Seroprevalences of Herpes Simplex Virus Type 2, Five Oncogenic Human Papillomaviruses, and Chlamydia trachomatis in Katowice, Poland

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Herpes simplex virus type 2 (HSV-2), human papillomaviruses (HPVs), and Chlamydia trachomatis are the most common pathogens causing sexually transmitted infections (STIs). There is limited information about the prevalences of these STIs in Poland. Here, we estimated the occurrence of immunoglobulin G (IgG) antibodies against HSV-2, HPV, and C. trachomatis in 199 blood donors and 110 patients of both genders attending an STI clinic in Katowice in southern Poland. The seroprevalences of HSV-2 were 5% for blood donors and 14% in the STI cohort. The seroprevalences of the five potentially oncogenic HPV types 16, 18, 31, 35, and 51 were 15%, 7%, 5%, 5%, and 17%, respectively, in blood donors and 37%, 8%, 12%, 5%, and 21%, respectively, in the STI cohort. The majority of HPV-infected individuals showed antibodies against more than one type, i.e., had been infected with multiple HPV types. Anti-C. trachomatis IgG antibodies were detected in 6% of blood donors and 13% of individuals attending the STI clinic. The relatively high prevalence of HPV-51 may have implications for future vaccine programs, as the newly introduced HPV vaccines are based on the potentially oncogenic HPV types 16 and 18.

Infections with herpes simplex virus type 2 (HSV-2), human papillomavirus (HPV), and Chlamydia trachomatis are spread sexually, causing considerable morbidity and socioeconomic problems. A number of severe complications are associated with these sexually transmitted infections (STIs). HSV-2 infects the genital mucosa and establishes a life-long infection in sensory ganglia. HSV-2 is the most common cause of genital ulcers, and a strong association between HSV-2 infection and the AIDS epidemic has been described. Local HSV-2 reactivation enhances both HSV-2 and human immunodeficiency virus (HIV) transmission by increasing the number of HIV target cells in the genital mucosa, i.e., cervical immature dendritic cells and CD4+ T cells (34). Furthermore, HSV suppressive therapy significantly reduces genital and plasma HIV-1 RNA levels in dually infected women (28). Seroepidemiological data from worldwide studies performed during the last decade have estimated the HSV-2 seroprevalence to range from 0% in children to more than 80% in selected populations such as STI cohorts in some African countries (14, 36). For Poland, data are scarce, but recently, the prevalence of HSV-2 infection was described to vary between 6.5% and 12% from random selected serum samples in four geographic regions in Poland, not including Katowice (37).

HPV is the most commonly spread STI (40). Infection with oncogenic HPV types is the dominating cause of cervical cancer, which is globally the second most common cancer among women. There is great variation in the prevalence of HPV infection depending on different population-based factors such as age, gender, number of sexual partners, and geographic region. The oncogenic HPV-16 and HPV-18 are the best-documented types, with reported seroprevalences of 3% to 52% in adult populations worldwide (9, 21, 43). Most studies show higher seroprevalences among women than men, and the highest seroprevalences are reported from STI cohorts (9). To our knowledge, there are no seroepidemiological data available for HPV from Poland. However, by using a PCR method, HPV-16 DNA was detected in 13% of pregnant women (11, 12), a prevalence similar to or higher than that described for unselected pregnant and nonpregnant women in Finland (38).

Finally, C. trachomatis is the most prevalent bacterial STI causing symptomatic and, more commonly, asymptomatic genital infections. In women, C. trachomatis is an important cause of cervicitis and salpingitis as well as pelvic inflammatory disease, which may lead to tubular factor infertility (16). C. trachomatis infection in men may be manifested as urethritis and epididymitis, and recently, immunoglobulin G (IgG) antibodies against C. trachomatis in men have also been shown to correlate with reduced pregnancy rates in couples (17). Although somewhat conflicting data, C. trachomatis has been suggested to be associated with cervix cancer as well (30). By using direct identification methods such as antigen detection assays or PCR, the prevalence of C. trachomatis in asymptomatic women living in Europe, not including Poland, varied from 1% to 17% (45). The corresponding prevalence in Poland was recently described to be 1.8% (22). For women residing in Poland with uncomplicated pregnancy and with no history of pregnancy failure or urogenital disorders, IgG antibodies against C. trachomatis were recently detected in 14.5% of them (31).

As with HSV-2 infections, HPV and C. trachomatis cause a
considerable burden of disease and present a challenging task for the health care system to reduce the spread of these STIs. A major problem is that the infection is mostly asymptomatic. This situation implies that the infections are silent and are frequently unrecognized by the individual, increasing the risk for complications and further transmission. Detection of IgG antibodies against specific STIs may offer limited insight about the time point of infection, frequency of reinfection in seropositive individuals, or effect of treatment. However, based on the assumption that IgG antibodies remain for longer periods after infection, serological data are important to monitor changes in prevalences of STI infections over time in follow-up studies of prevention and vaccination programs. In this study, we estimated the seroprevalences of HSV-2, HPV, and *C. trachomatis* in blood donors and patients with STI in Katowice, Poland.

**MATERIALS AND METHODS**

**Serum samples.** Sera were collected from individuals residing in Katowice, a city situated in the southern part of Poland, during 2005 and 2006. All sera were stored at −20°C until analyzed. Two different serum cohorts were included. The first group, 199 sequentially selected blood donors, consisted of 110 women aged 19 to 56 years and 88 men aged 21 to 56 years (median age, 31 years). All blood donors were shown to be HIV seronegative. In the second group, 110 sequentially selected sera were drawn from 37 male and 73 female patients with clinical symptoms of STI (STI cohort), with an age range of 20 to 77 years (median age, 37 years). Although HIV screening of blood is standard procedure, HIV testing is not regularly performed in Poland. Therefore, HIV serostatus for this cohort was not available. As information about age and gender was available only as pooled data for the blood donors and for the STI cohort, no stratification based on age or gender was performed. The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice.

**Commercial HSV-2 IgG antibody assays.** The HerpeSelect 2 IgG enzyme-linked immunosorbent assay (ELISA) (Focus2) (Focus Technology) was used for the detection of type-specific IgG antibodies against HSV-2. The assay was performed according to the manufacturer’s instructions. Samples giving index values of >1.1 were considered to be positive, while samples with index values of <0.9 were considered to be negative. Those samples showing repeated index values of ≤0.9 and ≤1.1 were considered to be equivocal and were excluded from the study. The Focus2 assay has been evaluated for sera from adult populations in Europe and the United States (15, 33). In those studies, the sensitivity varied from 94% to 100%, and specificity varied from 81% to 100%, depending on the population investigated.

**In-house HSV-2 assay.** An indirect ELISA, based on the mature portion of glycoprotein G of HSV-2 (mgG-2), was here designated mgG-2 ELISA. This assay was used as an additional assay for the detection of type-specific IgG antibodies against HSV-2. The assay was carried out as described recently (14). The performance of the mgG-2 ELISA has been evaluated for blood donors and an STI cohort resident in Tanzania, showing sensitivity and specificity of >90% (14).

**WB.** Western blotting (WB) is considered to be the “gold standard” for the detection of IgG antibodies against HSV-2. Antigens were prepared by infecting HEP-2 cells with a Swedish HSV-2 isolate, B4327UR, as described previously (23). Lysates of virus-infected cells were mixed with sample buffer containing 2% sodium dodecyl sulfate and 5% mercaptoethanol and subjected to polyacrylamide electrophoresis under reducing conditions by using NuPAGE 7% Tris-acetate gel (Novex). The proteins were then electrophoretically transferred onto an Immobilon-P transfer membrane (Millipore Corp.). Strips were washed for 30 min at 37°C by using Tris-buffered saline with 0.3% Tween 20 and blocked with 3% skim milk and 4% fetal calf serum in Tris-buffered saline (BLOC) for 30 min. Sera were added at a 1:100 dilution prepared in BLOC solution and incubated overnight at room temperature. An HSV-2-positive serum sample drawn from a culture-positive individual and a well-characterized anti-mgG-2 monoclonal antibody (O1.C5.B2) (24) were used for the correct identification of the carbohydrate-terminal intermediate portion of gG-2 and the mgG-2 protein. Peroxidase-labeled rabbit anti-human or rabbit anti-mouse IgG (Dako) at a 1:100 dilution in BLOC was used as the conjugate, and 4-chloro-1-naphthol was used as the substrate. A positive WB profile was defined as reactivity to mgG-2 (≥120 kDa) alone or in combination with the carboxyl-terminal intermediate (≥70 kDa).

**Interpretation of HSV-2 ELISAs.** All 309 sera were analyzed with both the mgG-2 ELISA and the Focus2 ELISA. Sera showing discordant results in both ELISAs were interpreted as true-positive or true-negative samples. Sera showing discordant results were resolved by WB.

**HPV ELISA.** IgG antibodies against HPV-16, -18, -31, -33, and -51 were detected by ELISA based on virus-like particles. The assay was performed as described previously, with modifications (19, 41). Briefly, wells of Polysorp microtiter plates (Nunc, Naperville, IL) were coated with virus-like particles of HPV-16 (66 ng/well), HPV-18 (50 ng/well), HPV-31 (30 ng/well), HPV-33 (20 ng/well), or HPV-51 (40 ng/well) in phosphate-buffered saline (PBS) (pH 7.4). The plates were blocked with 0.5% (wt/vol) polyvinyl alcohol (PVA), with a molecular weight of 30,000 to 70,000 (Sigma, St. Louis, MO), in PBS (0.5% PVA). Serum samples, diluted 1:100 in 0.5% PVA, were left to react for 1 h at 37°C. Antibogen-bound immunoglobulin (Ig) was detected with peroxidase-conjugated goat antibodies against human IgG (Zymed, San Francisco, CA) diluted 1:4,000 in a solution containing 0.5% PVA, 0.0025% Tween 20, and 0.8% (wt/vol) polyvinylpyrrolidone, molecule weight 360,000 (Sigma), in PBS. Color development was initiated by the addition of 2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonate) hydrogen peroxide solution (Kirkgaard & Perry, Gaithersburg, MD). The absorbance was measured at 490 nm with a reference wavelength of 700 nm in an automated microtiter plate reader (Molecular Devices, Menlo Park, CA). The cutoff value of the assay was determined by comparison with the distribution of values obtained for serum samples from 74 children, aged 4 to 5 years. An iterative statistical approach was used to exclude outliers in the distribution of control sera until no remaining values were greater than 3 standard deviations above the mean optical density value. Seropositivity was defined as 3 standard deviations above the mean optical density obtained for the negative control sera (minus outliers). The reported sensitivity of the HPV serology based on sera from HPV DNA-positive individuals varies from 65% to 75% (18, 39). The specificity of serological assays for genital HPVs has been estimated to be >98% (8). High specificity is supported by the following findings: (i) the low seroprevalence in children (1) and adolescent girls before sexual debut (3), (ii) stronger association of HPV seroreactivity with the detection of homologous HPV DNA in the genital tract than with the detection of DNA of other HPV types (44), and (iii) type-specific serocconversion in women with proven HPV-16 infection (6).

**Commercial C. trachomatis assay.** The microimmunofluorescence (MIF) test is considered to be the gold standard for chlamydial serological testing (42). IgG antibodies against *C. trachomatis* were assessed by the IgG Micro IF MIF test (Amp Lystems, Ltd., Oy, Vantaa, Finland). The assay is based on the broadly reactive serovar L2, which also cross-reacts with serovars D to K. As the type-common lipopolysaccharide component has been removed from the antigen, there is minimal cross-reactivity with other chlamydial species (5, 26). The assay was performed according to the instructions provided by the manufacturer using a cutoff titer of ≥32.

**Statistics.** Statistical calculations were performed with StatView 5.0 software using Fisher’s exact test and the chi-square test. A P value of <0.05 was considered to be statistically significant.

**RESULTS**

**Seroprevalence of HSV-2.** The HSV-2 seroprevalences for blood donors and patients with STI are shown in Fig. 1. Twenty-two samples were positive by both mgG-2 ELISA and Focus2, while 283 samples were negative by both assays. In addition, four sera showed discordant results between the mgG-2 ELISA and Focus2 assays. These sera were resolved by WB, resulting in three additional HSV-2-positive samples, giving a total seroprevalence of 8% (25/309) for all three cohorts. There was a statistical difference in HSV-2 prevalences between the two cohorts in that HSV-2 infection was more common in the STI cohort (15/110; 14%) than in blood donors (10/199; 5%) (P = 0.015).

**Performance of type-specific HSV-2 serology.** The performances of mgG-2 ELISA and Focus2 were evaluated based on the seroprevalence data presented in Fig. 1. Four sera presented discordant results. Two sera were Focus2 positive/mgG-2 ELISA negative and positive by WB, one serum sample...
was Focus2 positive/mgG-2 ELISA negative and negative by WB, and one serum sample was Focus2 negative/mgG-2 ELISA positive and positive by WB. Thus, mgG-2 ELISA presented two false-negative samples, while Focus2 presented one false-negative and one false-positive sample. As shown in Table 1, both ELISAs showed high performances, with sensitivities and specificities as well as positive predictive values and negative predictive values of ≥92%, with no significant differences between the assays.

**Seroprevalence of HPV.** The seroprevalences of the five oncogenic HPV types (HPV-16, -18, -31, -35, and -51) were evaluated by using HPV ELISA. Data for all HPV types are shown in Fig. 2. Antibodies against any of the five investigated HPV types were detected in 107/309 (35%) sera (data not shown). HPV of any type was more common in the STI cohort (52/110; 47%) than in blood donors (55/199; 28%) (P = 0.0007). Similarly, antibodies against HPV-16 were more common in the STI cohort (41/110; 37%) than in the blood donors (30/199; 15%) (P < 0.0001). In the STI cohort, the seroprevalence of HPV-51 was significantly lower than that detected for HPV-16. This was in contrast to the seroprevalence results from blood donors, where HPV-51 seroprevalence (34/199; 17%) was similar to that found for HPV-16 (30/199; 15%). Also, the seroprevalence of HPV-51 was significantly higher than that for HPV-18 in blood donors (14/199; 7%) (P = 0.003) and in the STI cohort (9/110; 9%) (P = 0.012). HPV-31 and HPV-35 were less common, showing seroprevalences of ≤12% for both cohorts. For all sera, a statistical trend was seen between seropositivity against HSV-2 and one or more HPV types (chi square, 3.6; P = 0.057).

Antibodies against multiple (more than one) HPV types were found in 59/107 (55%) HPV-positive sera. Two types were detected in 41/59 (69%), three types were detected in 14/59 (24%), and four types were detected in 4/59 (7%) multiply infected patients. Single infection with HPV-16, -18, -31, -35, or -51 was present in 19/107, 6/107, 11/107, 3/107, and 9/107 HPV-positive individuals, respectively (Fig. 3). Thus, we conclude that a majority of HPV-infected individuals have been infected with two or more HPV types.

**Seroprevalence of C. trachomatis.** Blood donors and the STI cohort were assayed for the presence of IgG antibodies against C. trachomatis by MIF. IgG antibodies against C. trachomatis were detected in 6% of blood donors and in 13% of the STI cohort (P value was not significant) (Fig. 1).

**DISCUSSION**

There are several factors affecting the seroprevalence in a population, such as the inclusion of individuals from different geographical regions, selection of the study population, age, gender, and the performance of the assay used (for a review, see reference 25). Direct comparisons of data from different studies may therefore be misleading. With these limitations in mind, we found that the seroprevalence of HSV-2 infection in blood donors (5%) was lower than or similar to the seroprevalence in populations in northern Poland (8.6% to 10.7% in women and 3.5% to 12.6% in men) (37) by using the same type-specific HSV-2 assay (Focus2). As described previously in other studies from different countries (4, 14, 29), the seroprevalence of HSV-2 infection in the STI cohort included here was significantly higher (14%) than that described for blood donors.

To our knowledge, there are no reports on HPV seroprevalence from Poland. This study presents seroprevalence data for five oncogenic HPV types (types 16, 18, 31, 35, and 51). Although the HPV ELISA presents a relatively low sensitivity (65% to 75%) (8, 18, 39), the seroprevalence for any HPV type was higher than that for HSV-2 and C. trachomatis (Fig. 2).

Thus, based on serology, HPV infections are overall the most common STIs in the Katowice region. As expected, HPV infection was significantly more common among the STI cohort than among the blood donors. Compared with data from the United States, Finland, and Sweden (2, 9, 21), we report similar seroprevalences for HPV-16 and HPV-18 in Poland. Consequently, our data suggest that HPV infections are more widespread than HSV-2 and C. trachomatis in Katowice. The reason for this is unknown but may be explained by a higher transmission rate for HPV than for HSV-2 and C. trachomatis and/or that transmission may occur through nonpenetrative sexual activity (46).

Surprisingly, we found that HPV-51 infections among blood donors were as common as HPV-16 infections. For the STI cohort, HPV-51 was the second most common HPV type next to HPV-16. These findings are in accordance with seroepidemiological data from a survey of women aged 14 to 59 years in

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**TABLE 1. Performance of mgG-2 ELISA and the Focus2 assay**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focus2</td>
<td>96.0</td>
<td>99.6</td>
<td>99.6</td>
<td>96</td>
</tr>
<tr>
<td>mgG-2 ELISA</td>
<td>92.0</td>
<td>100</td>
<td>99.3</td>
<td>100</td>
</tr>
</tbody>
</table>

*a Based on concordant results from Focus2 and mgG-2 ELISA and by reactivity in WB for discordant sera.

*b Negative predictive value (NPV) is calculated as true negative/(true negative + false negative).

*c Positive predictive value (PPV) is calculated as true positive/(true positive + false positive).
Moreover, data from worldwide observations showed that HPV-51 was the third most common HPV type, occurring in 11% of women with low-grade squamous intraepithelial lesions (7). Although infections with HPV-51 are relatively common, this HPV type seems to be less oncogenic (7), a suggestion that is supported by the finding that HPV-51 was the 11th most common HPV type, with an estimated contribution to cervical cancer of only 1% (27). Currently, there are no data indicating an increase in cervical cancer caused by HPV-51. However, as only the oncogenic HPV-16 and HPV-18 are included in the newly introduced HPV vaccines, it is possible that type-restricted protection might lead to the expansion of other oncogenic HPV types (35). From this point of view, HPV-51 infections as well as the relative contribution to cervical cancer may increase in the future. We therefore conclude that follow-up studies on HPV-51 seroprevalence as well as the occurrence of HPV-51 in cervical cancer are warranted, especially in HPV-vaccinated populations. Antibodies against multiple HPV types were detected in 55% of HPV-positive patients (Fig. 3). This finding is in agreement with other seroepidemiological studies where a majority of HPV-infected women harbored antibodies against multiple HPV types (43). Although antibodies against HPV persist for a relatively long time, reflecting cumulative HPV infections for the individual, we could not distinguish between multiple infections or infection by more than one HPV serotype at a time.

In our study, the seroprevalence of *C. trachomatis* among blood donors (6%) was lower than that described previously for pregnant women in Poland based on the *Chlamydia* IgG immunoassay (14.5%) (31). The difference may be caused by differences in the cohorts studied and/or in the different methods used for detection. The performances of several commercially available *Chlamydia* antibody tests have been evaluated recently (20). From this comparison, it was obvious that the performance varies considerably between different tests. One conclusion is that the cohorts studied and the detection method used must be identical to provide a direct comparison.

To our knowledge, the MIF test described here and used for the detection of IgG antibodies against *C. trachomatis* has not been evaluated previously for blood donors and STI cohorts. However, the same assay has been used for a cohort of 149 asymptomatic women who were screened for *C. trachomatis* infection by PCR. By using a cutoff value of ≥16, 32 of 43 (74%) women with a PCR-positive cervical sample were shown to have IgG antibodies against *C. trachomatis* (26). This finding together with earlier studies indicates that not all *C. trachoma*-infected individuals present detectable anti-*C. trachoma*

![FIG. 2. Seroprevalences for HPV-16, -18, -31, -35, and -51 in blood donors (BD) and in patients with STIs.](http://cvi.asm.org/)
tis IgG antibodies (32). In this study, we used a cutoff value of \( \geq 32 \), suggesting that the sensitivity of the MIF test may be lower. Anti-\( \text{C. trachomatis} \) antibodies are known to persist for many years after infection (13). These observations together with the somewhat low sensitivity of the MIF test suggest that the prevalence of \( \text{C. trachomatis} \) infection in seroepidemiological studies is probably underestimated. The prevalence of \( \text{C. trachomatis} \) infection in pregnant Polish women, detected by a direct antigen method, is low (1.8%) (22). As the correspond-

trachomatis infection in pregnant Polish women, detected by a
the prevalence of \( \text{C.}
lower. Anti-
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\text{tis} \text{IgG} \text{antibodies} (32). In this study, we used a cutoff value of

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