countries reported the absence of both a nationwide and a local protocol.

Almost all respondents (96%) from the 25 countries without nationwide guidelines to prevent neonatal GBS infection would give antibiotics to women with signs of infection during labor. In case of preterm birth (<37 weeks), ruptured membranes for >18 or 24 h, respectively, 53, 54 and 65% of the respondents would give antibiotics during labor. When GBS-positive mothers were adequately treated during labor, administration of antibiotics to infants without signs of infection would be decided for by 48% of the respondents if preterm and 19% if term. When mothers were not or inadequately treated during labor, 61% of the infants born before 37 weeks gestation and 35% of term infants would be treated with antibiotics after birth.

Discussion. The aim of the study was to establish the incidence of GBS colonization in pregnant women and the incidence of neonatal GBS infection and to determine policies for prevention of neonatal GBS infection in Europe. The response rate of 15% was low, but 29 of the 33 European countries involved were represented by at least 1 respondent. Therefore we believe that we have a reasonable impression of these incidences.

The data collected show that in most European countries the incidence of GBS colonization among pregnant women varies between 10 and 20% and the incidence of neonatal GBS infection ranges from 0.5 to 2 per 1000 live births. The differences found may be partly explained by the use of different data collection sources (regional, national), differences in definition of GBS sepsis used (probable and/or proven cases) and differences in the culture methods used.

Nationwide guidelines for prevention have been issued in only a few European countries thus far. About one-half of the respondents reported the existence of local hospital-based protocols in their institution, but the prevention practices vary among doctors and institutions. The policy of giving antibiotics to pregnant women with signs of infection during delivery is widely accepted. However, only about one-half of the respondents would give antibiotics in case of expected preterm delivery and after prolonged rupture of the membranes.

When pregnant women are identified as being colonized with GBS, most respondents would not give antibiotics to prevent early onset GBS infection to the infant in case of term delivery and absence of neonatal signs of infection, whether or not intrapartum antibiotic prophylaxis was given. However, about one-half of the respondents would do so after preterm delivery, especially if no or inadequate intrapartum prophylaxis was given.

Our study shows that only four European countries have a nationwide guideline for prevention of early onset GBS infection. Because of the lack of epidemiologic data and uniform early onset GBS infection prevention methods, a surveillance study in European countries would be needed for determination of the most appropriate prevention policy.

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AFFORDABLE DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN INFANTS BY P24 ANTIGEN DETECTION

The expense of PCR testing limits diagnosis of HIV infection in infancy in low resource settings.

The ultrasensitive p24 antigen assay has been proposed as an accurate substitute; however, its ability to detect different HIV viral subtypes remains to be determined. A sensitivity of 98.1% and specificity of 98.7% was obtained testing 203 samples from 24 HIV-infected and 66 uninfected infants born to HIV subtype C-infected women.

Antenatal clinic prevalence rates of HIV infection in South Africa were 25.9% in 2002, which means that more than one-fourth of children currently born in South Africa are vertically exposed to HIV.[1] In general guidelines for Prevention of Mother to Child Transmission (PMTCT) programs in low resource settings require all exposed children to be followed to 12 months of age or older before their HIV infection status can be determined by an HIV antibody test. The persistence of maternal anti-HIV antibodies and the cost and complexity of two HIV PCR tests precludes earlier testing.[2] In South Africa >280 000 HIV-exposed children per annum, of whom as many as 90% may be HIV-uninfected, must be followed for 12 months or more. A universal frustration of PMTCT programs in low resource settings is the lack of capacity to achieve this follow-up, thereby missing the opportunity to determine the HIV infection status of exposed children.[3, 4] This prevents access to available medical care known to improve the quality and quantity of life of HIV-infected children and their families (e.g. prophylactic medication, treatment of intercurrent infections, nutritional and emotional support). Assessment of the p24 antigen (Ag) assay for diagnosis of HIV infection in infants is listed among the urgent research priorities to improve pediatric HIV/AIDS care in developing countries.[5, 6]

The value of the p24 Ag assay in this context has been limited by its poor sensitivity.[7, 8] Recently a technically improved version of the assay has been as sensitive and specific as HIV PCR tests that detect viral nucleic acids. This ultrasensitive p24 Ag assay has been proposed for use in diagnosis of pediatric HIV infection and in viral load monitoring of patients receiving antiretroviral therapy.[5, 8–14] A proviso for widespread introduction of the p24 Ag assay is the requirement for further validation of the assay in non-B viral subtypes.[5, 9, 13] Viral subtype C accounts for 99% of HIV infections in South Africa.[15] The performance of the p24 Ag assay for early infant diagnosis in HIV subtype C is described.

Materials and methods. Infants born to HIV-infected women attending Coronation Women and Children's Hospital in Johannesburg, South Africa were enrolled between January and October 2002 in a study to assess an affordable infant diagnostic protocol in a low resource setting. The ethics committee of the University of the Witwatersrand approved the study. Mothers and infants each received a single dose of nevirapine for PMTCT as standard practice at the hospital.[16] Fewer than 3% of mothers reported breast-feeding their infants; this was consistent with an HIV transmission rate of <9% at 6 weeks and 3 months of age.[16] No infant received antiretroviral therapy after the first 72 h of age.

The p24 Ag ultrasensitive enzyme-linked immunosorbent assay (HIV-1 p24 Ag Ultra kit; PerkinElmer Life Sciences, Turku, Finland) was performed on 203 plasma samples belonging to 24 HIV-infected and 66 HIV-uninfected infants (Table 1). The plasma samples were collected prospectively in EDTA, separated and stored at –70°C within 8 h of collection. HIV DNA PCR tests were analyzed with the Roche Amplicor HIV-1 DNA test Version 1.5 (Roche Diagnostic Systems, Inc., Branchburg, NJ) at the time that the blood was sampled. The final HIV infection status was established by 2 concordant HIV DNA PCR tests done at 6 weeks and 3 months of age and seroreversion at 12 months of age. When seroreversion had not occurred by 12 months of age, an additional negative PCR result was obtained on stored samples at 4 or 7 months of age.[2] Laboratory staff performing the p24 Ag assay were blinded to the PCR results and the HIV infection status of the child. Viral load test results were not available for assessment of the quantitative accuracy of the p24 Ag assay. HIV subtyping was performed on 16 (67%) of the 24 HIV-infected children and 40 (44%) of the 90 mothers of these infants.

Results. The HIV DNA PCR test, regardless of the age at which it was performed, concurred with the final HIV infection status of the child in all 90 patients. The HIV DNA PCR results and final HIV infection status can therefore be used interchangeably as the standard against which the p24 Ag assay was assessed. Diagnostic sensitivity of the ultrasensitive p24 Ag assay was based on 52 plasma samples from 24 HIV-infected infants at various ages (Table 1). One false negative result was obtained in a sample taken at 6 weeks of age, giving an overall sensitivity of 98.1%. Diagnostic specificity of the p24 Ag assay was evaluated on 151 samples from 66 HIV-uninfected infants at varying ages. Two false positive

TABLE 1. Results achieved with the p24 Ag assay in comparison with the known positive and negative HIV DNA PCR results at different ages

The number of tests performed in each age group equals the number of patients tested except as noted. The majority (82%) of assays were performed at or before 3 months of age.

Age	No. of Positive p24 Ag Tests/No. of Positive PCR Tests	Sensitivity (%)	No. of Negative p24 Ag Tests/No. of Negative PCR Tests	Specificity (%)	Total Samples	PPV (%)	NPV (%)
6 wk (5.9 wk)*	22/23†	95.7	62/62	100	85 (42)‡	100	98.4
3 mo (3 mo)	20/20	100	62/62	100	82 (40)	100	100
4 mo (4.1 mo)	2/2	100	3/3	100	5 (3)	100	100
7 mo (7.4 mo)	7/7	100	22/24§	93.6	31 (15)	92.3	100
Total samples	52		151		203		
Total patients	24		66		90		

* Numbers in parentheses, median.
† 21 patients.
‡ Numbers in parentheses, percent.
§ 20 patients.
PPV, positive predictive value; NPV, negative predictive value.

results were obtained in samples taken at 7 months of age, giving an overall specificity of 98.7%. The positive and negative predictive values were 96.2 and 99.3%, respectively. Reanalysis of the 3 discordant samples and second aliquots of thawed plasma for each of the 3 samples, analyzed in a blinded manner, yielded correct results. In these instances the problem was that the test reading differed minimally from the cutoff reading, signifying a marginal negative or positive p24 Ag test result. When HIV genotyping was performed, all HIV-infected infants and all mothers of infants tested were infected with subtype C virus.

Discussion. Local experience with previous versions of the p24 Ag assay showing a poor sensitivity of 48% despite an excellent specificity of 100% (data not shown) has been as disappointing as reported elsewhere.[7, 9, 14] HIV-1 p24 Ag detection in plasma is problematic because the p24 antigen forms a complex with p24-specific antibodies resulting in underdetection or false negatives.[8, 9] Boiling plasma before antigen testing destroys the antigen-binding capacity of all antibodies releasing p24 Ag from the immune complex. The p24 Ag ultrasensitive assay assessed here uses heat denaturation and boosts p24 Ag detection by a signal amplification step that further increases the sensitivity of the assay. The improvement in technology has resulted in an assay that rivals the performance of qualitative and quantitative HIV PCR tests.[5, 8–14] The technology, in comparison with PCR is less costly and less complex, involving less expensive equipment already available in most laboratories. Technically the p24 Ag assay is simpler to perform and requires minimal training, making diagnosis and monitoring of HIV infection more affordable and accessible in low resource settings.

The reported sensitivity and specificity of the ultrasensitive p24 Ag assay in the context of pediatric HIV diagnosis ranges from 97 to 100% and from 99 to 100%, respectively.[5, 10, 11, 14] Viral subtypes A, B, D and E have prevailed in these studies.[5, 10, 11, 14] The sensitivity and specificity of 98.1 and 98.7%, respectively achieved in this study reaffirms how well the ultrasensitive p24 Ag assay performs in comparison with the current standard PCR test for infant diagnosis of HIV. The data show that the ultrasensitive p24 Ag assay detects viral subtype C as well as subtype B, the subtype that has been most extensively evaluated.[10] The timing of HIV testing in infancy to detect all cases of *in utero* and intrapartum HIV infection has been subject to debate.[2, 17] Initial studies concentrated on establishing the accuracy of the p24 assay in determining HIV infection status in HIV-exposed infants of varying ages and did not concentrate on younger infants.[10, 11] The efficacy of the ultrasensitive p24 Ag assay in early infant diagnosis requires testing at defined ages in the first 3 to 4 months of life with a view to establishing guidelines for the ages at which infants should be tested (Table 1).[14] In this cohort the majority of infants were tested at 6 weeks or 3 months of age (Table 1), at which time the specificity of the ultrasensitive p24 Ag assay was 100% with a single false negative result at 6 weeks of age.

The technical improvements that have enabled the ultrasensitive p24 Ag assay to perform as well as PCR for infant diagnosis can make early infant diagnosis of HIV a reality and transform health care for HIV exposed children of sub-Saharan Africa and other low resource settings worldwide.

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PSEUDOMONAS AERUGINOSA SEPTIC SHOCK SECONDARY TO "GRIPE WATER" INGESTION

We report the case of a 9-month-old girl who presented in septic shock after ingestion of a contaminated herbal supplement commonly used to treat colic. Herbal supplements are widely used by well-meaning parents for many common conditions. Pediatricians should be aware that the variable manufacturing and packaging conditions of herbal supplements can lead to contamination with infectious agents.

Herbal medicines are popular in many countries worldwide, including the United States, for treatment of infant colic and gassiness. Commercial preparations containing seed oils from dill, fennel, anise, mint, ginger and other herbs are widely believed to relieve abdominal distress in babies. Although generally safe some herbal supplements can lead to significant and potentially lethal complications for children. We report a case of an infant who developed septic shock, severe absolute leukopenia, neutropenia and subcutaneous nodules caused by *Pseudomonas aeruginosa* after ingestion of contaminated "Gripe Water," an off-brand Ayurvedic medicine brought from India.

Case report. A 9-month-old previously healthy infant presented to our emergency department with fever and fussiness for 2 days. The day before admission she began to vomit repeatedly. On the day of admission, she became progressively lethargic and was taken to the emergency department. At presentation the infant was febrile (39.9°C), with a pulse of 181 beats/min, respiratory rate of 45 breaths/min, blood pressure 94/50 mm Hg and oxygen saturation at 96% in room air. The infant's skin was mottled. She received immediate fluid resuscitation and laboratory assessment for infection of urine, blood and cerebrospinal fluid, after which, ceftriaxone and vancomycin were administered intravenously. The initial total leukocyte cell count was $1.6 \times 10^3/\mu l$ ($1.6 \times 10^9/l$) with an absolute neutrophil count of zero. The hemoglobin was 8.2 g/dl (82 g/l). Analysis of electrolytes and pH showed hyponatremia (125 mmol/l), hypokalemia (2.2 mmol/l) and a nonanion gap metabolic acidosis. The patient was admitted to the pediatric intensive care unit.

Past history. The infant was a previously healthy full term infant, born in the United States and cared for at home by her mother. She received all immunizations according to schedule. Growth and development were normal. The family history was unremarkable. There were no siblings, no one else was ill at home and there were no pets.

Hospital course. On arrival to the pediatric intensive care unit, the patient remained lethargic with abnormal vital signs. Twelve hours into her hospital course she developed several 1-cm erythematous subcutaneous nodules on her extremities. Six hours later the blood culture contained Gram-negative rods identified as *P. aeruginosa.* Anti-*Pseudomonas* therapy (ceftazidime and tobramycin) was initiated. The subcutaneous nodules were in retrospect consistent with those seen during *Pseudomonas* sepsis.

The parents revealed that the child had been given, since early infancy, a daily preventative dose of an herbal solution called *chvarka,* which had been purchased in India. *Chvarka* is an Ayurvedic medicine version of gripe water, used by parents around the world to treat or prevent infant colic. Three days before onset of the infant's illness, the parents opened a new bottle of *chvarka* which had recently been purchased in India. A sample of the *chvarka* was cultured, and numerous bacterial colonies on the agar were identified as *P. aeruginosa* the following day.

The infant's clinical condition improved slowly. She was discharged from the hospital in good condition after 3 weeks of anti-*Pseudomonas* therapy.

Discussion. Gripe water is a traditional herbal remedy said to effectively treat or prevent "flatulence, teething pain and tummy upsets in babies." Despite many enthusiastic anecdotes no formal evaluation of gripe water has ever been undertaken. First formulated in England in 1851, it became commonplace for English nannies to use the product liberally. The original Woodward's Gripe Water contained 3.6% alcohol, dill oil, sodium bicarbonate, sugar and water.[1] Over time many companies have modified the ingredients in gripe water. Typical ingredients in commercial formulations include some combination of "herbal oils" (e.g. dill, cardamom, fennel and/or anise), sugar, bicarbonate and sometimes alcohol.[2,3] Recipes for making gripe water at home are also readily available over the Internet.[4] In 1993, the Food and Drug Administration (FDA) ordered automatic detention of all shipments of gripe water because it fit the definition of a new drug without having FDA approval.[5] However, companies now market and sell gripe water in the US as a dietary supplement, instead of a medicine, bypassing FDA regulation. A typical single dose is 2.5 to 5 ml, depending on the age of the baby. It can be repeated several times daily. The product, from the United Kingdom, India and other countries, can be purchased in many ethnic grocery stores. Domestic gripe water can also be purchased in specialty stores and over the Internet.

We were unable to find any previous reports of sepsis caused by bacterial contamination of gripe water or documented cases of adverse affects from gripe water. To test locally purchased products, we purchased gripe water made by two Indian companies and one Egyptian company. Cultures from the contents of the three bottles were sterile.

Because of the lack of regulation of dietary supplements from the FDA, there can be an increased risk of contamination of improperly manufactured herbal liquid preparations. Given the increased frequency of use of herbal supplements in children, clinicians should be aware of the potential dangers of ingesting products with loosely regulated manufacturing standards.

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