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Deep Sequence and Microarray Techniques of Salmonella Enterica Serovar Typhimuriumin Horses

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ABSTRACT

My research can detect this transmitted gene that usually cause of antibiotics resistance to help control this type of salmonella stain by treatment and control in animal as well as human Sero typing has been the core of public health monitoring of Salmonella infections for over 50 years. Now, Diagnosis use DNA testing to further divide each serotype into more subtypes and to detect more outbreaks. With the next generation of sequencing technology, advancements continue as the laboratory can find information about the species, serovar, and subtype of bacteria in just one test. Availability of resources will determine the extent of research carried out by me, versus research conducted at collaborating labs.

Keywords: *salmonella, horses, gene, antibiotics, serotype*

INTRODUCTION

Importance of these bacteria of the Salmonella genus is widespread in nature. They can be detected in many cold and warm-blooded animals across the globe. In numerous countries today they are the most important bacterial diarrhea-causing pathogens in man. As they are mostly transmitted from animals to man through consumption of foods of animal origin, salmonellae are classified as zoonosis ("zoonosis" is the scientific term for all diseases which can be transmitted by pathogens from animals to man).They transmit genetic material (DNA) in resistant strains.

ETIOLOGY

Smith et al (1978)

Reported that Salmonellosis, a serious infectious disease of equids, caused by different serovars of Salmonella enterica subspecies enterica, often precipitates as fatal septicemia and severe diarrhea in foals and colitis/typhilitis in equids of all ages. Salmonellae are the most frequent causes of acute diarrhoea in horses and the incidence seems to be increasing (Powell et al., 1988, Traub- Dargatz et al., 1990).

Michael et al (2005)

Indicated that Salmonellae may be a primary cause of infection in foals under 6 months old. The serovar abortus equi (groupB with O ANTIGEN epitopes 4and 12) belonging to salmonella enterica subspp entrica is acause of equine paratyphyoid although salmonella typhimurium has been reported (Gall et al 2006).

Niwa et al (2009)

Reported that the equine affected with different seovar of salmonella but the most sever one is the salmonella typhimurium.

EPIDEMIOLOGY

Horses recovering from acute salmonellosis may act as a source of environmental contamination with Salmonella spp for a variable period of time (Palmer and Benson, 1985) monitored 81 horses recovering from acute enteritis for a period of 30 to 718 days.

Although 31 horses were lost to follow-up, the median period of fecal shedding of Salmonella spp was <30 days. In another population, 2 of 50 horses on a farm exposed to 43 horses recovering from acute salmonellosis transiently shed Salmonella spp.

Outbreaks of equine salmonellosis are generally associated with a high population density of horses such as occurs on large horse farms and in equine veterinary hospitals Horses admitted to hospitals are more susceptible to Salmonella infection because of multiple stresses, which may include predisposing illness, transportation and

physical treatment (Hird et al., 1986; Traub-Dargatz et al., 1990; van Duijkeren et al., 1994).

Walker et al (1991)

Estimated that the frequency of salmonellosis varies with age, 25% of foals 0-7 days of age have diarrhea compared to 40% and 8% of foals aged 8-31 days and 32-180 days respectively.

Hartmann et al (1996)

Recorded that Case fatality rates up to 38%.during occurring Salmonella outbreaks in several hospitals in the United States, including the Universities of California, Georgia and Wisconsin and Colorado and Michigan State Universities

Murray (1996)

Reported that infection of horses by Salmonella organisms is a serious health issue. It is particularly troubling when outbreaks occur in hospitalized patients because these outbreaks can result in substantial economic losses and have a major impact on the welfare and Establishments with a high-density of horses.

Traub-Dargatz et al (2000)

Stated that the prevalence of fecal shedding of Salmonella spp among healthy horses is less than 1% However, the prevalence in hospitalized horses ranges between 5% and 10%.

Ward Et Al (2004)

Claimed that Salmonella spp can be transmitted rapidly either directly or indirectly among highly susceptible and stressed horses and the outbreaks of salmonellosis in hospitalized horse populations have been associated with severe economic loss.

Clinical Signs

Owen et al (1983)

Found that Infection of horses with Salmonella can have arranged of outcomes from asymptomatic carriage to life threatening septicaemia.

Hird et al (1986)

Recorded that Salmonella in horses causing diarrhea, large colon impaction, colic, and distance of transportation without preventive programs, nosocomial salmonellosis may result in substantial morbidity, mortality, and economic loss.

Equine Salmonellosis is a common serious condition in the horse; several clinical forms are recognized. The peracute form, usually seen only in foals, is characterized by fever, tachycardia, hperpnoea, leucopenia, anorexia, and septic shock.

The second and most common form is characterized by fever, anorexia, neutropenia, and diarrhea Chronic diarrhea is typical of the third manifestation and is often a sequela to an acute episode Ward et al (2005)

A mild form of salmonellosis is characterized by fever, anorexia, and depression without diarrhea or with only mild changes in fecal consistency Various presentations of colic are also attributed to Salmonella infections, due to acute colitis with severe diarrhea Salmonellae may cause septicemia in addition to diarrhea in foals that may also localized in joints or other organs, particularly in young foals (extra intestinal disease as a consequence of bacteremia as uveitis, infectious synovitis, osteomyelitis, pneumonia, and meningitis).

The pyemic form of Equine Salmonellosis has been reported furthermore, has described a syndrome characterized by fever, anorexia and depression, without diarrhea, in both young and adult horses.

Diagnosis of Diarrhea

Edwards and Ewing (1972)

Serotyped the isolates of salmonella spp using standard tube agglutination test with factor-specific and group-specific Salmonella ‘O’ and ‘H’ antisera.

Begg et al (1988)

Concluded that survey of 2 horse populations was done to detect the number of asymptomatic faecal excretors of Salmonella sp. 1201 faecal samples from 250 horses hospitalised at the University of Sydney were cultured. Three serotypes, S. typhimurium (4 horses), S. anatum (2) and S. tennessee (1) were isolated from 7 horses (2.8%).

None was detected in 75 mares similarly examined at a thoroughbred stud farm. In retrospect, S. typhimurium was also the most common (70%) of the 19 serotypes recovered from 171 horses with clinical salmonellosis seen at Camden, 1969 to 1986.

Forty cases occurring since 1983 were reviewed in detail; the mortality rate was high (60%) and an increased proportion was due to S. bovis-morbificans.

Five horses developed salmonellosis while hospitalized and it was usually impossible to be certain whether these cases developed from the carrier state into overt disease or resulted from infections acquired in horse.

Genomic DNA of Salmonella was Extracted Using Phenol

Chloroform and Puri®ed Salmonella DNAwas digested with 20 U of PstI and the DNA fragments were separated by electrophoresis through 0.8% (w/v) agarose gels at 0.7 V/cm for 22 h. The DNA was transferred to Hybond. Nylon membranes (Amersham Pharmacia Biotech) using capillary tran under alkaline conditions (Sambrook et al., 1989).

Amavisit et al (2001)

Used a rapid method for PCR typing of Salmonella strains was developed that ampli®ed the intervening DNA sequences between IS200 elements located on the genome. Pair of primers complementary to IS200 forward and IS200 reverse primers (IS200 forward complement, 50-AGTTCCGTCGGGTGTGCGCT-30, and IS200 reverse complement, (50-TAAAGCACCAG CTTG AAGAG-30) was constructed. The PCR conditions were optimized to 40 cycles of 958C for 10 s, 628Cfor 30 s and 728C for 1 min to predominantly amplify the regions between closely spaced IS200 elements.

The resultant PCR fragments were separated by electrophoresis through2% (w/v) agarose gels and seven isolates had patterns B, C, D, E or F, and there was one isolate each with patterns A and A1.

Ward et al (2004)

Cultured faecal samples for Salmonella spp. were streaked onto brilliant green and xylose-lysine-tergitol plates, and approximately 10 g of fecal material was put into 100 mL of tetrathionate Hajna broth. Brilliant green plates were incubated at 35˚ to 37°C for 18 to 24 hours, and xylose-lysine-tergitol plates were incubated for 24 to 48 hours. Tetrathionate broth was incubated at 35˚ to 37°C for 24 to 48 hours and then streaked to brilliant green and xylose-lysine-tergitol plates.

Gall et al (2006)

Demonstrated that an indirect enzyme- linked immunosorbant assay (IELISA) was developed for the detection of equine serum antibodies to lipopolysaccharide of salmonella enterica sub sppenterica serovarabortus equi acaustive agent of equine paratyphoid.

Van Duijkeren et al (2007)

Studied the presence of Salmonella genomic island 1 (SGI1) or its variants was by PCR and nucleotide sequencing and PFGE was used to genotype the isolates.

Niwa et al (2009)

Applied several genetic methods, namely polymerase chain reaction (PCR) for detecting class 1 integrons, multiplex PCR for detecting multidrug resistant S. Typhimurium definitive phage type 104 (MR-DT104), and fluorescent amplified-fragment length polymorphism (FAFLP).

Antibiotic Sensitivity Test

Wray et al (1981)

Reported resistance of equine salmonellae was only for streptomycin and the sulfonamides. Expanding resistance among Salmonella to antimicrobial agents has been reported by several authors (Mathewson et al., 1981, Gupta et al., 1983, Benson et al., 1985).Since 1977, however, the prevalence of resistance to other drugs e.g. ampicillin, tetracyclines and chloramphenicol has increased (Prescott et al., 1984).From 1973 to1979, Multiple resistances are most commonly associated with certain *Salmonella typhimurium* strains such as phage types 204 and 193 (Wray, 1985).However, multiple resistance of other *Salmonella* serotypes like agona (Donahoe, 1986),*saint paul* (Ikeda and Hirsh, 1985; Powell et al., 1988).

Prescott and Baggot (1993)

Reported that susceptibility testing, combined with knowledge of the pharmacokinetic and toxicological data of the drug is essential in choosing an effective drug for antimicrobial therapy. A rational antibacterial therapy should produce and maintain effective concentrations of the chosen drug at the site of infection for a sufficient time to kill or inhibit the organism without toxic effects on the host.

Engeline et al (1995)

Found that all salmonellae tested were susceptible to the cephalosporin CFI and susceptibility to the quinolones FLU. S.Heidelberg strain was resistant to ampicillin, streptomycin, tetracycline, chlor amphenicol, sulphonamides and spectinomycin, but the resistance genes of the former are not found on plasmids (Ridley and Threlfall, 1998; Sandvang et al., 1998; Bolton et al., 1999)

Amavisit et al (2001)

studied the concentrations of the antimicrobial suesptability drugs for salonella spp were ampicillin at 32 mg/ml,streptomycin at 5 and 25 mg/ml, tetracycline at 20 mg/ml, chloramphenicol at 10 mg/ml, sulphathiazole at 550 mg/ml, trimethoprim at 50 mg/ml, kanamycin at 10 mg/ml, nalidixic acid at 50 mg/ml, spectinomycin

at 50 mg/ml, gentamicin at 2.5 mg/ml and cipro¯oxacin at 0.06 mg/ml. The bacteria were added to the agar at an inoculum density of 105 colony forming units (cfu) per inoculum spot and plates were incubated overnight at 37C

TREATMENTS, CONTROL AND PREVENTION

Treatment is usually symptomatic involving electrolytes, fluid therapy, with occasionally antibiotics, spasmolytics and anti-ulcer preparations. In recent years, prophylactic treatment has been extended to the use of oral plasma containing antibodies. (Dugdale 1992; Dugdale 1995)

Veterinarians should be aware of the limitations of antimicrobial therapy in horses with salmonellosis and should weigh the benefits expected from antibiotic drugs against the risk of their side-effects.

Oral treatment with furazolidone at a dosage of 10 mg/kg given twice daily has been used for the treatment of salmonellosis. Regarding the susceptibility data of this study, this seems to be a reasonable alternative. However, resistance to nitrofurans is almost exclusively chromosomal and develops in a gradual matter and even slight increases.

In addition; furazolidone shows a very strong first pass effect resulting in low plasma concentrations and therefore cannot be advised in horses suspected of bacteraemia and horses that require systemic treatment for other reasons. In addition, the use of furazolidone is limited by its toxicity (Engeline et al 1995).

The first 18 hours of the foal's life are critical when it comes to the absorption of colostrums. It during that window of opportunity that the foal's gastrointestinal system can absorb the antibodies found in the colostrums. The mare produces antibodies against bacteria and viruses by vaccination or exposure to these organisms in her environment.

The colostrums protection is essentially the only protection a foal has against harmful germs. Foals are born with an immature immune system that has to develop for about 30 days in order for it to produce antibodies on its own (Roberta Dwyer, 1999).

Treatment for foals less than 30 days of age with serious diarrhea often consists of intravenous fluids. This not only replenishes the lost fluids but can help correct electrolyte imbalances such as low potassium, sodium, and chloride, Glucose is also provided in many IV fluid solutions. If serious electrolyte imbalances are not corrected, other organs can be adversely affected, such as the heart. Also, a protectant.such as equine version of Pepto Bismol, can be used to coat and soothe the gastrointestinal tract (Mackay, 2001)

Good farm management is key to preventing diarrhea. These practices include the following: (Michael et al, 2005).

- The higher the concentration of animals on land, the higher their risk of exposure to organisms that cause diarrhea. Do not exceed more than two horses per acre.
- Make sure the foal gets good-quality maternal colostrums in sufficient amounts in the first 18 hours of life.
- Do not move new mares and foals in with the resident population immediately. Either group might be carrying an infectious disease.
- If you are in an area where rotavirus is known to be a problem, you should vaccinate the mare before she gives birth in order to pass on the antibodies to the foal through the mare's colostrums. The vaccination, which can be obtained from your veterinarian, should be administered to the mare at eight, nine, and 10 months of gestation.
- Chain harrow pastures where horses are kept to break up manure piles and expose parasites and other organisms to environmental conditions.
- Make sure the foal is born into a clean environment. Disinfect the stall in which the foal is born to protect the foal from bacteria and viruses using a phenolic disinfectant.
- If you are moving a pregnant mare to a different barn, make sure to transport her four to six weeks before she foals. This time will allow her body to build up antibodies to the local pathogens in her new environment, which will then be passed on to the foal in the colostrums.

Asai et al (2006)

Reported that most of the recent salmonella isolates in Japanese livestock of equine are suucesbitable to fluroquinolone and gentamicin. Owners should not reach for antibiotics when they discover a foal with diarrhea. Indiscriminate use of antibiotics can complicate some diarrhea cases by killing off "good" bacteria found in the foal's gut. Antibiotic decisions should be left to the veterinarian (Kirsten, 2007).

Niwa et al (2009)

Reported that Fluoroquinolones may become a powerful candidate for use in treating severe salmonellosis in horses

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