrichment, Capillary PCR and capillary Gel Electrophoresis. *J. Clin. Microbiol* **39**: 1871-1876.

Carter, J.D., Hird, D.W., Farver, T.B. and Hjerpe, C.A., (1986). Salmonellosis in hospitalized horses: Seasonality and case fatality rates. *J. Am. Vet. Med. Assoc.* **188**: 163-167.

Chandra, M., Singh, B.R., Shankar H., Agarwal M., Agarwal, R.K., Sharma, G. and Babu, N. (2007). Prevalence of *Salmonella* antibodies among goats slaughtered for chevon in Bareilly (Northern India). *Prevent. Vet. Med.* 80: 1-8

Gentry-Weeks C., Hutcheson, H.J., Kim, L.M., Bolte, D., Traub-Dargatz, J., Morley, P., Power, B. and Jessen, M. (2002). Identification of two phylogenetically related organisms from faeces

by PCR for detection of *Salmonella* spp. *J. Clin. Microbiol.* **40**: 1487-1489.

Cohen, N.D., Wallis, D.E., Neibergs, H.L., Hargis, B.M., Simpson, R.B. and McGruder, E. B. (1994). Genus specific detection of *Salmonellae* in equine species by use of polymerase chain reaction. *Am. J. Vet. Res.* **55**: 1049-1054.

Cohen, N.D., Martin, L.J., Simpson, R.B., Wallis, D.E. and Neibergs, H. L. (1996). Comparison of polymerase chain reaction and microbiological culture for detection of *Salmonellae* in equine species and environmental samples. *Am. J. Vet. Res.* **57**: 780-786.

Edwards, P.R. and Ewing, W.H. (1972). Identification of enterobacteriaceae, (4th edn.), Elsevier Science, Burgees Pub. Co., USA.

Feder, I., Jerome, C.N., John, G., Teresa, Y., Jan, M.S., Richard, O., Mark, L.T. and John, B.L. (2001). Comparison of cultivation and PCR-hybridisation for detection of *Salmonella* in porcine fecal and water samples. *J. Clin. Micriobiol.* **39**: 2477-2484.

Gouws, P.A., Vesser, M. and Brozel, V.S. (1998). A Polymerase chain reaction procedure for the detection of *Salmonella* spp within 24 hours. *J. Food. Prot.* **61**: 1039-1042.

Gupta, B.R. and Verma, J.C. (1993). Monograph on Animal Salmonellosis. National *Salmonella* Centre (Vet.), Indian Veterinary Research Institute, Izatnagar, India.

House, J.K., Smith, B.P., Dilling, G.W. and Roden, L.D. (1993). Enzyme linked immunosorbent assay for the serological detection of *Salmonella* Dublin carriers on a large dairy. *Am. J. Vet. Res.* **54**: 1391-1399.

Hudson, L. and Hay, F.C. (1991). Practical Immunology (3rd edn.), Oxford Blackwell Scientific Publication, London.

LoFo-Wong, D.M.A., Dahl, J., van der Wolf, P.J., Wingstrand, A., Leontides, L. and von Altrock, A. (2003). Recovery of *Salmonella enterica* from seropositive finishing pig herds. *Vet. Microbiol.* **97**: 201-214.

Rogan, W.J. and Gladen, B. (1978). Estimating prevalence from the results of a screening test. *American J. Epidemiol.* **107**: 71-76.

Singh, B.R. (2005). Prevalence of *Salmonella* serovars in animals in India. http://www. aclisassari.com/ acli-openlearning/ uploads/lectures/Methods

Singh, B.R., Babu, N., Jyoti, J., Shankar, H., Vijo, T., Agrawal, R., Chandra, M., Kumar, D. and Teewari, A. (2007). Prevalence of multi-drug-resistant *Salmonella* in equines in organized and unorganized sectors in India. *J. Equine Vet. Sci.* **27**: 266-276.

Singh, B.R. and Verma, J.C. (2003). A study on factors influencing 'O' and 'H' agglutinin titres of hyper immune serum raised in rabbits. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **24**: 49-53.

Smith, B.P., Reina-Guerra, M., Hardy, B.S., 1978. Prevalence and epizootiology of equine salmonellosis. *J. Am. Vet. Med. Assoc.* **172**: 353-356.

Soumet, C., Ermel, G., Rose, V., Rose, N., Droin, P., Salvat, G. and Colin, P. (1999). Identification by a multiplex PCR based assay of *Salmonella* Typhimurium and *Salmonella* Entritidis strains from environmental swabs of poultry houses. *Lett. Appl. Microbiol.* **29**: 1-6.

Stone, G.G., Oberst, R.O., Hay, M.P., Mc Vey, S. and Chengappa, M.M. (1994). Detection of *Salmonella* serovars from clinical samples by enrichment broth cultivation-PCR procedure. *J. Clin. Microbiol.* **32**: 1742-1749.

Thrusfield, M., (1995). Veterinay Epidemiology. (2nd edn.), Blackwell Science Ltd., Oxford, pp. 276-282.

Veling, J., Barkema, W., Schans, J., Van Zijderveld, F. and Verhoff, J. (2002). Herd level diagnosis for *Salmonella enterica* subsp. *enterica* serovar Dublin infection in bovine dairy herds. *Prev. Vet. Med.* **53**: 31-42.

Veling, J., Van Ziderveld, G., van Zijderveld-van Bemmel, A.M., Barkema, H.W. and Schukken, H.V. (2000). Evaluation of three newly developed enzyme linked immunosorbent asays and two agglutination tests for detecting *Salmonella enterica* subsp. *enterica* serovar Dublin infections in dairy cattle. *J. Clin. Microbiol.* **38**: 4402-4407.

Widjojoatmodjo, M.N., Fluit, A.C., Torensma, R., Verdonk, G.P.H.T. and Verhoef, J. (1992). The magnetic immunopolymerase chain reaction assay for direct detection of *Salmonellae* in fecal samples. *J. Clin. Microbiol.* **30**: 3195-3199.