A Contribution Towards Diagnosis and Virulence of Streptococcus Suis in Swine

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Summary

A survey to detect Streptococcus suis (S. suis) serotype 2 in 75 weaned pigs were examined at the time of slaughter from El- Basatin Abattoir. 150 swab specimens of the nasal cavity and tonsils of pigs were obtained for bacteriologic culture, Streptococcus suis serotype 2 was detected by using brain heart infusion agar containing a Streptococcus selective supplement and 5% goat antiserum raised against serotype 2. The precipitation zone diameter (PZD) on a quantitative basis led to the proposal of a simple and reliable technique to screen swine herds for S. suis serotype 2 in weaned pigs. The organism was found in both sites in nasal and tonsils specimens with a percentage of 41.33 and 60%, respectively, of all S. suis serotype 2 isolated. In order to study host – specific of virulence of Streptococcus suis types for mice and pigs, it was found that when a group of mice was inculated intraperitoneally with 10^7 CFU/ml of 13 different S. suis serotype 2 appeared that the S. suis strains that were highly virulent for pigs caused high morbidity (100%) and an intermediate mortality (20-60%) in mice, the Streptococcus suis strains that were weakly virulent for pigs caused high morbidity (40%) but low mortality (0%) and the strains that were non- virulent for pigs, induced highest morbidity and mortality (100%) in mice. Streptococcus suis serotype 2 was more commonly isolated from pigs older than 4 week. Most isolates were susceptible to Amoxicillin + Clavulanate, Ampicillin, Ceftiofur, Enrofoxacin, Penicillin, Spectinomycin, Spiramycin, Tiamulin and Trimethoprin + Sulphadiazine. A high frequency (30%) of resistance to tetracycline was observed. Among isolates of serotype 2, 9.7% were resistant to Lincomycin and 12.9 % to Spiramycin.

1-Introduction

Streptococcus suis type 2 is an important swine pathogen. It is associated with meningitis, arthritis, endocarditis, septicemia, pneumonia and sudden death[1, 5]. Most infections occur in piglets at the age of 3-12

weeks, especially after weaning [6]. Streptococcus suis type 2 colonize the tonsil of both clinically affected and healthy pigs [7]. Subclinical carrier animals are the important source from which the bacteria are transmitted to susceptible young pigs [8]. Streptococcus suis type 2 strains differ in virulence for pigs [2, 3, 8, 9, 13, 14, 15]. Study of such strains can be an aid to identify virulence factors and immunogenic antigens [2, 3, 9, 13, 14 15].

At present several potential virulence factors have been described [17]. But, despite the availability of pig and murine models. It has not been demonstrated if and how these bacterial factors function in the pathogenesis of Streptococcus suis infections in pigs. Various experiment in pig [2, 9, 13, 16, 18] and murine models have been used to study S. suis infections. In these models different parameters with respect to microbiological status (e.g. germ- free, specified- pathogen free), route of inoculation, age, breed and inoculums size were used [16, 19, 20, 21, 22].

Antibiotics, especially penicillin are commonly used to cure or control infection caused by S. suis.

The increased mortality rate among young pigs due to pneumonias and septiceamia at Al Basatin abattoir, so this studies was carried out to

a- Diagnosis of the causative agent with special reference S. suis.

b- Study the virulence of the isolated Strain for mice

c- Study the antibiotic sensitivity test for the isolated strain.

2- Material and Methods

2.1. Specimens:

150 specimens from El- Basatin slaughter house for bacteriologic culture were obtained from the nasal cavity and soft palatine tonsils of pigs using swabbing technique. The sterile swabs were not humidified before

use, but after specimen acquisition, nasal and tonsil swabs were placed immediately in sterile test tubes containing a few drops of 0.6 % glucose. Specimens were kept refrigerated until laboratory procedures were performed.

2.2. Isolation and identification:

Brain – heart infusion (BHI) agar containing Streptococcus selective supplement and 5% goat anti- S. suis serotype -2 serum was used. Nasal and tonsil swabs from each pig were directly seeded on each half of the same selective medium plate. The plates were incubated aerobically for 24 hours at 37°c and then for 72 hours at 4°c plates were examined, using indirect illumination, for the presence of well – defined precipitation zone around some colonies. Representative colonies were chosen according to their morphologic features and precipitation zone. The precipitation zone diameter (PZD) was measured in millimeters, using a transparent ruler. The suspected colonies were subcultured on bovine blood agar, A slide agglutination test was performed, using rabbit antisera against S. suis serotypes 1and 2; this procedure distinguished serotype 1 from serotype 2. Phosphate- buffered saline solution (PBSS) was used as a control rule out - agglutination.

2.3. Virulence of Streptococcus suis type 2 for mice and pigs:

2.3.1. Bacterial strains and inocula:

Thirteen, S. suis type 2 strains were selected based on the degree of virulence for pigs. 4 strains were highly virulent for pigs (HV- group), 4 strains were weakly virulent for pig (WV-group) and 5 strains were non – virulent for pigs (NV- group). All the selected strains were biochemically and serologically typed as S. suis type 2. The phenotype of S. suis type 2 strains was determined by the presence of muramidase – released protein (MRP), extracellular factor protein (EF). All strains of the HV group had

phenotype MRP+ EF+, all weakly virulent strains belonged to phenotype MRP+ EF* and all non – virulent were phenotypically MRP- EF-

The procedure for inoculums preparation was done as described for the pig model of S. suis infection, inoculums size was determined using a spiral plater (Spiral systems model CU, lameris) and contained 1.1 (±0.3) x 10^7 CFU/ml, equivalent to the minimal lethal dose MLD for mice.[22].

2.3.2. Experimental design of the murine model of infection:

25 female, 28 days- old specified – pathogen free (SPF) mice were randomly divided to 5 groups, each group consisting of 5 mice, mice of four groups were inoculated intraperitoneally with one ml inoculums (10^7CFU/ml) containing one of the 13 selected S. suis type 2 strains, one group of mice (negative control) was injected with one ml of sterile Tris – Hcl buffer (pH 8.0). All groups were housed in separate cages. Mice were observed twice daily for clinical signs of disease, such as depression, lameness and nervous signs.

Pathological and bacteriological examinations were done immediately on severely diseased or dead mice. Bacteriological examination was performed by plating tissue specimens of the liver, brain and the thoracal and abdominal serosae, on Columbia blood agar base with 6% horse blood (CM331, Oxoid). Plates were incubated overnight at 37°c, identification of colonies as S. suis type 2 [25]. Streptococci recovered from the tissues were examined by double – antibody sandwich (DAS) enzyme-linked immunosorbent assays (ELISA) [4] to check whether they had the same phenotypes as the original inocula.

2.4. Antimicrobial susceptibility testing:

Susceptibility to antimicrobial agents was determined by a disc diffusion test on Mueller – Hinton II agar supplemented with 5% bovine blood, using the following antimicrobial agents (diffusible amount),

Ampicillin + Clavulanate (30+15 µg), Enrofloxacin (10 µg), Lincomycin (19 µg), Penicillin (5 µg), Spectinomycin (200 µg), Spiramycin (200 µg), Tetracyclines (80 µg), Tiamulin (30 µg) Trimethoprin + Sulphadiazine (52+240 µg) according to [24].

The degree of sensitivity was determined by measuring the visible and clear zone of growth inhibition produced, [28]

3-Results

Table 1: The percentage of isolation of Streptococcus suis serotype 2 from nasal and tonsils specimen sites in 4-8 week old weaned pigs:

Site	No. of examined samples	No. of positive samples		No. of negative samples	
		No.	%	No.	%
Nasal	75	31	41.33	44	58.7
Tonsils	75	45	60	30	40
Total	150	76	50.7	74	49.3

% calculated according to the number of examined samples (75 nasal and tonsils sample = 150 total samples)

Table 2: Differentiation between slide agglutination zone and PZD to positive isolates from S. suis serotype 2 and others serotype.

Isolates	Slide agglutination		PZD	
	No.	%	No.	%
S. suis serotype 2	40	52.67	28	36.84
Others serotype	36	47.33	48	63.16
Total	76	100	76	100

% calculated according to the number of serotypes (40 S. suis serotype 2 and 36 others serotype to Slide agglutination and 28 suis serotype 2 and 48 others serotype to PZD.

Table 3: Determination of the virulence of 13 S. suis type 2 strains by morbidity and mortality in mice

Groups of mice	Virulence strains to pig	Morbidity in mice		Mortality in mice	
		No	%	No	%
1st group	H.V	5/5	100	3/5	60
2nd group	H.V	5/5	100	1/5	20
3rd group	W.V	2/5	40	0/5	0
4th group	N.V	5/5	100	5/5	100
Control group	-	0/5	-	0/5	0

Abbreviation: N (mice): 25 H.V.: highly – virulent for pigs W.V.: weakly-virulent for pigs N.V.: non virulent for pigs Morbidity: is the number of mice per group with clinical signs

Mortality: is the number of mice per group that died.

Table 4: Sensitively of S. suis to different antimicrobial agents

Antimicrobial	Susceptible		Intermediate		Resistant	
	Reading	%	Reading	%	Reading	%
Amoxycillin+ clavulanate	+++	100	-	-	-	-
Ampicillin	+++	100	-	-	-	-
Ceftiofur	+++	100	-	-	-	-
Enrofloxacin	+++	95.2	+	3.2	+	1.6
Lincomycin	+++	90.3	-	-	+	9.7
Penicillin	+++	98.4	+	1.6	-	-
Spectinomycin	+++	98.4	+	1.6	-	-
Spiramycin	++	82.3	+	4.8	+	12.9
Tetracycline	++	53.2	+	6.5	++	40.3
Tiamulin	+++	98.4	-	-	+	1.6
Trimethoprim+ sulfadiazine	+++	96.8	-	-	+	3.2

% calculated according to the reading of zone inhibition surrounding the antimicrobial disc

All isolates were susceptible to Amoxycillin + Clavulanate, Ampicillin and Ceftiofur whereas a limited number of isolates were resistant to Enrofloxacin, Penicillin, Spectinomycin, Tiamulin and Trimethoprim + Sulphadiazine. A high frequency of resistance to tetracycline was observed. A lower frequency of resistance to spiramycin and lincomycin.

4-Discussion

In this study S. suis serotype 2 was the most common serotype associated with Pneumonia and Septicaemia 50.7% (76 out of 150) nasal and tonsils examined samples (Table 1) this results agree with some other studies as [26, 27] which found that most infection with S. suis (types 1-8) were associated with pneumonia (55%), Septicaemia (32%) and only a limited number of isolates were recovered from meningitis (2%) or endocarditis (2%) according to [26]. Also S. suis was the most commonly associated with pneumonia (57%) followed by Septicaemia (16%). However, cases of meningitis (16%) and endocarditis (8%) have become more common than previously results according to [27].

Swabbing technique was considered to be the best technique to use with slaughtered pigs, in this study for determination of Streptococcus suis serotype 2. An important technical point was to rub the tonsil thoroughly with swab when obtaining specimens to collect a maximal number of bacteria; S. suis serotype 2 seems be more confirmed in the crypt lumen [7, 8].

Table 1: revealed that tonsils were reported to be the main carrier site of S. suis serotype 2 where as 45 (60%) of the examined samples positive to S. suis type 2 and the nasal cavity was found 31(41.3%) of the examined samples positive to S. suis type 2, this result agree with [29] .they found that nasal and tonsillar specimens yielded 55.3% and 65% respectively of all S. suis serotype 2 isolates.

Table 2: It was found that 40 (52.6%), 28 (36.8%) of S. suis type 2 were detected by slide agglutination test and precipitation zone diameter (PZD), respectively. This result agree with [29] where they found that 133(24.7%) of S.suis serotype 2 were detected by slide agglutination test and PZD respectively.

In conclusion, the measurement of PZD appears to be a reliable technique to identify S. suis serotype 2 in comparison with the slide agglutination test.

Table 3: It was found that S. suis strains which were highly virulent for pigs caused high morbidity (100%) and an intermediate mortality from 20% to 60 % in mice, while the strains which were weakly virulent for pigs caused high morbidity 40% but low mortality 0% and the strains that were non – virulent for pigs induced highest morbidity and mortality 100% in mice. Some observation in these studies such as similar lesions in pigs and mice indicated that mice may be suitable as experimental animals for studying S. suis in infections in pigs [21]. The obtained results in table 3 revealed that the non- virulent strains for pigs were highly virulent for mice and the highly virulent strains for pigs tested appeared completed non- virulent for mice, mice inoculated with weak virulent strains showed frequently signs of disease or died. This appear host- specific of virulence of S. suis type 2 for mice and pigs

In general, virulence of isolated strains were determined by the mortality in mice, mortality in this study were sometimes low, while morbidity was high. This agreed with [21]

Table 4: It was found that most isolates were susceptible to amoxicillin + clavulanate , ampicillin , ceftiofur , enrofloxcin , spectinomycin, tiamulin and Trimethoprim+ sulphadiazine, where as a relatively high frequency of isolates were resistant to tetracycline, lincomycin and spiramycin.

These results are in general agreement with some other studies as [10]. However, most other studies have reported higher levels of resistance [11, 12].

5-References

1- Vecht, U., Van Leengoed, L.A,M.G., Verheijen, E. R. M., (1985). Streptococcus suis infections in pigs in the Netherlands (Part one). Vet. Quart. 7, 315-321.

2- Vecht, U., Arends, J. P., Van der Molen, E. J., Van Leengoed, L. A. M. G., (1989). Difference in virulence between two strains of Streptococcus suis type 2 after experimentally induced infection of newborn germ- free pigs Am. J. Vet. Res. 50, 1037-1043.

3- Vecht, U., Wisselink, H. J., Van Dijk, J. E., Smith, H. E., (1992). Virulent of Streptococcus suis type 2 strains in newborn germ- free pigs depends on phenotype. Infect. Immun. 60, 550-556.

4- Vecht, U., Wisselink, H. J., Anakotta, J., Smith, H. E., (1993). Discrimination between virulent and non- virulent Streptococcus suis type 2 strains by enzyme- linked immunosorbent assay. Vet. Microbiol. 34, 71-82.

5- Reams, R.Y., Glickman, L. T., Harrington, D. D., Thacker, H. L., Bowersock, T. L., (1994). Streptococcus suis infection in swine a retrospective study of 256 cases. Part II. Clinical signs, gross and microscopic lesions and coexisting microorganisms. J. vet. Diagn. Invest. 6, 326-334.

6- Lamont, M. H., Edwards, P. T., Windsor, R.S., (1980). Streptococcal meningitis in pigs: results of a five- year survey. Vet. Rec. 107, 467-469.

7-Arends, J. P., Hartwig, N., Rudolphy, M., Zanen, H. C., (1984). Carrier rate of Streptococcus suis capsular type 2 in palatine tonsils of slaughtered pigs. J. clin. Microbial. 20, 945-947.

8- Clifton- Handley, F. A., Alexander, T. J. L., Upton, I., Duffus, W. P. H., (1984). Further studies on the subclinical carrier state of Streptococcus suis type 2 in pigs. Vet. Rec. 114, 513-518.

9- Clifton- Handley, F. A., Alexander, T. J. L., Enright, M. R., Lindsay, H. J., (1986). Monitoring herds Streptococcus suis type 2: cross reactions and variations in virulence. In: Proc. Int. Pig Vet. Soc., Barcelona, P. 359.

10- Estoepangestie, S., Lammler, C. H., (1993). Distribution of capsular types 1 to 28 and further characteristics of Streptococcus suis isolates from various European countries. Zbl. For Bakt 279, 394- 403.

11- Hariharan, H., Bryenton, J., St. onge, J., Mcnair, N., Long, J. R., (1989). Antimicrobial drug susceptibility of Streptococcus suis type 2. Ir. Vet. J. 42, 113-114.

12- Cantin, M., Harel, J., Higgins, R., Gottschalk, M., (1992). Antimicrobial resistance patterns and plasmid profiles of Streptococcus suis isolates. J. Vet. Diag. Invest. 4, 170-174.

13- Robertson, I. D., Blackmore, D. K., (1990). Experimental studies on the comparative infectivity and pathogenicity of Streptococcus suis type 2. Porcine and human isolates in pigs. Epidemiol. Infect. 105, 469-478.

14- Jacobs, A. A. C., Loeffen, P. L., Van den Berg, A. J., Storm, P. K., (1994). Identification, purification and characterization of a thiol- activated hemolysin (suilysin) of Streptococcus suis. Infect. Immun. 62, 1742-1748.

15- Quessy, S., Dubreuil, J. D., Caya, M., Letourneau, R., Higgins, R., (1994). Comparative of pig, rabbit and mouse IgG response to Streptococcus suis serotype 2 proteins and active immunization of mice against the infection. Can. J. Vet Res. 58, 220-223.

16- Quessy, S., Dubreuil, J. D., Caya, M., Higgins, R., (1995). Discrimination of virulent and avirulent Streptococcus suis capsular type 2 isolates from different geographical origins infect. Immun. 63, 1975-1979.

17- Chanter, N., Jones, P. M., Alexander, T. Y. L., (1993). Meningitis in pigs caused by streptococcus suis- a speculative review. Vet. Microbiol. 36, 39-55.

18- Galina, L., Pijoan, C., Sitjar, M., Christianson, T., Rossow, K., Collins, J. E., (1994). Interaction between Streptococcus suis serotype 2 and porcine reproductive and respiratory syndrome virus in specific pathogen- free piglets. Vet. Res. 134, 360-364.

19- Williams, A. E., Blackmore, W. F., Alexander, T. J. L., (1988). A murine model of Streptococcus suis type 2 meningitis in the pig. Res. Vet. Sci. 45, 394-399.

20- Kebede, M., Chengappa, M. M., Stuart, J. G., (1990). Isolation and characterization of temperature – sensitive mutants of Streptococcus suis: Efficacy trial of the mutant vaccine in mice. Vet. Microbial. 22, 249-257.

21- Kataoka, Y., Haritani, M., Mori, M., Kishima, M., Sugimoto, C., Nkazawa, M., Yamamoto, K., (1991). Experimental infections of mice and pigs with S.suis type 2. J. Vet. Med. Sci. 53, 1043-1049.

22- Beaudoin, M., Higgins, R., Harel, J., Gottschalk, M., (1990). Studies on a murine model for evaluation of virulence of Streptococcus suis capsular type 2 isolates. FEMS Microbiol. Lett. 99, 111-116.

23- Foster, N., Staats, J. J., Chengappa, M. M., (1994). Isolation, characterization and protection studies in mice of a streptomycin- dependent mutant of Streptococcus suis type 1/2. Vet. Res. Commun. 18, 155-163.

24- Casals, J. B., Pringler, N., (1991). Antibacterial sensitivity lesting using Neo- Sensitabs. Taastrup. Denmark, Rosco Diagnostica.

25- Divriese, L. A., Ceyssens, K., Hommez, J., Killiper- Bàlz, R., Schleifer, K. H., (1991). Characteristics of different Streptococcus suis ecovars and a description of a simplified identification method. Vet. Microbiol. 26, 141-150.

26- Perch, B., Pedersen, K. B., henrichsen, J., (1983). Serology of capsulated streptococci pathogenic for pigs: six new serotypes of Streptococcus suis. J. Clin. Microbiol. 17, 993-996.

27- Aarestrup, F. M., Jorsal, S. E., Jensen, N. E., (1998). Serological characterization and antimicrobial susceptibility of Streptococcus suis isolates from diagnostic samples in Denmark during 1995 and 1996. Vet. Microbiology, 60, 59-66.

28- Quinn, P. J., Carter, M. E., Markey, B. K., Carter, G. R., (1994). "Clinical Veterinary Microbiology". Wolfe, Publishing Livestock, London.

29- Alain Moreau, DVM., Robert Higgins, DVM, phD., Michel Bigras- Poulin, DVM phD., Maria Nadeau, DVM, MSc (1989) Rapid detection of Streptococcus suis serotype 2 in weaned pigs. Am. J. Vet. Res., Vol. 50, No. 10, 1667-1671.

إسهام نحو تشخيص وضراوة الميكروب السبحي في الخنازير

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تم عمل مسح شامل لتواجد الميكروب السبحي (الخاص بالخنازير) تصنيف نوعي رقم 2 في عدد 75 عينة من فطام الخنازير والتي تم فحصها وقت الذبح في مجزر البساتين بالقاهرة ، تم اخذ 150 مسحة من تجويف الانف واللوز في الخنازير وذلك للفحص البكتريولوجي المعملي .

الميكروب السبحي تصنيف نوعي رقم 2 تم عزله وتصنيفه بإستخدام آجار من مستخلص المخ والقلب والذي يحتوي على مدعم منتقى خاص بالميكروب السبحي وايضا يحتوي على 5% ضد سيرم الماعز ليكون رافع ضد التصنيف النوعي رقم 2 للميكروب السبحي . وقد وجد ان قطر حيز الترسيب (PZD) على الاساس النوعي الذي يؤدي الى طريقة بسيطة ومتاحة لعمل فحص شامل لقطعان الخنازير لوجود الميكروب السبحي تصنيف نوعي رقم 2 في فطام الخنازير .

والميكروب السبحي وجد في عينات كل من التجويف الانفي واللوز بنسبة مئوية 41.33%، 60% على الترتيب في كل معزولات الميكروب السبحي تصنيف نوعي رقم 2 .ولدراسة ضراوة الميكروب السبحي التخصصية لكل عائل من فئران التجارب والخنازير وجد ان : عند حقن مجموعة من فئران التجارب في التجويف البريتوني بعترة الميكروب السبحي تركيز 10^{7} CFU / لكل 1مم من 13 عترة (تم عمل بولنج لها) من الميكروب السبحي تصنيف نوعي رقم 2 بعدها تبين ان الميكروب السبحي له ضراوة عالية في الخنازير وسبب ظهور اعراض مرضية بها بنسبة 100% وايضا كان بنسبة متوسطة لظهور اعراض مرضية في فئران التجارب بنسبة 20-60%. والميكروب السبحي في الخنازير والذي كان ضعيف الضراوة للخنازير سبب اعراض مرضية عالية 40% ونسبة صفر % نفوق في فئران التجارب ، والعترة التي كانت غير ضارة للخنازير اصبحت اكثر ضراوة من حيث احداث المرض والنفوق بنسبة 100% في فئران التجارب. الميكروب السبحي تصنيف نوعي رقم 2 تم عزله من الخنازير التي تجاوز عمرها 4 اسابيع. وكان معظم المعزولات من الميكروب السبحي حساسة للمضادات الحيوية الاتية : اموكساسلين ، كلافيولانت ، امبسلين، سيفتوفور، افروفوكساسين، بنسلين، سبكتينوميسين، سبراميسين، تيميولان بالاضافة الى تراميثوبريم و السلفاديازين. ايضا وجد ان 30% من العترات المعزولة من الميكروب السبحي مقاومة للمضاد الحيوي تتراسيكلين . وايضا من بين المعزولات وجد ان 9.7 % من الميكروب السبحي مقاومة للمضاد الحيوي لينكومايسين، 12.9% للمضاد الحيوي سبراميسين.