Chapter 1

General Introduction

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Group B Streptococcus (GBS, Streptococcus agalactiae) has been recognized as an important cause of neonatal infection and early neonatal mortality within the first seven days of life.(1-3)

Vertical transmission of GBS from mother to child occurs during labor. The gastrointestinal tract of the mother has been recognized as the source of vaginal GBS colonization. The frequency of GBS colonization ranges from 10% to 35% in women of reproductive age.(4;5) Studies on vertical GBS transmission in colonized mothers during labor report incidences of colonization of the infant between 16 and 69%.(6-11) Early-onset group B streptococcal disease (GBS-EOD) occurs in approximately 1% of newborns who are colonized with GBS and typically presents with sepsis, pneumonia or meningitis.(12) Risk factors for acquiring GBS-EOD are prelabor rupture of membranes, preterm labor, intrapartum fever, GBS bacteriuria during pregnancy or a previous child with GBS.(13)

Clinical trials in the USA during the 1980s showed that GBS-EOD may be prevented by administering antibiotics during labor to mothers who are colonized with GBS.(14) After introduction of national guidelines in 2002, in which culture based screening of all pregnant women and intrapartum antibiotic prophylaxis (IAP) in all GBS carriers was recommended, the incidence of GBS-EOD in the USA has declined from 1.8 per 1000 live births to 0.32 cases per 1000 live births in 2003.(5;15) Overall mortality from GBS-EOD, as high as 50% in 1970s, fell to 5% in 2003.(1;16-18)

In Europe little data have been published on national incidence rates of GBS-EOD. The incidence of neonatal early-onset GBS disease in some European countries varies from 0.5 to 1.15 per 1000 live born infants.(19-21) In the United Kingdom, studies reported incidence rates of GBS-EOD of 0.5 per 1000 live births in the absence of screening,(19;22) with a case-fatality rate of 10.6%.(22)

In the Netherlands, the incidence of GBS-EOD before implementation of a nationwide guideline during a 2-year period (1997-1998) was 1.9 per 1000 live born infants. This is the total incidence of proven sepsis and probable sepsis. In proven sepsis, streptococci are isolated from blood and/or from cerebrospinal fluid combined with physical signs of infection in the neonate. In probable sepsis GBS is detected in serious ill children at various sites, but not in blood and/or cerebrospinal fluid. The incidence of proven GBS-EOD alone was 0.54 per 1000 live births. After the Dutch Society of Obstetrics and Gynecology and the Dutch Society of Paediatrics approved modified risk factor based guidelines for prevention in 1998, there has been a limited decrease in the incidence of proven GBS-EOD in the Netherlands from 0.54 per 1000 live births to 0.36 per 1000 live births.(22) There was no decrease in the incidence of probable early-onset GBS sepsis, meningitis or case fatality rate. According to the Netherlands Perinatal Registry, incidences of (proven and probable) GBS sepsis and GBS meningitis seemed to be stable until 2008, with respectively 108 and

15 reported cases in 2008. In 2009 an unexplained increase was seen, with 172 cases of GBS-EOD (0.93 per 1000 live births). Between 2000 and 2009 a case fatality rate for GBS-EOD of 6.3% was found.

Since the overall effect of the Dutch guideline on the incidence of GBS-EOD is disappointing, revision of the Dutch guidelines was considered in 2006. Because of the presumed lack of evidence to change towards an alternative strategy, the Dutch prevention strategy remained as it was. However, given the on-going burden of GBS-EOD, adaptation of the Dutch guidelines should be reconsidered, particularly concerning the fact that perinatal mortality in the Netherlands is high compared to other European countries.(23) The aim of this thesis is to contribute to the information needed for the establishment of an optimal prevention strategy for GBS-EOD.

BACKGROUND AND SCOPE

The Pathogen

Group B streptococcus (GBS, Streptococcus agalactiae) has been known as a human pathogen since 1938.(24) GBS are facultative anaerobic Gram-positive cocci growing in chains or as diplococci.(Figure 1) On sheep blood agar they cause a characteristic zone of beta-hemolysis around colonies, because of destruction of red blood cells.(Figure 2).

With serological techniques using capsular polysaccharides as type-specific antigens, GBS can be distinguished from other types Streptococci (A, C, D and G). When using surface proteins as additional antigenic markers, GBS can be divided into nine serotypes (Ia, Ib and II-VIII). Recently a tenth serotype has been proposed.(25)

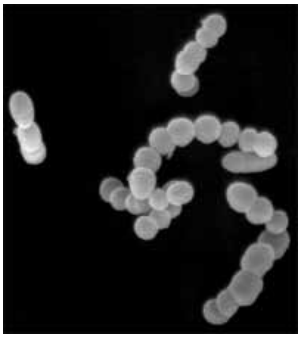

Figure 1 GBS: Gram-positive cocci growing in chains

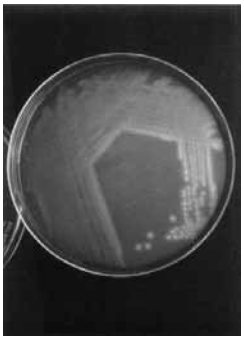

Figure 2 Blood Agar Plate with Streptococcus Agalactiae and typical haemolytic zone around colonies

Differences in the expression of polysaccharides and surface proteins account for differences in the pathogenesis of infections.(26) Factors playing a role in the development of an asymptomatic or invasive infection have not yet fully been elucidated. Capsular polysaccharides expressed by GBS assist in bacterial evasion of host defense by interfering with their ingestion by phagocytes. More virulent strains of GBS can produce increased amounts of polysaccharides. Another important virulence factor of GBS is related to its ability to attach to endothelium and epithelium, particularly of vaginal tissue and chorionic membranes and to the neonatal lungs.(27) The more virulent invasive strains of GBS have been found to have a greater capacity for adherence to endothelium and epithelium, and this has been particularly evident in studies of serotype III GBS.(27;28)

GBS disease is caused mainly by serotypes I, II and III.(27) Serotype III is the most prevalent serotype in asymptomatic carriers.(30-32)

Colonization and transmission

The gastrointestinal tract is the human reservoir of GBS. Women may carry GBS temporary, intermittent or persistent.(33-36) Colonization has not always been studied with the use of optimal microbiologic methods such as specific growth medium in a cohort that was studied over months. Detection methods for GBS that were used in earlier studies might have missed lightly colonized women.(28)

A cross-sectional study among healthy male and non-pregnant female students reported on colonization with group B Streptococcus. With adequate detection methods, GBS was isolated from one or more sites (vaginal, anal and urine specimens) in 34% of women and 31% of men. The prevalence was associated with sexual activity, tampon use, milk consumption, and hand washing done < 4 times per day.(29) Although GBS can be sexually transmitted, colonization has not been associated with frequency of sexual activity or numbers of partners.(30).

Since in the USA Caribbean Hispanics and black women were reported to be GBS carriers more frequently than white women, a role for ethnic or genetic factors is presumed. (33;40)

Colonization with GBS is described to occur in 10-35% of pregnant women.(4;5)

Studies on vertical GBS transmission in colonized mothers during delivery report incidences of colonization of the infant between 16 and 69%.(6-11) The majority of infants who are exposed to GBS are colonized on skin or mucous membranes but remain asymptomatic.

Of the colonized newborn infants, 1-2% develops serious neonatal infection.

Persistent carriage and high-level maternal colonization are important risk factors for vertical transmission.(31)

Intrauterine infection of the fetus results from ascending spread of GBS from the vagina of colonized women into the uterine cavity. This vertical transmission from mother to child usually occurs after rupture of the membranes, when GBS enters the amniotic fluid and colonizes the skin and mucocutaneous areas of the fetus. Aspiration of infected amniotic fluid may cause pneumonia, and when GBS entries the bloodstream, sepsis may occur. Entry in the cerebrospinal fluid after hematogenous spread may cause meningitis. (Figure 3)

The ability of GBS to attach and invade the chorioamniotic membranes has been demonstrated in vitro.(32) GBS may also penetrate the intact chorionic membranes, leading to cases of intra-amniotic infection or abortion.(33)

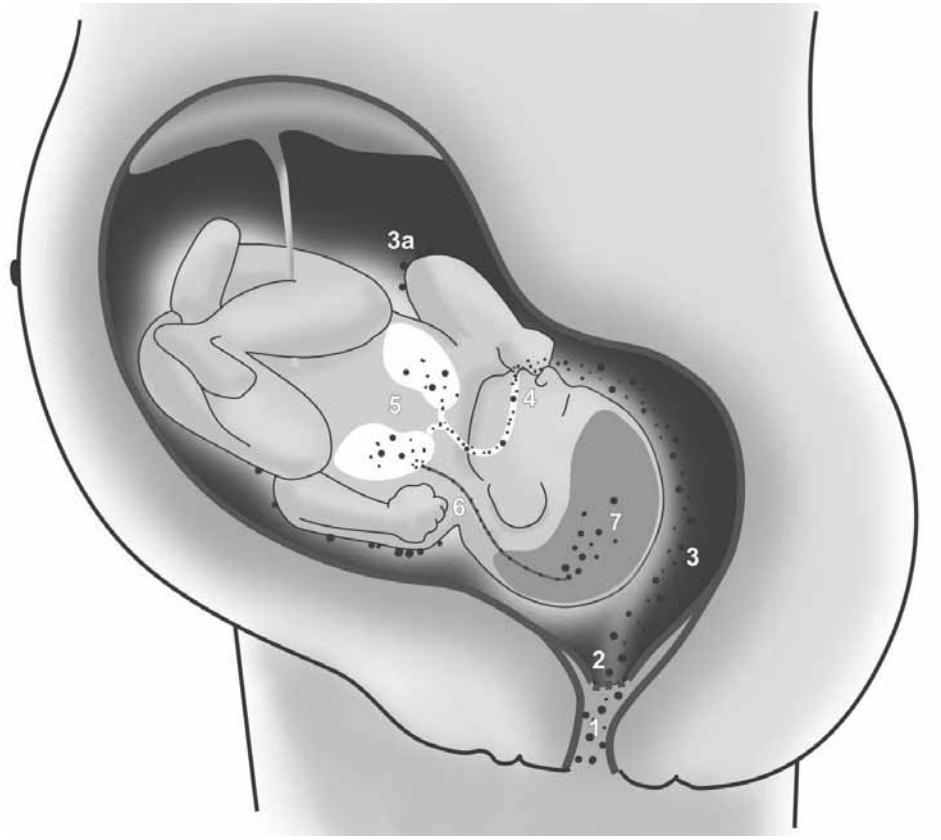

Figure 3: Hypothesized pathogenesis of GBS-EOD (Designed by Vincent Khouw, VMK- designs) 1 Colonization in the rectovaginal compartment; 2 Rupture of the membranes; 3 GBS enters the amniotic fluid; 3a GBS colonization of skin and mucocutaneous areas; 4 Aspiration of infected amniotic fluid; 5 Infected amniotic fluid causes pneumonia (if the bacterial load is high enough); 6 Entry of GBS in the bloodstream (sepsis or bacteraemia); 7 Entry in cerebrospinal fluid after hematogenous spread (meningitis). Derived from thesis A.E. Muller; Population pharmacokinetics of antibiotics to prevent group B streptococcal disease: from mother to neonate; 2009

Intra-amniotic bacterial colonization or progression to infection depends on the number and pathogenicity of the colonizing bacteria and the effectiveness of the amniotic fluid antibacterial mechanisms.(34) In addition, it is conceivable that maternal genetic variation plays a role in the response to occurrence and severity of intra-uterine infections. Romero et al. speculated that it is not the presence of the bacterial organism itself, but the response of the host that is the critical step in this chain of events. When the host defence system is inadequate, bacterial growth may become excessive and lead to an ascending infection into the uterus.(35)

GBS disease in the newborn

Neonatal GBS is diagnosed as *culture-proven* if streptococci are isolated from blood and/or from cerebrospinal fluid combined with physical signs of infection in the neonate. The diagnosis *probable* GBS-EOD is used for cases of serious neonatal disease when GBS is detected at various sites, but not in blood and/or cerebrospinal fluid.(36) A different group is the "asymptomatic" GBS bacteriemia, defined as positive blood cultures for GBS in neonates without clinical signs of infection.(37-39)

Most infections in newborns occur in the first week of life and are designated early-onset GBS disease (GBS-EOD). The majority of cases of GBS-EOD occur within 24 hours after delivery(22) and present as a rapidly progressive septicaemic illness (Figure 4).

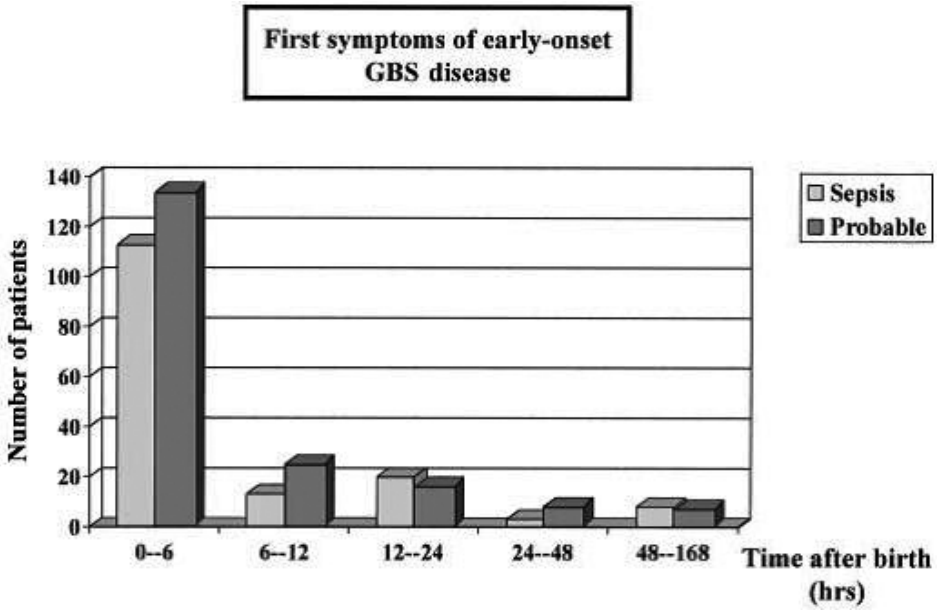

Figure 4: Onset of neonatal early-onset GBS disease(18)

Newborns with GBS-EOD usually present with sepsis or pneumonia, and less often contract meningitis, osteomyelitis, or septic arthritis.(18;27)

In late-onset neonatal GBS infections (GBS-LOD), illness occurs between 8 and 90 days after birth. In contrast to GBS-EOD, late-onset infection is not always acquired from the mother. Horizontal transmission during the perinatal period may occur from mother to infant or from nosocomial or community sources. GBS-LOD is more often associated with localized infections (especially meningitis and pneumonia), which are less rapidly progressive than GBS-EOD. The incidence of meningitis in late onset disease is approximately 30%.(15)

Case reports demonstrate that maternal milk, in cases of either clinical or subclinical mastitis, may be a source of GBS infection resulting in either late onset or recurrent neonatal GBS disease.(40-44) GBS may initially colonize the neonatal oropharynx mucosa during delivery, infecting maternal ducts during breastfeeding. The organism multiplies in the milk ducts and as the microbial concentration increases in the milk, the infant might be reinfected during breastfeeding.(40)

There is significant long-term morbidity after GBS-EOD and GBS-LOD.(45) Survivors may suffer from hearing or visual loss, uncontrolled seizures, impaired psychomotor development and/or mental retardation.(46;47) A prospective study with 5-year follow-up showed that GBS meningitis results in children with neurological deficiencies in 34.8% of cases ranging from partial sensory loss to profound mental retardation, blindness and deafness. (48)

Incidence and causes of neonatal sepsis

The reported incidence of overall neonatal sepsis varies from 7.1(49) to 38(50) per 1000 live births in Asia, from 6.5(51) to 23(52) per 1000 live births in Africa and from 3.5(53) to 8.9 per 1000 live births(54) in South America and the Caribbean. Incidence rates reported in the United States and Australia range from 1.5 to 3.5 per 1000 for early onset sepsis (EOS) and up to 6 per 1000 live births for late onset sepsis (LOS), a total of 6-9 per 1000 for neonatal sepsis.(55-58)

Neonatal surveillance in *developed* countries generally identifies GBS and E. coli as the dominant EOS pathogens and coagulase negative staphylococci (CONS) followed by GBS and Staphylococcus Aureus as the dominant LOS pathogens.(55;57) The pathogens most often implicated in neonatal sepsis in *developing* countries differ from those seen in developed countries. Overall, Gram-negative organisms are more common in developing countries and are mainly represented by Klebsiella, E.coli, Pseudomonas and Salmonella. Of the Gram-positive organisms in developing countries, Staphylococcus Aureus, coagulase

negative staphylococci, Streptococcus pneumoniae and Streptococcus pyogenes are most commonly isolated.(59;60)

The difference in occurrence of invasive pathogens in developed and developing countries might be explained by true differences in pathogens across the world, but might also be explained by an epidemiological bias linked to the fact that most EOS babies in developing countries die at home before reaching health care facilities and they do not appear in the statistics.(59) In a review of studies from developing countries reporting on etiology of community-acquired infections, authors stated that hospital-based studies suggest that most infections in the first week of life are due to Gram-negative pathogens, and many may be environmentally rather than maternally acquired, owing to unhygienic delivery practices. (60)

Risk factors for GBS-EOD

In general and for several reasons neonates are at risk for infections, and this is in particular for preterm neonates.(61;62) In the first place, preterm babies are at risk for infections because of decreased barrier function of skin and mucocutaneus tissue. Necessary invasive treatment of preterm neonates, such as intravenous and umbilical venous infusion, may contribute to infection. Secondly, the immune system in neonates is impaired. Considering the humoral immune response, adequate numbers of B lymphocytes are present, but antibody production is delayed compared with the adult. Decreased levels of complement components contribute to the increased vulnerability to infection of neonates. Infection is a major problem for infants born before 28 weeks. Their serum IgG antibody levels are low and the deficiencies in complement levels and chemotactic responses noted in full-term neonates are even more marked and persist for longer.(63)

For cellular immune response, compared with older children and adults, neonates have an intrinsic limitation in their capacity to produce neutrophils and a subsequent susceptibility to exhaustion of marrow reserves during times of increased use, such as sepsis. (64) In addition, the neutrophils have impairments of numerous functions important to the clearance of microbes, including marrow egress, adhesion to the microvascular endothelium, chemotaxis and antibacterial function.(61;64-67) Deficiencies in circulating levels of GBS-specific antibody in the context of neutrophil dysfunction heighten the neonatal susceptibility to GBS infection.(61)

Both host related factors and bacterial properties may increase the risk on GBS-EOD. A number of factors are known to increase the risk of neonatal early-onset infection in the presence of maternal GBS carriage. (Table 1)

Table 1 Risk Factors for GBS-EOD

Author	Country	Year	Studydesign	Prevalence	No of patients	OR for risk factor with 95% CI								
						Prematurity < 37 weeks	PROM > 18 hrs$	Fever	ROMBOL®	LBW%	IU-FM*	PProm^	Amnionitis	Frequent VE#
Oddie(13)	UK	1998-2000	Case Control	0.57/1000	37-147	10.4 (3.9-27.6)	25.8 (10.2-64.8)	10.0 (2.4-40.8)	11.1 (4.8-25.9)					
Benitz(74)	USA	1999	Review		NM	5.80 (2.15-15.7)	7.28 (4.42-12)	4.05 (2.17-7.56)		7.37 (4.48-12.1)			6.43 (2.32-17.8)	
Schuchat(56)	USA	1995-1996	Case Control	1.4/1000	41-200			4.1 (1.2-13.4)						2.9 (1.1-8.0)
Adair(73)	Canada	1993-1997	Case Control	0.25/1000	90-489	3.89 (2.08-7.27)		4.65 (2.48-8.69)		3.60 (1.68-7.65)	2.24 (1.22-4.13)	2.39 (1.38-4.14)	15.03 (5.58-41.89)	

$Prolonged rupture of membranes
@Rupture of membranes before onset of labour
%Low birth weight
*Intrauterine fetal monitoring
^Premature Prelabour Rupture of Membranes
Frequent Vaginal Exam
NM Not Mentioned

Preterm delivery (before 37 weeks of gestation)

The excess risk of GBS-EOD in preterm and LBW infants has been well recognized for many years. Early reports noted that preterm and low- birth-weight infants were overrepresented among infants with early-onset disease.(68;69) This is confirmed by more recent case-control studies.(70;71)There is a progressive increase in risk for neonatal sepsis in general with decreasing gestational age.(72) Reported odds ratio's for GBS-EOD in prematurity differ from 3.89 (95% CI 2.08-7.27)(73;74) to 10.4 (95% CI 3.9-27.6).(13)

Prolonged rupture of the membranes (more than 18-24 hours)

Prolonged rupture of the amniotic membranes for > 18(72;75) to 20(76) hours before delivery substantially increases the risk of neonatal GBS-EOD.(77)

Odds for GBS-EOD in prolonged rupture of the membranes vary from 7.28 (95% CI 4.42-12) to 25.8 (95% CI 10.2-64.8).(13;74)

Intrapartum fever

Intrapartum temperature > 37.5°C(75) or fever without additional definition of temperature are associated with an increased risk of neonatal GBS infection.(27)There are no objective data to quantitate that higher maternal fever confers a higher risk. Odds of 4.1 (1.2-13.4) to 10.0 (95% CI 2.4-40.8) for GBS-EOD in patients with intrapartum fever were reported, but studies differ in definition of fever (either > 37.5°C or > 38°C).(13;56;73;74)

Chorioamnionitis

Intrapartum fever accompanied by two or more additional signs of chorioamnionitis, including fetal tachycardia, uterine tenderness, foul-smelling vaginal discharge, or maternal leukocytosis is associated with higher neonatal GBS attack rates.(72;78-81) One study provided sufficient data for estimation of OR, which was 6.42 (95% CI 2.32-17.8).(74)

One case control study reported that at least one of the risk factors preterm delivery, intrapartum fever and membrane rupture of at least 18 hours is found in 49% of GBS-EOD cases. (56)

Maternal GBS bacteriuria

The incidence of GBS in quantities $>10^5$ colony forming units (cfu) /ml urine in pregnant women has been reported to be between 0.4 and 7%.(82-84) Infants born to women with GBS bacteriuria during pregnancy are more frequently and more heavily colonized with GBS and may be at increased risk for invasive GBS disease.(85;86)

Two studies investigating the relation between bacteriuria and genital colonization reported a positive predictive value of GBS bacteriuria in the first trimester of pregnancy for positive GBS genital culture at the time of labor of 30.2%(87) and 61%.(88)

In another study urine was sampled at admission for labor. Of 1786 women whose urine was sampled during labor, GBS were isolated from 128 (7%), in 22 of whom (1% of the total) GBS were present in quantities greater than or equal to 10^4 colony forming units (cfu)/ml urine. Neonates born to women with greater than or equal to 10^4 cfu GBS/ml urine were apparently at greater risk for neonatal infection, as they were more commonly and more heavily colonized than were the newborns of women with lower quantities of GBS in urine. (84)

It has been shown that only 60% of bladder punctured pregnant women whose urine specimens contained $>10^5$ cfu/ml urine harboured GBS in the bladder.(84) Therefore, a high quantity of GBS in urine is assumed to reflect heavy colonization of urethra, vulva and vagina instead of cystitis. However, there are no studies with quantative cultures to confirm this assumption.

History of a previous child with invasive neonatal GBS disease.

Although having had a sibling with invasive GBS disease is widely accepted as a risk factor for GBS-EOD, only few reports show neonatal GBS infection followed more than one pregnancy in the same mother.(89-91) In some but not all of the cases, mothers received antibiotics during a subsequent pregnancy and delivery. Therefore it is difficult to determine what the exact risk for GBS-EOD in a subsequent pregnancy is.

Women may remain colonized with the same strain of a virulent subtype of GBS for prolonged periods and may fail to develop protective levels of type specific serum antibodies despite long term colonization.(92;93)

To conclude, there may be an increased risk of GBS-EOD in subsequent pregnancies in women who have had a child with GBS-EOD disease, but this risk has not been quantified.

Other factors

Bacterial virulence properties might influence the risk on GBS-EOD(94), as well as low levels of maternal antibodies.(95) Race or ethnicity,(96;97) maternal age,(96;97) neonate gender, multiple gestation, (98-101) internal monitoring> 12 hours, (72) increased number of vaginal exams, (56) meconium staining,(77) asphyxia(70) and fetal acidosis(102) may be associated with increased risk. These variables may be covariant with GBS colonization, gestational age at delivery, duration of ruptured membranes or other factors. Therefore, their independent contributions are not delineated readily.

However, 30-40% of GBS-EOD occurs in the absence of one of the five major risk factors (prelabor rupture of membranes, preterm labor, intrapartum fever, GBS bacteriuria during pregnancy or a previous child with GBS).(18;103)

GBS isolation and detection

The detection rate of GBS from clinical specimens depends on several factors. Culturing specimens from both the anorectum and the vaginal introitus increases the likelihood of GBS isolation by 5-27% over vaginal culture alone.(75;104;105) Internal examination or visualization of the cervix by speculum examination should not be performed for collection of screening cultures, since studies show much lower isolation rates from cervical swabs than low vaginal swabs.(5;106;107)

After collection of swabs, it is important that Group B streptococci from rectovaginal swabs will survive during shipment from satellite clinics to a central microbiology laboratory.

In 1967, Amies published about a transportation device that would preserve clinical samples from collection sites to the testing lab that could be many hours or even days away.(108) Until today, this "Amies transport medium" is internationally recommended for throat, wound and genital tract samples.(109) Amies broth with charcoal is a non-nutritional, phosphate buffered type medium used to maintain the viability of microorganisms without a significant increase in growth. Charcoal was added to the formulation to neutralize the toxic effects of fatty acids that are toxic to microorganisms.

The CDC guidelines state specifically that the viability of GBS can be maintained for up to 4 days in appropriate transport medium.(109) There are few data, which support this statement. One study showed that there will be a loss of positive culture results if the GBS colony density is low or if the room ambient temperature is relatively high (> 30^0C).(110) GBS was recovered from 92-100% of swabs containing 10 or more colony forming units (cfu) when stored either 3^0C or 24^0C for 4 days. However, GBS recovery decreased significantly when the swabs were stored at 30^0C. After 6 days, sensitivity of 96-100% was observed only for the swabs held at 4^0C and 24^0C and containing the high density of 100 or more cfu.(110)

Another study showed that among initially positive swabs kept at 21^0C in Amies transport medium, GBS were recovered after 24h, after 48 h, after 72 h and after 96 h in 95%, 88%, 85% and 71% of the specimens respectively.(111)

To conclude, viability of GBS is not fully preserved by storage of vaginorectal swabs in Amies transport medium. There are effects of time and temperature and these effects are greater for lower GBS concentrations.

When swabs arrive in a laboratory and culturing starts, the use of selective broth medium (i.e. a broth containing antimicrobial agents to inhibit competing organisms) is essential because it can increase the yield of screening cultures by as much as 50%.(112;113) Examples of selective enrichment broths include Todd-Hewitt broth supplemented either with gentamicin (8 microgram/ml) and nalidixic acid (15 microgram/ml) (called TransVag broth) or with colistin (10 microgram/ml and nalidixic acid (15 microgram/ml) (called Lim broth).(114)

Although TransVag and Lim broth media are often available without blood, the addition of 5% sheep blood can increase the recovery of GBS. Selective enrichment broth can also contain chromogenic substrates that provide for a change in color in the setting of beta-hemolytic GBS; however, nonhemolytic isolates will not be detected by these broths alone. (115-120)

Rapid tests

Nowadays, several non-culture-based tests are available which enable rapid point-of care diagnostics for intrapartum screening, allowing optimal targeting of IAP to women carrying GBS.

As well as being accurate, the ideal test should be rapid enough to allow adequate time for IAP to be effective, and should require minimal preparatory steps and be easily interpretable to enable routine use on busy delivery suites.

Polymerase chain reaction

Polymerase chain reaction (PCR) involves the logarithmic amplification of specific areas of the bacterial chromosome using an iterative process of hybridisation of replication primers, amplification from these primers of the target DNA and separation of the nascent DNA so that the process can be repeated. Real-time detection of the amplified DNA is by incorporation of a fluorescent marker, which is quantitatively measured within a PCR thermocycler. The net effect of this is to reduce the results turnaround time from 12–24 hours to less than 2 hours. One of the main disadvantages of current PCR technology is that laborious preparative steps are required to extract DNA before the thermocycling process can be undertaken.(121)

Optical immunoassay

In the optical immunoassay (OIA) an antibody specific to a GBS surface carbohydrate is coated on a sample well. In the presence of GBS carbohydrate the optical substrate of the test well reflects differently and can be detected visually using a luminometer. Again a preparative step is needed, to extract the carbohydrate antigen from GBS.(121)

DNA hybridisation

Nucleic acid hybridization tests are based on the ability of complementary nucleic acid strands to specifically align and associate to form stable double-stranded complexes. Commercially available kits use a single-stranded DNA probe with a chemiluminescent label that is complementary to GBS ribosomal RNA. A preparatory step releases the RNA from the organism, to which the labeled DNA probe combines to form a stable DNA:RNA hybrid. A specific reagent enables the differentiation of hybridized probe from unhybridized probe

and measurement in a luminometer, with a positive result being one that is greater than a predefined threshold.(121)

Enzyme-linked immunosorbent assay

Similar to the OIA, the enzyme-linked immunosorbent assay (ELISA) employs antibodies to GBS surface carbohydrate, both coated on a sample well and in soluble form linked to an enzyme. The GBS binds first to the sample well and then the soluble form of the antibody binds to the GBS. The enzyme then produces a reaction in a colored substrate, which can be detected by eye or quantitated in a luminometer.(121)

Latex agglutination

The most easy to use of all the available rapid tests is based on antibodies bound to latex particles. If GBS is present, the antibodies bind to its surface and the attached latex agglutinates into visible clumps.

Each of the tests currently available has advantages and disadvantages. Even laboratory-based use of these tests is limited, and there has been no proper evaluation of any of these tests in the point-of-care setting.(121)

Accuracy of rapid tests for GBS

In a recent systematic review on rapid tests for GBS colonization in laboring women 23 relevant papers of 29 test accuracy studies assessing a total of six tests were analyzed.(122)

This review shows that many of the GBS tests, with the exception of real-time PCR and optical immunoassay (OIA) either took too long to produce a result on time or were not of sufficient accuracy to be feasible for maternal intrapartum testing. The review focused on studies in which selective media were used for gold standard culture. PCR had a median sensitivity and specificity of 96% (Range 88–99%) and 98% (Range 96–98%) respectively. Median sensitivity for optical immunoassay (OIA) was 48% (Range 37–72%) and median specificity was 97% (Range 95–97%). Positive likelihood ratio for PCR was 38.80 (95% CI 6.05-248.720), negative likelihoodratio was 0.06 (95% CI 0.03-0.11). The positive likelihood ratio for OIA was 14.7 (95% CI 10.6-20.3) and negative likelihood ratio was 0.47 (95% CI 0.31-0.73).

Although OIA seems less accurate than PCR, it was more rapid (30 minutes compared to 40 minutes) and less complex to perform, making it more feasible as a near-patient test.

However, the real time PCR was only evaluated in 2 relatively small studies. Authors state that with regard to the poor methodological quality of the existing studies and the imprecision of the evidence for PCR, a robust technology assessment comparing the most promising tests (PCR and OIA) is needed before reaching recommendations for practice.(122)

Recently, in a primary test accuracy study swabs were obtained at the onset of labor from 1400 women from two large maternity units to compare the results of vaginal and rectal PCR and OIA (index tests) with the reference standard of enriched culture of combined vaginal and rectal swabs.(121) PCR was significantly more accurate than OIA for the detection of maternal GBS colonization. The sensitivity for PCR was 84% (95% CI 79-88%), with a specificity of 87% (95% CI 85-89%). Authors conclude that PCR performed better than OIA, but results of their economic analysis demonstrate that both rapid tests should not yet be used in practice, since both are not clearly cost-effective methods of screening women for GBS colonization.

Preventive measures

Vaccination

Vaccination of pregnant women offers the opportunity for primary prevention of GBS disease of the newborn by two mechanisms. First, a vaccine that induces mucosal immunity would decrease maternal colonization and consequently the risk of transmission to the fetus. Second, and potentially more important would be the transplacental transmission of protective antibodies to the baby. Babies with high concentrations of antibodies to GBS proteins have an OR of 0.002 (95% CI 0.000-0.57) of developing GBS-EOD compared with those with low levels.(123) Protective maternal antibodies are believed to persist in the fetus for about 3 months after birth affording additional passive protection against GBS late onset disease.(124)

The search for a suitable candidate molecule for vaccination has been ongoing for two decades, but a vaccine has yet to be licensed for use and evaluated for effectiveness in reducing neonatal GBS disease. Initial developments involved carbohydrate-based vaccines of which immunogenic efficacy has been demonstrated in women.(124) The problem with each vaccine is that there are five major, and several minor, serotypes of GBS, each with a different outer carbohydrate. The current vaccine preparations for GBS are based on the serotypes prevalent in the USA and Europe. However, these vaccine preparations are not as effective in women of other regions of the world because of the prevalence of different serotypes expressing a different repertoire of surface proteins. To ensure effective vaccine development, it will be important to monitor the distribution pattern of the prevalent serotypes and sequence types in all regions of the world, thereby ensuring the inclusion of the most relevant components in a global GBS vaccine.(124) Any vaccine would have to be multivalent and appropriate to the serotype prevalence within the population. Focus therefore shifted to an ubiquitous protein that is present on the outer surface of all GBS serotypes.(125) Protein-based antigens are inherently more immunogenic than carbohydrates, are less likely to cross-react with human tissues and can more readily be manipulated by molecular techniques.

Chlorhexidine

Vaginal disinfection with chlorhexidine during labor has been suggested as a simple, cheap and safe alternative for IAP to prevent vertical transmission and subsequent GBS-EOD. Chlorhexidine is without risk of bacterial resistance and with no risk of allergic complications.

In a systematic review in 2004, analysis of 5 studies (including 2190 term and preterm infants) comparing vaginal disinfection with chlorhexidine during labor versus no treatment or placebo resulted in a statistically significant reduction in GBS colonization of neonates (RR 0.72, 95% CI 0.56 to 0.91), but the studies were not large enough to draw conclusions regarding reduction of GBS infections.(126) There was no statistically significant reduction in EOD including GBS infection, GBS pneumonia, GBS meningitis or mortality.

In a randomized controlled study with 244 GBS colonized mothers at term (screened at 36-38 weeks), the efficacy of intrapartum vaginal flushings with chlorhexidine was compared with ampicillin intravenously (IV) in preventing GBS transmission to neonates. The rate of neonatal GBS colonization was not statistically different in both groups (chlorhexidine, 15.6%; ampicillin, 12%). However, colonization with Escherichia coli was significantly more prevalent in the ampicillin (7.4%) than in the chlorhexidine group (1.8%, $p < 0.05$).(127)

In a large trial in Soweto, South Africa, 8011 women were randomized to chlorhexidine vaginal wipes or external genitalia water wipes during active labor, and their babies were assigned to full body (intervention) or foot (control group) washes with chlorhexidine at birth, respectively.

Rates of neonatal sepsis did not differ between the groups. Rates of colonization with GBS in newborn babies born to mothers in chlorhexidine and control groups did not differ. (128)

In conclusion, studies with chlorhexidine showed promising results regarding reduction of transmission of GBS colonization but a definite conclusion on the effect of this treatment on the incidence of GBS-EOD cannot be drawn.(126;129;130)

Antibiotic treatment of the mother

Since vaginal re-colonization from the gut may occur, antibiotic treatment of GBS carriers during pregnancy is inadequate as prophylaxis.(5) In addition, prolonged prophylactic treatment can result in resistance of other microorganisms and disturbance of the intestinal and vaginal flora.

In a small double-blind randomized controlled trial, prenatal oral amoxicillin (500 mg 2 times daily during 5 days) or placebo was given to GBS carriers identified by rectovaginal culture at 35-37 weeks of gestation. Persistence of GBS colonization after treatment with

amoxicillin compared to placebo was 43% and 67% of the women at the time of labor (difference statistically not significant).(131)

Intrapartum antibiotic prophylaxis (IAP) of the mother during delivery is regarded as the most effective method to reduce the number of neonatal GBS infections.(14)

In the absence of IAP in vaginal deliveries, neonates born from GBS colonized mothers were colonized in 16%-69% at one or more surface areas.(6-11;132;133) After administration of IAP with ampicillin in randomized studies, a lower neonatal colonization rate has been found after vaginal delivery, varying from 0% to 10%.(9;14;133;134)

The most direct indicator for efficacy of IAP is the bacterial load in neonatal blood cultures.

Recently, the Cochrane collaboration conducted a systematic review on the impact of maternal intrapartum antibiotics for maternal Group B streptococcal colonization on neonatal GBS infection. Only three randomized controlled trials, evaluating the effects of IAP versus no treatment, conducted more than 20 years ago in three different countries and enrolling a total of 500 women have been published.(14;132;135)

Overall quality of the included studies was poor and the risk of bias high. No study reported on a pre-set sample size and no placebo was used in the three studies comparing one antibiotic versus no treatment; care-givers and researchers were not blinded to group assignment.(9)

Despite serious concerns about bias in the three included trials, studies were combined. A statistically significant reduction in GBS-EOD was found. (RR 0.17, 95% CI 0.04-0.74) The authors concluded that IAP reduces GBS-EOD, but there is lack of evidence from recent well designed and conducted trials to recommend IAP to reduce GBS-EOD.(11)

Antibiotics and dosing regimens

There are only a few well designed studies in which the efficacy and side effects of different antibiotics and dosing regimens have been examined. Adequate antibiotic concentrations in amniotic fluid are likely to be involved in eradication of GBS from surface areas. Since there is some time needed to achieve adequate amniotic fluid concentrations and eradicate GBS from these areas, the time interval between IAP and delivery is essential. De Cueto et al. found for ampicillin that when this interval is at least 2 hours, vertical transmission of GBS was minimized to 1.5%.(133)

Muller et al collected blood samples from mothers in labor, umbilical cord and neonates after administration of an intrapartum dose of amoxicillin of 2 gram. With these data a multicompartment model to describe the overall concentration versus time profile in maternal plasma, umbilical cord and neonatal plasma was developed. Peak concentrations in umbilical cord and neonatal serum were lower and delayed compared to the maternal peak concentration. Approximately one hour after the start of the antibiotic administration the

neonatal concentration reached its highest level, and thereafter exceeded the concentrations in venous umbilical cord. Simulation of a 2 gram infusion on basis of the developed pharmacokinetic model demonstrated that amoxicillin concentrations in maternal, venous umbilical cord and neonatal serum exceeded the minimal inhibitory concentration for > 90% of the dosing interval of 4 hours.(136)

The CDC guideline advises intravenous prophylaxis with 5 million IU penicillin G or 2 gram amoxicillin or ampicillin, followed by respectively 2.5 million IU penicillin or 1 gram amoxicillin or ampicillin every four hours until delivery.(5) Dutch guidelines deviate from this guideline, advising an initial dose of 2 million Units benzylpenicillin and subsequent doses of 1 million Units every four hours. (Table 2)

Muller et al. described in a simulation model in women with PPROM that a dosing regimen of bolus injections of 1 gram amoxicillin every 6 hours was predicted to be adequate for the prevention of GBS infection in pregnant patients.(137) This regimen was described as the usual regimen in a former review of the Cochrane Library(138), which now is withdrawn. The new Cochrane review on this topic doesn't describe recommendations for antibiotic dosing regimens anymore. A two gram loading dose does not seem to be beneficial and the 1 gram doses can safely be administered by bolus injection increasing the comfort of the patient and facilitating prophylaxis.(137)

Penicillin is the first choice because of the narrow spectrum and less risk of selection of resistant bacteria.(139) Resistance to penicillin in GBS has been described (140;141), but is very rare. Resistance to erythromycin and clindamycin has been reported in 7.4% and 3.4% of invasive GBS isolates respectively.(142;143) This has clinical consequence as these agents are recommended for intrapartum prophylaxis in women with a history of penicillin allergy. For this reason in the last report of the CDC the recommendation has been changed.

In case of penicillin allergy with low risk of anaphylaxis, cefazolin instead of erythromycin or clindamycin is recommended. In case of allergy to penicillin with high risk of anaphylaxis, clindamycin or erythromycin is given after susceptibility to one of these agents is proven. In case of allergy to penicillin with high risk of anaphylaxis and resistance to clindamycin and erythromycin, vancomycin can be given.

Dutch guidelines advise either clindamycin or erythromycin in case of history of penicillin allergy without establishing low or high risk of anaphylaxis or the susceptibility of GBS to one of these antibiotics. (Table 2)

Antibiotic treatment of the neonate

Complete absence of GBS-EOD was reported as an unexpected benefit of a policy of routine administration of intramuscular penicillin to neonates to prevent gonococcal ophthalmia.

Table 2 Antibiotic Dosing regimen as recommended by the CDC(5)

Antibiotic	Initial Dose	Subsequent Dose	Dosing Interval	Patients
Benzylpenicillin	5 million Units*	2.5 million Units*	4 hours	Not penicillin allergic
Ampicillin	2 g	1 g	4 hours	Not penicillin allergic
Cefazolin**	2 g	1 g	8 hours	Allergic to Penicillin; Low risk of anaphylaxis
Clindamycin	-	900 mg	8 hours	Allergic to Penicillin; High risk of anaphylaxis, susceptibility to clindamycin proven
Erythromycin	-	600 mg	6 hours	Allergic to Penicillin; High risk of anaphylaxis, susceptibility to erythromycin proven
Vancomycin**		1g	12 hours	Allergic to Penicillin; High risk of anaphylaxis, resistant to clindamycin and erythromycin

* Dutch guidelines differ from these CDC guidelines, advising an initial dose of 2 million Units and subsequent doses of 1 million Units

** Dutch guidelines do not mention cefazolin nor vancomycin as alternatives for Benzylpenicillin in case of allergy

(144;145) Subsequent observational studies have suggested that the administration of intramuscular penicillin to the newborn immediately following delivery may be an effective strategy to reduce the incidence of GBS-EOD. However, these studies were uncontrolled, retrospective and non-randomized.(146-149) A Cochrane review on intramuscular penicillin for the prevention of early onset group B streptococcal infection in newborn infants included only one study. In this randomized controlled trial of 1187 infants of birth weight 501 to 2000 grams, there were no significant differences found for the outcomes of GBS-EOD (RR 0.73, 95% CI 0.32-1.62) or neonatal mortality (RR 0.78, 955 CI 0.55-1.11). Other outcomes such as GBS-LOD, neonatal sepsis and secondary outcomes such as neurodevelopmental status and length of hospital stay could not be assessed.(150)

Adverse effects of antibiotics

The administration of antibiotics as prophylaxis against GBS-EOD should have minimal risks for both mother and child. For the mothers, an important adverse effect of an increased use of antibiotics is the increasing incidence of potential severe adverse reactions including anaphylaxis to penicillin.(151;152) The incidence of anaphylaxis after administration of penicillin is estimated to be 0.01% with a mortality rate of 9%.(153;154)

Neonatal risks include an increase in incidence of non-GBS-EOD. The use of ampicillin rather than penicillin for intrapartum GBS prophylaxis has been reported to be associated with an increase in the incidence of neonatal sepsis caused by ampicillin-resistant Gram-negative micro-organisms.(55;155;156)

One study reported an association between the use of intrapartum antibiotics and LOD.(157) The incidence of postnatal yeast infections may increase with the use of intrapartum antibiotics(158) and possibly acquired abnormalities in early-life bacterial colonization may affect the development of the immune system and change the pattern of initial colonization of the gut in the first days of life which may be linked to later development of allergic disease.(157;159-161)

Burden of the disease

The incidence of GBS-EOD *before* implementation of prevention strategies ranged from 0.2 to 3 or even more cases per 1000 live births with substantial geographical variations.(162) In Europe, prior to 2000, incidence varied between 0.2 and 0.3 in Denmark(21) to 0.76 in Finland(163), 0.69 to 4.5 in France(164), 1.9 in the Netherlands(18), 3.25 in the Czech Republic(165) and 2.4 in Spain.(166)

Wide variations in incidence could be correlated with differences in women's GBS carriage rate, with differences of ethnic or racial susceptibility to GBS infection, with differences in definition of diagnosis of GBS-EOD and with differences in virulence among the prevalent GBS strains.

Evolution of Guidelines for prevention of GBS-EOD

During the past two decades, various initiatives have been established to prevent GBS-EOD. These were mainly promoted in the USA and transferred and adapted into national guidelines in some European countries.

Clinical trials in the 1980s showed that GBS-EOD can be prevented by administering intrapartum antibiotic prophylaxis (IAP) to mothers who are colonized with GBS.(14;17)

Prevention starts with a strategy to identify mothers at risk for having a baby with GBS-EOD.

The simplest strategy, IAP in all deliveries, has not been considered because of unnecessary exposure to antibiotics.

In the USA, two different options of IAP, either based on the presence of maternal factors associated with increased risk of GBS-EOD (risk factor based screening) or on maternal antenatal GBS-positive screening cultures (culture based screening), were established in 1996.(46) Although neither option was optimal or properly implemented, surveillance studies already documented from the early 1990s to 1999 a decline in GBS-EOD of 65%.(17)

In the risk factor based approach, women presenting at the time of labor with clinical risk factors for disease (ie preterm delivery before 37 weeks of gestation, prolonged rupture of the membranes, intrapartum temperature > 37.5°C, maternal GBS bacteriuria during pregnancy and a history of a previous child with GBS-EOD) are offered intrapartum antibiotic prophylaxis.

In the screening approach, women are screened for carriage of group B streptococcus between 35 and 37 weeks of gestation, and IAP is offered to all carriers as well as in case of unknown GBS carriage and delivery at < 37 weeks gestation or amniotic membrane rupture > 18 hours.

In both strategies, antibiotics are given during labor to women who had group B streptococcal bacteriuria during their current pregnancy, or who have had an infant with GBS-EOD previously.(46)

In 2002, a population-based retrospective study of 5144 births, including 312 in which the newborn had GBS-EOD, showed that culture based screening was 50% more effective in prevention of GBS-EOD compared to risk factor based screening.(167) In this study, to identify candidates for intrapartum prophylaxis, either screening of pregnant women for GBS colonization by means of cultures (screening approach) or assessing clinical risk factors (risk factor approach) was used as both recommended by the former CDC guidelines in the USA.(46) Antenatal screening was documented for 52 percent of the mothers in the study. The risk of GBS-EOD was significantly lower among the infants of screened women than among those in the risk factor group (RR 0.46, 95% CI 0.36-0.60).

Thereafter, national guidelines in the USA were changed, shifting from a recommendation of either of these strategies to a recommendation of only culture based screening at 35-37 weeks of gestation for all pregnant women and IAP for any woman with GBS colonization. The risk factor based approach was reserved for women in labor with an unknown maternal colonization status.(5) Culture based screening was expected to result in further declines in the incidence of GBS-EOD.(167)

Since the end of the 1990's, in several European countries a culture based screening strategy has been recommended, including Spain (1998 and 2003), Italy (1996), France (2001), Belgium (2003), Germany (1996, 2008) Switzerland (2007), Poland(2008) and Czech Republic (2008). The risk factor based strategy has been recommended in the UK and in the Netherlands. In some other European countries, for example in Bulgaria and Denmark, there are no guidelines.(168)

Epidemiology

In the USA, after widespread implementation of prevention strategies, the incidence decreased from 1.8 cases per 1000 live births in 1993 to 0.6 per 1000 live births in 1998 and 0.32 cases per 1000 live births in 2003.(17;109;169)

In European countries in which a prevention strategy was adopted, an important drop of the incidence of GBS-EOD was observed as well. In Spain for example, incidence declined from 2.4 cases per 1000 live births in 1996 to 0.59 in 2005.(166) Table 3 shows GBS colonization during pregnancy and incidence of GBS-EOD in various European countries after 2000.

The initial reported case fatality rate associated with GBS-EOD in the USA dropped from > 50% in the 1970s to 4-10% in recent years.(1;16-18;109;170-173)

Intrapartum administration of antibiotic prophylaxis (IAP) to the mother may have contributed to above named declines, by the antibiotic effect on the infant and by decreasing the severity of the disease. It is unclear to what extent the decrease in GBS-EOD can be attributed to the administration of antibiotics. Other factors may contribute as well, among which are early recognition of infection and improved neonatal care(174), as well as natural changes in prevalence of maternal colonization and variation in GBS subtype distribution.

From Norway an, unexplained, marked increase in case fatality was reported. In this country a risk factor based strategy is applied. From an average of 5.8% in 2000-2005 a significant increase to 33% in the first 6 months of 2006 and a slightly increased incidence of invasive GBS disease in neonates using a risk factor based strategy has been reported.(175) Such changes in case fatality rates without alterations in antibiotic policy might be explained by changes in the virulence characteristics of circulating GBS.

In all countries the case fatality rate in preterm born infants remains substantially higher than in term born infants, with case-fatality rates of approximately 20% - 30% among infants born before 33 weeks' gestation, compared with 2%-3% among term born infants.(173;176;177)

Remaining burden of the disease

In 2009, evaluation of the implementation of the screening guidelines in the USA was performed over the period 2003-2004. This revealed that the rate of screening for GBS during pregnancy increased from 48.1% in 1998-99 to 85% in 2003-2004; the percentage of infants exposed to IAP increased from 26.8% to 31.7%.(169)

IAP was given in 87.0% of the women who were screened positive for GBS and who delivered at term, but in only 63.4% of women with an unknown colonization status who delivered preterm.

Table 3 GBS colonization during pregnancy and incidence of GBS-EOD in various European countries after 2000. Derived from Trijbels-Smeulders et al(168), Barcaite et al(4) and Edmond et al(3)

		GBS colonization (% of Pregnant Women)		Incidence of GBS-EOD/1000 Live Births
Eastern Europe				
Lithuania	2012	15.3(179)		
Czech Republic	2004	29.3(180)	2004	0.80(181)
Poland	2003	19.7(182)		
Western Europe				
France	2001	14.3(183)		
France	2003	15.4(184)		
France			2010	0.75(185)
Germany	2006	16(186)	2005	0.21(171)
United Kingdom	2006	21.3(187)	2011	0.67(188)
The Netherlands	2002	21(189)	2007	0.36(22)
			2010	0.70(190)
Ireland	2004	11.8(191)		
Scandinavia				
Denmark	2004	36(192)	2004	0.34(193)
Iceland	2003	24.3(194)		
Sweden	2008	25.4(195)	2004	0.69(196)
Norway			2006	0.85(175)
Southern Europe				
Greece	2003	6.6(197)		
Italy	2007	17.9(198)	2007	0.50(199)
Spain			2005	0.59(166)
Turkey	2003	10.6(200)		
Turkey	2004	6.5(201)		
Turkey	2005	32(202)		
Turkey	2005	9.2(203)		

The overall incidence of GBS-EOD in the USA in 2004 was 0.31 cases per 1000 live births. Preterm infants had a higher incidence of GBS-EOD than term infants (0.73 vs. 0.26 cases per 1000 live births). However, 74.4% of the cases of GBS disease occurred in term infants. Missed screening (i.e. "forgot to screen" at 35-37 weeks of gestation and no IAP) among mothers who delivered at term accounted for 13.4% of group B streptococcal disease. (34 of the 254 cases) A total of 61.4% of the term infants with GBS disease were born to women who had tested negative for GBS before delivery.(169)

From 2003 to 2006, an increase in GBS-EOD in the USA was seen towards 0.40 per 1000 live births. When stratified by race, incidence among black infants increased significantly (0.53 to 0.86 cases per 1000 live births), whereas incidence among white infants did not change significantly (0.26 to 0.29 cases per 1000 live births.(178) The reason for this difference remains unexplained.

Strategy for prevention and incidence of GBS-EOD in the Netherlands

In the Netherlands, the Dutch Society of Obstetrics and Gynecology (NVOG) and the Dutch Society of Pediatrics (NVK) approved the guidelines for prevention of GBS-EOD in 1998. These guidelines do not recommend universal screening nor prophylaxis in case of risk factors for GBS-EOD alone, but were based on a sort of the risk factor based approach. (46) IAP is recommended following a previous delivery of an infant with GBS-EOD, or heavy maternal GBS colonization (which may present as GBS urinary tract infection or GBS bacteriuria during current pregnancy). In the case of onset of labor before 37 weeks of gestation or prolonged rupture of membranes (>18 hours before delivery), screening for GBS carriage is performed first, followed by prophylaxis when the culture is positive. When delivery occurs before the result of the culture is available, the gynecologist should decide about antibiotic prophylaxis, based on the severity of the risk factor(s). (Figure 5) In this approach, the Dutch strategy differs from the risk factor based strategy. The choice for this risk factor-based strategy in 1998 was made with the intention to reduce the number of women that receives prophylactic antibiotics, taking into account the Dutch organisation of obstetrical care with approximately 30% home deliveries.

The Dutch guidelines advise to discontinue prophylactic administration of antibiotics to the mother after delivery, unless symptoms of maternal infection persist. In that case prophylaxis is changed to therapy with a change of antibiotic strategy.

A differentiated sequential management of newborn infants of GBS carriers who had an indication for antibiotic prophylaxis seems to be justified. It should be established whether adequate prophylaxis was given during delivery. Despite lack of evidence for existing dosing schedules, IAP is regarded as adequate if the antibiotics were given intravenously in the right dosage and at least 4 hours before delivery, so that two doses have been given. After adequate prophylaxis, observation during 48 hours, of which at least 24 hours in hospital, is recommended. If no or inadequate prophylaxis was given and gestation was more than 35 weeks, the same strategy is followed. If no or inadequate prophylaxis was given and gestation was less than 35 weeks, a culture is taken from blood and cerebrospinal fluid and treatment for sepsis is started. If the cultures of blood and of cerebrospinal fluid are negative and no clinical signs of infection are present, treatment can be stopped.

If clinical signs of infection exist, an extensive diagnostic approach for infection is indicated and the infant is treated as in sepsis, independently of the prophylaxis and the gestational age.(22) (Figure 6)

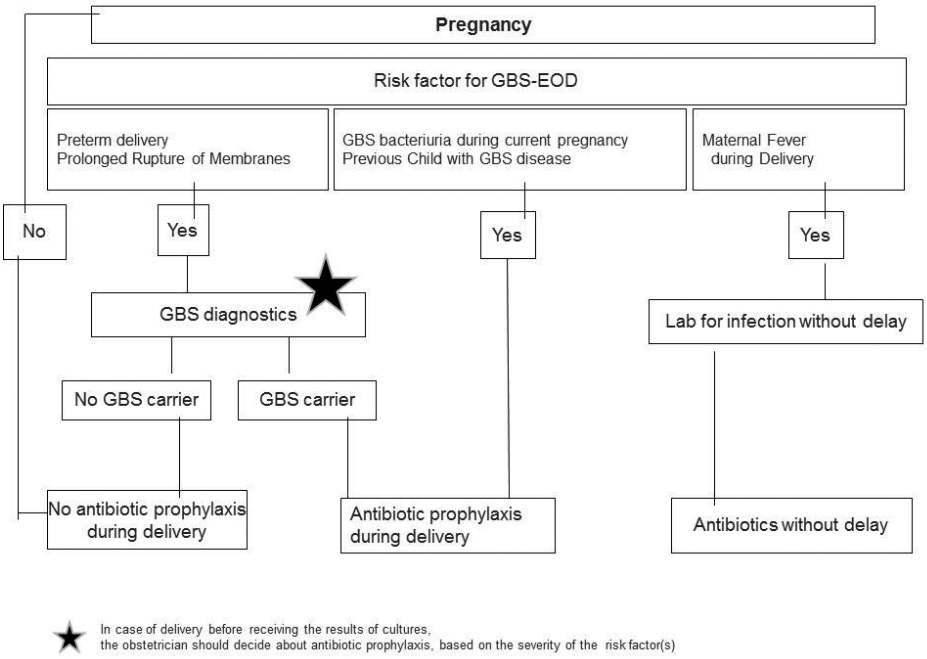

Figure 5: Scheme on prevention strategy of GBS-EOD in the Netherlands

After the introduction of above named prevention guidelines based on risk factors in 1999, there has only been a limited decrease in the incidence of proven GBS-EOD from 0.54 per 1000 live births to 0.36 per 1000 live birth in the Netherlands.(22)

There was no decrease in the incidence of probable early-onset GBS sepsis, respectively 1.3 and 1.4 per 1000 live births. In the small category of late onset GBS sepsis (> 7 days after birth), there was no decrease in the incidence of proven cases.(22)

Table 4 shows incidence figures of GBS meningitis and GBS sepsis and Table 5 shows deaths due to perinatal GBS infections in the Netherlands between 2000 and 2009, as registered by the Netherlands Perinatal Registry. This registration is estimated to cover 70% of the total population of the Netherlands and data therefore only show a trend over years. Incidences of (proven and probable) GBS sepsis and GBS meningitis seemed to be stable until 2008, with respectively 108 and 15 reported cases in 2008. In 2009 an unexplained increase was seen, with 172 cases of GBS-EOD (0.93 per 1000 live births). Between 2000 and 2009 a case fatality rate for GBS-EOD of 6.3% was found.

Revision of the Dutch guidelines was considered in 2006, but recommended prevention strategy remained as it was. Because of the ongoing burden of GBS-EOD, adaptation of the Dutch guidelines should be reconsidered, particularly with regard to the fact that perinatal mortality in the Netherlands is high compared to other European countries.(23)

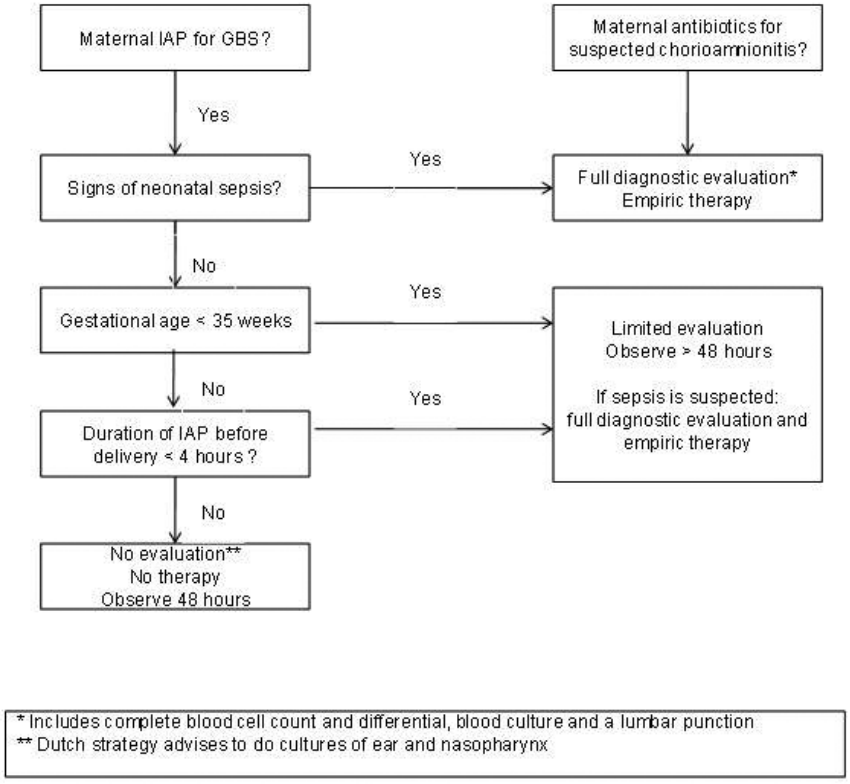

Figure 6: Postdelivery management of newborn infants of GBS carriers who received IAP as recommended by the CDC(5)

OUTLINE OF THE THESIS

The aim of this thesis is to contribute to the information needed for the establishment of an optimal preventive strategy for GBS-EOD.

In **chapter 2** a study is described that was performed to determine the prevalence of GBS and to identify risk factors for GBS carriage in a multicultural population of pregnant women in The Hague in the Netherlands. Several factors are known to increase the risk of perinatal GBS-infection in women that carry GBS. In **chapter 3** we report on the relationship between prolonged rupture of membranes (PROM, >24 hours) and labor before 37 weeks of gestation and GBS, to evaluate whether occurrence of these risk factors can predict prenatal GBS status. In GBS strains from the studied multicultural obstetric population, phenotypic and genotypic antibiotic susceptibility patterns and putative epidemicity was assessed, which is described in **chapter 4**.

Chapter 5 describes the results of a systematic review in which the relation between maternal colonization with Group B Streptococcus and preterm birth was determined and

Table 4 Incidence of GBS meningitis and GBS sepsis in the Netherlands, as registered by the Netherlands Perinatal Registry

Year	Number of cases	
	GBS meningitis	GBS sepsis
2000	15	158
2001	18	132
2002	19	137
2003	27	133
2004	28	151
2005	24	121
2006	18	117
2007	25	115
2008	15	108
2009	20	172
Total 2000-2009	209	1344

Table 5 Deaths due to perinatal GBS infections in the Netherlands between 2000 and 2009, as registered by the Netherlands Perinatal Registry

Diagnosis	Number of deaths	Percentage
GBS meningitis (n=209)	12	5.7%
GBS sepsis (n=1344)	87	6.5%
GBS meningitis or GBS sepsis (n=1171)	92	6.3%

in **chapter 6** we present results of a meta-analysis to assess the best timing of antenatal cultures, which may help to establish optimal prevention of GBS-EOD in neonates. With regard to the remaining burden of disease in the world, we list opportunities for improvement of prevention of GBS-EOD in **chapter 7**, followed by considerations in finding an optimal prevention strategy for the Netherlands in **chapter 8**. Finally, conclusions of earlier chapters are summarized and future perspectives and directions of research in the prevention of GBS-EOD are discussed in **chapter 9.**

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