# SEASONAL FLUCTUATIONS IN THE CERVICAL SMEAR RATES FOR (PRE)MALIGNANT CHANGES AND FOR INFECTIONS: A MIRROR-LIKE PATTERN OF DATA FROM THE NORTHERN AND THE SOUTHERN HEMISPHERE

W.J. Rietveld[1]

M.E. Boon[2]

J.J. Meulman[3]

[1] Department of Physiology, Division of Medical Chronobiology, Leiden University, P.O. Box 9604, 2300 RC Leiden, The Netherlands

[2] Leiden Cytology and Pathology Laboratory, P.O. Box 16084, 2301 GB Leiden, The Netherlands

[3] Department of Data Theory, Faculty of Social Sciences, Leiden University, P.O. Box 9555 2300 RB Leiden, The Netherlands

**Abstract**

Many forms of health and diseases exhibit seasonal fluctuations. In the present study, seasonal fluctuations are studied in cervical smears. Over a 9-year observation span (January 1983-January 1992) a series of 504,093 cervical smears obtained from a routine cytology laboratory in the Netherlands and a series of 2,405,271 cervical smears obtained from an equivalent laboratory in Australia, were examined for infections (Monilia, Trichomonas, Actinomyces, Human papilloma Virus (HPV), Chlamydia and Herpes) as well as for mild, moderate, and severe dysplasias, carcinoma in situ, and squamous carcinoma.

Statistical analysis (principal component analysis) demonstrates clear seasonal rhythms in the incidence of infections and precursor lesions. Comparing the data from the Northern hemisphere with those from the Southern, we found that the various infections and precursor lesions exhibit seasonal rhythms that are fully mirror imaged. The order in which the various infections and (pre)malignancies occur during the year is the same in both hemispheres. These findings suggest that we are dealing with "true" rhythms. To the best of our knowledge this is the first time that mirror-like fluctuations in the Northern and Southern hemisphere have been shown.

Keywords: Seasonal rhythms; Cervical carcinoma; Screening.

## Introduction

Rhythm is a fundamental characteristic of life. From the single-celled creatures to the higher primates, form and function are subjected to periodic changes which, in the end, cause the organism to synchronize with the geophysical rhythms of the surrounding environment. The change from day to night, from season to season - these can all be detected in living organisms on the Earth. These rhythms are endogenous with period lengths slightly deviating from those of the environment. This implicates that synchronizing signals from the surrounding (light, temperature), entrain the rhythms exactly to the environment, each day, each season.

Daily, or circadian, rhythms are well known. Sleep wakefulness, deep body temperature, and hormone levels fluctuate with a 24 hours pattern. To date we know that seasonal rhythms are also present in animal and man. We all know that SAD (seasonal affective disorders) only occur during fall and winter. Many infectious diseases, especially viral ones, are often seasonally bound. Also genital tract infections detected in cervical smears of women who were either subjects of cytology screening programs or visited a general practitioner (GP) during birth control, fluctuate with the seasons. Sodhani et al. (1) studied the smears of 62,757 women in Delhi (India), and found that Trichomonas vaginalis showed a high incidence in winter (November-February) and a low incidence in summer (March-June). The rainy season (July-October) gave intermediate values. In this case the yearly and exogenous rhythmicity of the monsoon might interact with the endogenous rhythm of the immune system. The detection of cervical cancer also proves to be seasonally bound. Hermida et al. (2), report a yearly pattern in the incidence of uterine cervix cancer for data obtained in Mexico for a

time span of 10 years (1978-1987). The incidence between the end of fall and the beginning of winter was higher than in the rest of the year.

No data are yet available on seasonal patterns detected through cervical screening in countries with a more moderate climate and with more gradual changes of the various seasons over the year. The study reported in this paper was undertaken to document a seasonal variation in genital infections as detected in cervical smears and for precursor lesions of cervical carcinoma in the Netherlands where summer and winter are separated by spring and fall, each lasting about the same amount of time. To compare the data from the Northern hemisphere with those from the Southern hemisphere, data have been collected as well from Southern Australia at a comparable latitude.

**Materials and Methods**

Over a 9-year observation span (January 1983-January 1992) a series of 504,093 cervical smears obtained from a routine cytology laboratory in the Netherlands and a series of 2,405,271 cervical smears obtained from an equivalent laboratory in Australia, were examined for infections (Monilia, Trichomonas, Actinomyces, Human Papilloma Virus (HPV), Chlamydia and Herpes, as well as for mild, moderate, and severe dysplasias, carcinoma in situ and squamous carcinoma. Diagnoses were based on the criteria described in Boon and Suurmeijer (3). The data needed for the analysis were obtained from a computerized database used in both laboratories. Mild, moderate, and severe dysplasias were grouped into "dysplasia" (DYS), and the carcinomas in situ and invasive carcinomas as "Carcinoma" (CAR). The data were first normalized for factors influencing the number of screenings per month by expressing the rates observed as those per 1,000 smears. At this point, the data still contain various sources of variation.

Rates vary per year, per type of infection, and per country. Because we are not interested here in these particular differences, but primarily in differences between seasons in the Netherlands and in Australia, rates were normalized per infection over each 12 month period to have a total sum of 1.0. Finally, normalized rates were averaged over nine years and four seasons. In the Netherlands, winter has been defined from January - March, spring from April - June, summer from July - September, and fall from October - December. In Australia, winter is from July - September, and the other seasons have been composed accordingly.

The null-hypothesis of nonexistent seasonal effects is expressed by defining expected infection rates that are equal for all seasons. The differences between observed and expected rates are the data to be analyzed. To reveal the structure among the four seasons on the one hand, and dysphasia, carcinoma, and the five infections (Herpes was omitted because of extreme small frequency) on the other hand, a Principal Component Analysis (PCA) was applied to the (observed minus expected) data. The present data can be perfectly displayed in three dimensions; PCA finds a solution in fewer dimensions, maximizing the proportion of total variance accounted for.

In addition, we could stratify the Australian data according to age groups in the following way: <20, 20-25, 25-30, 35-40, 40-45, 45-50 and >50 years of age. Accordingly, we were able to establish for these age groups relationships between type of infection (Manila, Trichomonas, Actinomyces, Human Papilloma Virus (HPV), Chlamydia and Herpes) and (pre)malignancy. This was necessary to exclude influence of referral patterns and access to care in relation to age.

**Results**

Table 1 pictures the raw data from the Netherlands and Australia. The level in both countries differs for the infections and cervical (pre)neoplasias under consideration. Trichomonas, Actinomyces and Chlamydia are higher in the Netherlands, whereas Monilia and HPV are higher in Australia.

Table 1. Raw data (averages over years; sums within seasons; rates per 10,000).

| | TRI | MON | ACT | HPV | CHL | DYS | CAR | |
|---|---|---|---|---|---|---|---|---|
| | | | | Netherlands | | | | |
| WIN | 272 | 598 | 283 | 113 | 75 | 402 | 36 | Jan, Feb, March |
| SPR | 241 | 520 | 286 | 126 | 46 | 448 | 45 | April, May, June |
| SUM | 232 | 660 | 267 | 186 | 71 | 551 | 56 | July, August, Sept |
| FAL | 258 | 661 | 284 | 147 | 90 | 446 | 37 | Oct, Nov, Dec |
| | | | | Australia | | | | |
| WIN | 114 | 1114 | 92 | 636 | 13 | 457 | 53 | July, August, Sept |
| SPR | 103 | 1209 | 92 | 695 | 13 | 496 | 55 | Oct, Nov, Dec |
| SUM | 117 | 1383 | 91 | 738 | 16 | 513 | 54 | Jan, Feb, March |
| FAL | 114 | 1163 | 86 | 625 | 14 | 472 | 56 | April, May, June |

In Figure 1, two typical examples are given: of data on dysplasia in the Netherlands and on monilia in Australia, comparing summer and winter over the various years. Although the level fluctuates, there are systematic differences between summer and winter scores.

Since we are interested in the relative differences 1) between infections, dysplasia and carcinoma scores in different seasons and 2) between scores in the Netherlands and Australia, the data have been normalized. In each year proportions were normalized to a total of 1.00.

After the expected (equal) frequencies were subtracted from the normalized data, PCA was applied in two dimensions. The data for the Netherlands and Australia were analyzed separately. The PCA solution assigns scores to the seasons, and scores to Monilia (MON), Trichomonas (TRI), Actinomyces (ACT), Human Papilloma Virus (HPV), Chlamydia (CHL), dysplasia (DYS),

and carcinoma (CAR); these scores can be used to display the analysis results graphically. The solution for the Netherlands is given in Figure 2, displaying 99% of the total variance in the table.

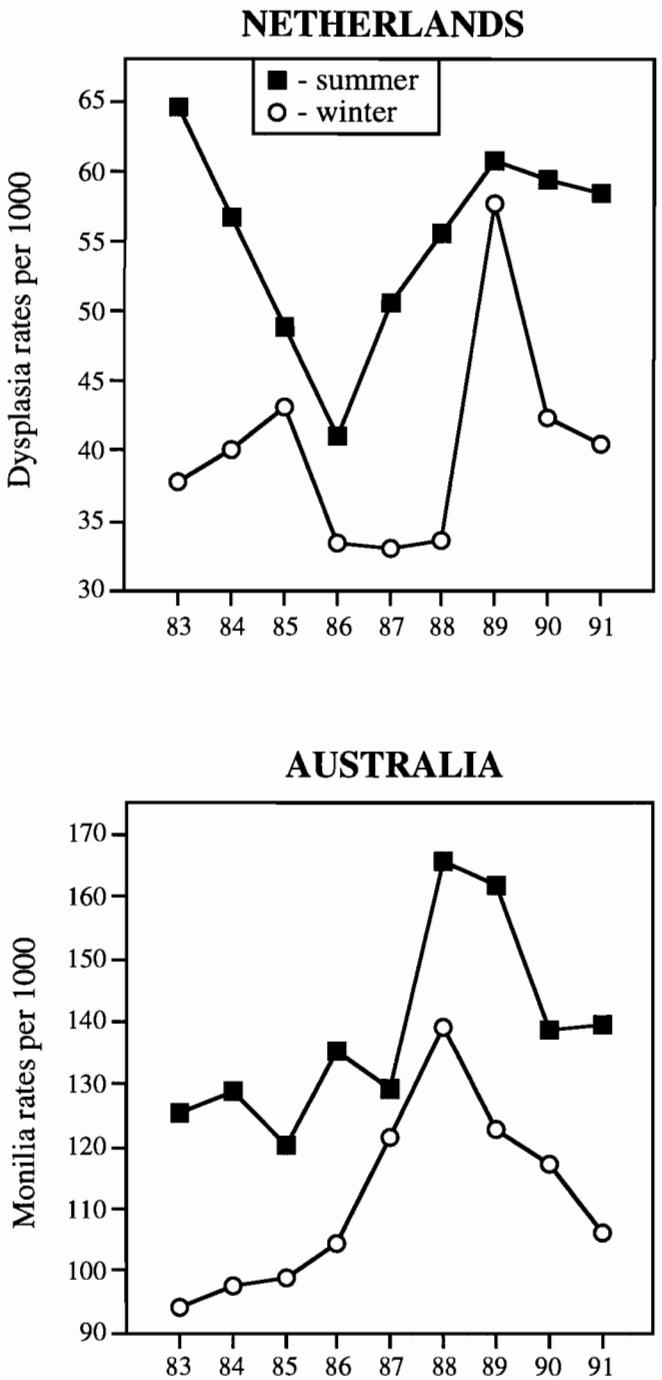


Figure 1. Typical examples of rates per 1,000 in summer and winter over a nine-year period.

First we describe the nature of the display. The points are located in such a way that the distances from the center of the graph, the origin, indicate the size of the seasonal variation. ACT and TRI, for example, have little seasonal variation, and are located close to the origin. In contrast, CHL, HPV and CAR have large seasonal variation. Arrows have been drawn through the points for the five infections and DYS and CAR, and the origin. The orthogonal projections of the season points on an arrow, represented by the lower case symbols w (for winter), p (spring), s (summer), f (fall), indicate the relative frequency of occurrence.

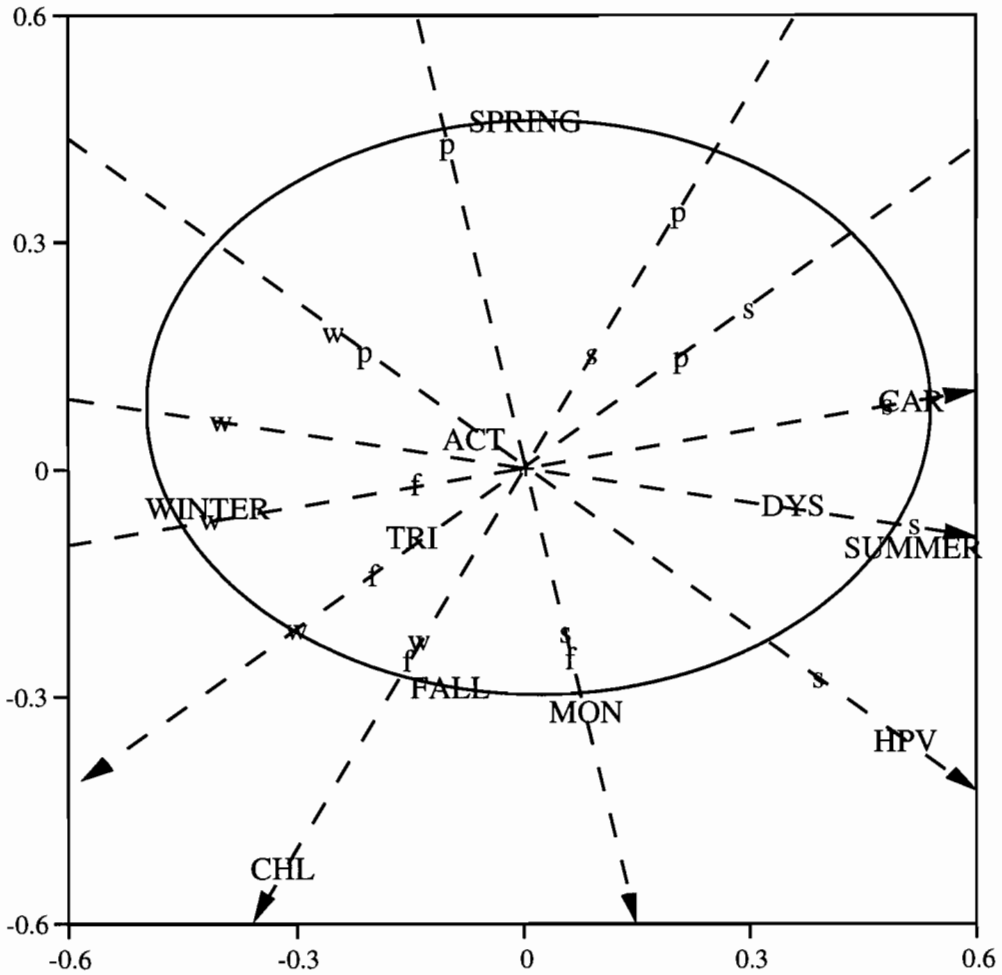


Figure 2. Graphical display of the analysis results for the Dutch data. The emerging seasonal cycle is expressed by an ellipse, starting with SPRING at the top, and going clockwise crossing SUMMER, FALL and WINTER.

Labels were omitted if the projection was very close to the origin (indicating scores close to the average of 0.0); the closer a label to the arrow head, the greater the frequency of occurrence.

The solution has revealed a winter versus summer dimension (the horizontal axis) and a spring versus fall dimension (on the vertical axis). this empirical seasonal structure has been emphasized by connecting the season points in the figure through an ellipse. The arrows, (the infections, DYS and CAR), appear to cross this ellipse always in between two seasons. Starting at the bottom-left, the figure shows that CHL occurs most in fall and winter (and less than average in summer and especially spring), MON in fall and summer (and not in spring), HPV especially in summer (and not in spring and winter) and DYS and CAR especially in summer (and not in winter).

The analysis results of the data for Australia are graphically represented in Figure 3, accounting for 95% of the total variation in the table. Here too a winter versus summer dimension and spring versus fall dimension has been found, but winter and fall are closer than in the solution for the Netherlands. In the Australia data, there is no seasonal effect for CAR, and little for ACT. By contrast, TRI occurs most in fall (followed by winter and summer), and noticeably less in spring. As was true for the Netherlands, MON HPV, DYS and CHL also occur most frequently in Australia in summer, and the frequency is noticeably less in winter.

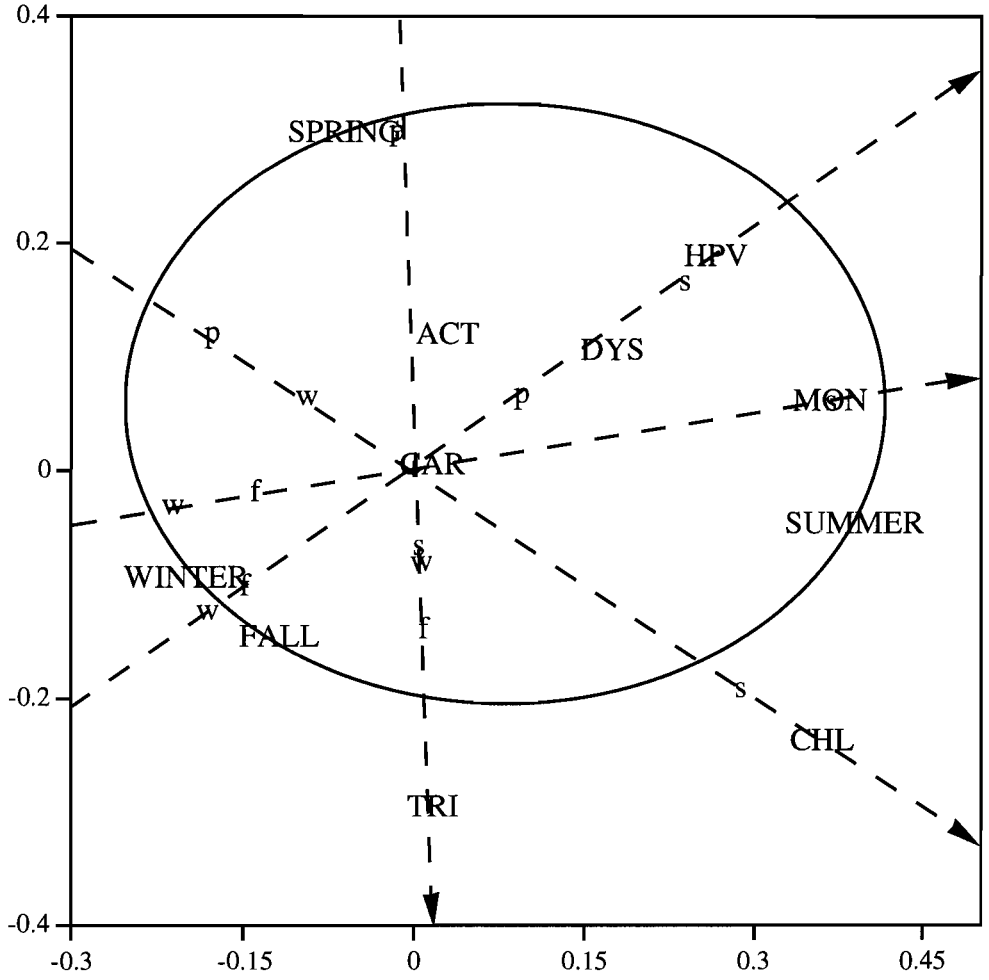


Figure 3. Graphical display of the analysis results for the Australian data. Similar seasonal structure as in the analysis of the Dutch data, as expressed by an ellipse.

Table 2 summarizes the cycles for infections and (pre)neoplasias that emerged from the analyses of the Dutch and the Australian data. Infections, DYS, and CAR have been ordered in Table 2 according to their position in the Figures 2 and 3. The seasonal structure was followed clockwise, starting in summer with CAR in the Netherlands and with DYS in Australia, and CAR-H indicates high occurrence. At the bottom of the upper part of the table we find TRI-L, indicating a low occurrence for Trichomonas. The order in which the various infections and (pre)neoplasias occur during the year is remarkably similar in both countries.

The lower part of Table 2 reports on the statistical significance of the contrasts found in the analyses. The p-values were obtained by a permutation procedure. The differences between the seasons were computed (after the data were aggregated as described in the materials and methods section) after assigning each month randomly to one of the four seasons, and this process was repeated 10,000 times. The contrasts in the analyses are significant when the values of the statistic under the hypothesis of randomness are larger than the actual observed values in less than 500 out of the 10,000 cases, corresponding to the conventional p-value of 0.05.

Table 2. Cycles and contrasts for infections and (pre)neoplasias that emerged from the analyses.

| | | NETHERLANDS | AUSTRALIA |
|---|---|---|---|
| CAR | –H | summer | – |
| DYS | –H | summer | summer |
| HPV | –H | summer | summer |
| MON | –H | summer/fall | summer |
| CHL | –H | fall/winter | summer |
| TRI | –H | winter | fall |
| CAR | –L | winter | – |
| DYS | –L | winter | fall/winter |
| HPV | –L | winter/spring | fall/winter |
| MON | –L | spring | fall/winter |
| CHL | –L | spring | winter/spring |
| TRI | –L | spring/summer | spring |

| | NETHERLANDS | | |
|---|---|---|---|
| | HIGH | LOW | |
| TRI | winter | summer | p = .002 |
| MON | fall | spring | p = .002 |
| ACT | winter | summer | p = .094 |
| HPV | summer | winter | p = .001 |
| CHL | fall | spring | p = .001 |
| DYS | summer | winter | p = .001 |
| CAR | summer | winter | p = .002 |
| | AUSTRALIA | | |
| TRI | fall | spring | p = .011 |
| MON | summer | winter | p = .001 |
| ACT | spring | fall | p = .070 |
| HPV | summer | winter | p = .004 |
| CHL | summer | spring | p = .044 |
| DYS | summer | winter | p = .000 |
| CAR | summer | winter | p = .371 |

To compare the magnitude of the difference between high and low occurrence, the aggregated original data were normalized to have an average score in winter of 100 (see Table 3). In those cases where a statistically significant rhythm was found, the differences between the maximum and the minimum in the original data ranged from 15% (TRI) to 58% (CHL) in the Netherlands, and from 12% (DYS) to 25% (CHL) in Australia. Overall the rhythms were much more pronounced in the Dutch data.

Table 3. Normalized data (average score in winter set to 100).

| | TRI | MON | ACT | HPV | CHL | DYS | CAR | |
|---|---|---|---|---|---|---|---|---|
| | | | | Netherlands | | | | |
| WIN | 100 | 100 | 100 | 100 | 100 | 100 | 100 | Jan, Feb, March |
| SPR | 89 | 87 | 101 | 111 | 61 | 112 | 125 | April, May, June |
| SUM | 85 | 110 | 94 | 164 | 94 | 137 | 157 | July, August, Sept |
| FALL | 95 | 110 | 100 | 130 | 119 | 111 | 103 | Oct, Nov, Dec |
| | | | | Australia | | | | |
| WIN | 100 | 100 | 100 | 100 | 100 | 100 | 100 | July, August, Sept |
| SPR | 90 | 109 | 100 | 109 | 96 | 109 | 103 | Oct, Nov, Dec |
| SUM | 103 | 124 | 99 | 116 | 121 | 112 | 102 | Jan, Feb, March |
| FALL | 100 | 104 | 94 | 98 | 105 | 103 | 104 | April, May, June |

Table 4. Normalized data Australia (average percentage for each age group in winter replaced by the score 100).

| | <20 | 20-25 | 25-30 | 30-35 | 35-40 | 40-45 | 45-50 | >50 | |
|---|---|---|---|---|---|---|---|---|---|
| WIN | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | July, August, Sept |
| SPR | 98 | 99 | 99 | 99 | 100 | 100 | 99 | 105 | Oct, Nov, Dec |
| SUM | 102 | 103 | 105 | 101 | 97 | 95 | 96 | 99 | Jan, Feb, March |
| FALL | 97 | 96 | 97 | 100 | 101 | 102 | 101 | 106 | April, May, June |

To evaluate the possible effect of age differences in the population of women screened during the various seasons, the percentages of women in each group (Australian data) were compared on the basis of normalized data with the average percentage for each age group in winter replaced by the score 100. The results are given in Table 4.

In the age groups up to 35 years, the largest positive difference is always between the scores for summer and fall, and amounts at most to 8%, which is for women in the age group 25-30. This percentage is much less than the seasonal fluctuations in amplitude that were observed for the infections and (pre)neoplasias in Table 3. Thus, we conclude that the latter fluctuations cannot be dominantly attributed to the larger occurrence of women in younger age groups in the population of women screened in summer.

To confirm this conclusion, percentages of women in the age group 25-30 screened in summer were associated for each year with the rate per 1,000 for the infections MON, HPV and CHL, which have a high occurrence in summer. This relationship is graphically displayed in the Figures 4, 5 and 6.

The horizontal axis displays the percentage of women in the age group 25-30 screened in summer. The nine years are thus reordered according to an ascending percentage of the 25-30 age group over these years. The vertical axes displays the incidence of MON, HPV, and CHL, respectively. Such graphs should display monotonic functions if higher infection rates in summer were caused by the fact in that particular season a higher percentage of younger women were screened.

It is clear from the completely nonlinear curves that there is no evidence for the hypothesis of an influence in referral pattern and access to health care related to age.

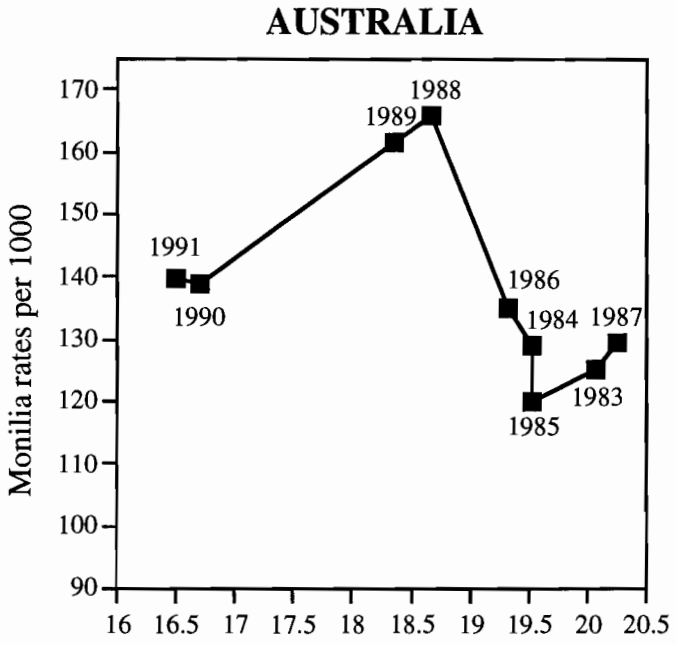


Figure 4. Infection rate for Monilia (high occurrence in summer) displayed at vertical axis. Rates for nine years versus percentages of women in the age group 25-30 in the population of women screened in summer.

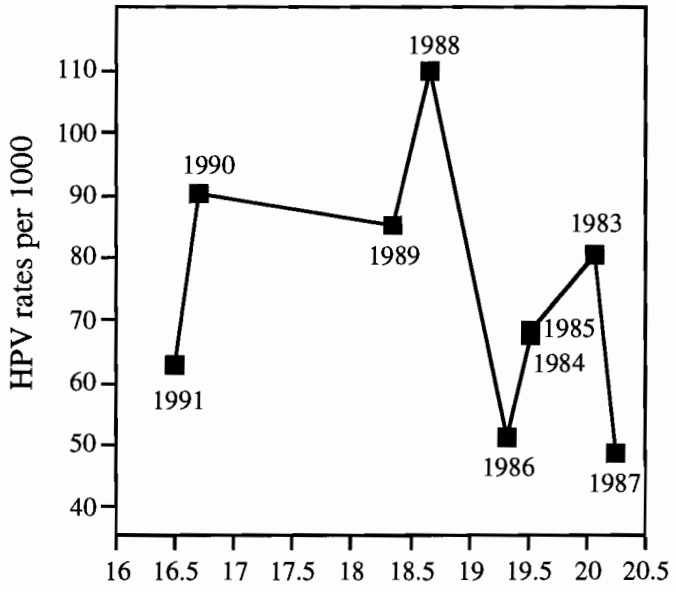


Figure 5. Infection rate for HPV (high occurrence in summer) displayed at vertical axis. Rates for nine years versus percentages of women in the age group 25-30 in the population of women screened in summer.

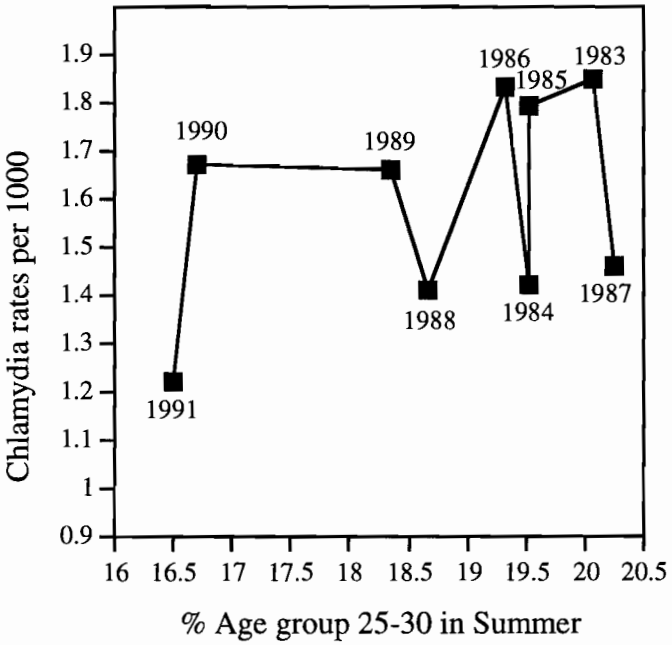


Figure 6. Infection rate for Chlamydia (high occurrence in summer) displayed at vertical axis. Rates for nine years versus percentages of women in the age group 25-30 in the population of women screened in summer.

**Discussion**

The data show clear seasonal effects, in the incidence of infections as well as in precursor lesions. Comparing the data from the Northern hemisphere with those from the Southern it is striking to note that the various infections and precursor lesions exhibit mirror-like seasonal rhythms; the order in which the various infections and (pre)malignancies occur during the year is the same in both hemispheres. These findings suggest that we are dealing with "true" rhythms.

The question arises to what extent these seasonal fluctuations reflect an endogenous rhythmicity, or are due to masking by either the environmental changes, like changes in temperature and humidity during the year, or to differences in sexual behavior. Another possibility is that general factors rather than biological ones are involved, which are associated with the health behavior of women and the health services (such as holidays). An argument against such nonbiological effects is the fact that the measured rates for infections (MON, HPV and CHL) are not correlated with the percentage of women in a particular age group studied. The last factor that might be involved is a seasonal change in attention of the screeners. An argument against this hypothesis is the detection of the various infections and precursor lesions peak in different seasons.

To date we are not able to explain the present seasonal fluctuations in infections and detection of (pre)malignancies. A rather complex interaction between host and infective agents, immunological factors, exfoliation pattern of the (pre)malignant cells, etc., could be involved. As mentioned before, data on possible seasonal rhythms in the detection of (pre)malignancies of the cervix are not published yet for countries with four seasons. For other malignancies scare, but convincing data are available. For example, Sankila et al. (4) state that the month of diagnosis in female breast cancer patients is a significant prognostic factor. After correction for age, period, and stage, an excess risk of death was higher when cancer was

diagnosed in July and August. The lowest risk occurred in March and November. Similar results were obtained while studying the survival pattern of colorectal cancer.

On the other hand, Joensuu and Toikkanen (5) describe differences in histological appearance of invasive breast cancer throughout the year; two series of unilateral breast cancer were studied, a first one with data from 1945-1965, and a second one from 1980-1984. In the first series mortality was greater in January, February, and August to October, in the second series mortality was greater in July to September. During the unfavorable months, the cancer had more tumor necrosis, a higher mitotic count and a larger tumor size. Similar data were obtained by Mason et al. (6, 7). According to these authors, the increased incidence of detection reflects cyclic influences on tumor growth due to hormonal fluctuations.

Holdaway et al. (8) attempt to relate the changes in the detection of breast cancer to changes in the plasma melatonin levels. Winter detectors of breast cancer showed an abnormal reduction of serum melatonin. The relative normal seasonal profile of summer detectors could be related to an increased ovarian steriodgenesis in spring and summer resulting in an increase in tumor growth. Our analysis proves that it is possible to detect mirror-like seasonal fluctuations in the Northern and the Southern hemisphere. To the best of our knowledge this is the first time that this has been shown.

**Acknowledgment**

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