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# A prospective study of influenza infections during pregnancy

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SUMMARY Serological evidence of infection with influenza A and B viruses was sought during three successive winters. Paired sera from 1595 pregnant women were studied and 79 infections occurred in 77 women (4·8%). A further 77 women who had no serological evidence of recent influenza infection were selected from the study population to serve as a control group. Cases and controls were comparable with respect to age, race, marital status, and number of previous pregnancies. Their offspring had virtually identical mean birth weights, skull circumferences, lengths, and incidences of neonatal jaundice. Although all the infections occurred in either the second or the third trimesters of pregnancy, the cases delivered more babies with congenital abnormalities than did the controls. The possibility was considered that the presence of an abnormal fetus made these women more susceptible to influenza infection. Unexpectedly, the women experiencing influenza infection during pregnancy delivered an excess of male babies, and an excess of females was born to the controls. Although this difference was statistically highly significant ($P < 0{\cdot}01$), a biological explanation for the results was not readily apparent and it is suggested that future studies of influenza during pregnancy should particularly look for evidence of an altered sex ratio.

Many untoward effects have been attributed to influenza infection during pregnancy including congenital abnormalities,[1 2] abortion or stillbirth,[3] low birthweight,[4] and even the subsequent development of leukaemia in the child.[5] Most of these studies have been epidemiological, so they have relied upon a clinical diagnosis of influenza. Such an approach would not only fail to document mild or asymptomatic infections with influenza viruses but, in addition, other respiratory viruses causing similar clinical syndromes would readily be confused with influenza infections.

In order to diagnose influenza infections more accurately, we decided to undertake a prospective study of pregnant women for serological evidence of recent infection with these viruses. It was hoped that an improvement in the diagnosis of influenza would clarify the postulated relationships between these viruses and neonatal problems, although it would be impossible to study any possible teratogenic effects of influenza because women generally do not present early enough to allow paired sera to be taken during the first trimester of pregnancy. However, the presence of congenital abnormalities was recorded because of the suggestion[6] that women bearing a malformed fetus may be more susceptible to infections with influenza viruses.

## Materials and methods

### *Population studied*

During this three-year study sera were obtained from all women booked for antenatal care at St. Bartholomew's Hospital and postpartum specimens were subsequently obtained from the majority. Additional sera taken at various gestational stages were also available from most women. All sera were stored at −20°C until required.

The study was restricted to women who were pregnant during the parts of the study years when influenza was prevalent by using epidemiological data provided in the Communicable Disease Reports of the Public Health Laboratory Service. The dates selected for study therefore varied from year to year and the study was enlarged each year as more experience was gained in the laboratory techniques required for the simultaneous assay of many hundreds of sera. In the first winter, women were

eligible for study if their last menstrual periods fell between 1 May 1975 and 1 November 1975. The corresponding dates for the second and third winters were 1 December 1976 to 30 June 1977 and 1 August 1977 to 30 April 1978. Women were studied only if a postpartum specimen had been received in the laboratory. It was not possible to determine why postpartum specimens had not been received from some women, so those who had abortions or stillbirths were not included in the study.

*Viruses*

The following strains of influenza A viruses were used: A/Victoria/3/75 ($H_3N_2$); A/Texas/1/77 ($H_3N_2$); and A/USSR/0090/77 ($H_1N_1$).

Only two strains of influenza B viruses were used: B/England/847/73 and B/Hong Kong/8/73.

*Serology*

The haemagglutination-inhibition (HI) test was performed by a standard microtechnique. Sera were pretreated with receptor-destroying enzyme from *Vibrio cholerae* (Wellcome Reagents Ltd.) to remove non-specific inhibitors, and with chicken erythrocytes to remove non-specific agglutinins. The treated sera were then titrated using eight haemagglutinating doses of each virus and 0·5% chicken erythrocytes. In each year of the study, all sera were titrated against each individual virus in a single batch.

Fourfold or greater increases in antibody titre between paired sera were taken to indicate infection with that virus. All of these infections were confirmed by retitrating freshly prepared sera at a later date. Fourfold decreases in antibody titre were not taken as evidence of recent infection.

*Selection of controls*

For each woman experiencing a confirmed influenza infection, the woman with the closest laboratory number who had no evidence of influenza infection was selected to act as a control. All control women possessed HI titres of less than 64 against all viruses in order to reduce the chances of their having had influenza in the first trimester.

*Clinical details*

Clinical information about the cases and controls was obtained by reviewing hospital records. All the pregnant women and their babies had therefore been seen by a large number of medical staff, but there is no reason to believe that cases had been treated any differently from the controls.

*Statistical analysis*

The $\chi^2$ test was used to assess the significance of differences between the cases and the controls.

## Results

*Number of women studied*

In the first year of the study, 445 women were eligible for inclusion because they were pregnant while influenza was prevalent. Twenty-two of these women aborted and two had stillbirths. Postpartum specimens were obtained from only 251 (59·6%) of the remaining 421 women. Of the 519 eligible women in the second year of the study, 25 aborted and three had stillbirths. Postpartum specimens were obtained from 463 (94·3%) of the remaining 491 women. In the final year, 931 women were eligible, 32 aborted and there were five stillbirths. Postpartum specimens were obtained from 881 (98·5%) of the remaining 894 women. The shortfall in the receipt of postpartum sera in the first year was due to collection difficulties in the initial stages of the study.

*Susceptibility of the population to influenza infection*

Table 1 shows the number of women possessing HI antibodies against the several strains of influenza viruses tested. The vast majority of women (80-90%) lacked antibodies at a level usually associated with protection (>32) so they were probably susceptible to influenza infection.

*Incidence of influenza infection*

Table 2 shows that the number of women experiencing influenza infection during pregnancy was surprisingly high, with an overall infection rate of

Table 1 *The susceptibility of the pregnant women to influenza infection during the three successive years of the study*

| *Years sera taken* | *Challenge viruses* | *No. of women tested* | *Women with HI titres <32* | |
|---|---|---|---|---|
| | | | *No.* | *(%)* |
| INFLUENZA A VIRUSES | | | | |
| 1975–6 | Victoria/3/75 | 251 | 232 | (92·4) |
| 1976–7 | Victoria/3/75 | 463 | 406 | (87·7) |
| 1977–8 | Texas/1/77 | 881 | 701 | (79·6) |
| 1977–8 | USSR/0090/77 | 881 | 797 | (90·5) |
| INFLUENZA B VIRUSES | | | | |
| 1975–6 | England/847/73 | 251 | 226 | (90·0) |
| 1976–7 | Hong Kong/8/73 | 463 | 385 | (83·2) |
| 1977–8 | Hong Kong/8/73 | 881 | 806 | (91·5) |

5%. However, as expected, the incidence during each of the three years was very variable: 22% of women were infected in the first winter but less than 2% in the second or third winters. In each year, influenza A virus infections always predominated over influenza B infections.

*Women selected as controls*

As described earlier, these women were selected solely because they had no serological evidence of influenza infection during pregnancy. The two groups of women were virtually identical with respect to age, race, civil state, and number of previous abortions or viable pregnancies.

*Clinical findings*

Table 3 shows that a few antenatal differences were noted between cases and controls—for example, amniocentesis was performed because of a raised serum alphafetoprotein more frequently in the

Table 2 *The number of influenza infections detected serologically in the pregnant women during three successive winters*

| | | *Number (%) of influenza infections detected in pregnant women* | | | |
|---|---|---|---|---|---|
| | | *Influenza A* | | | |
| *Year of study* | *No. of women studied* | *$H_2N_2$ strains* | *$H_1N_1$ strain* | *Influenza B strains* | *Total* |
| 1975–6 | 251 | 43* (17) | nd | 13* (5) | 56 (22) |
| 1976–7 | 463 | 4* (0·9) | nd | 2* (0·4) | 6 (1·3) |
| 1977–8 | 881 | 13 (1·5) | 3 (0·3) | 1 (0·1) | 17 (1·9) |
| Total | 1595 | 60 (3·8) | 3 | 16 (1·0) | 79 (5·0) |

nd = not done
* One woman in each of two years was infected with both influenza A and influenza B viruses.

Table 3 *Clinical findings in the 77 women experiencing influenza infection, in the 77 control women, and in all of their offspring*

| | *Number (%) of women or babies in each group* | |
|---|---|---|
| *Clinical findings* | *Cases* | *Controls* |
| EVENTS DURING PREGNANCY | | |
| Nil | 34 (44·2) | 33 (42·9) |
| Amniocentesis performed due to raised AFP | 6 ( 7·8) | 1 ( 1·3) |
| Hypertension | 10 (13·0) | 18 (23·4) |
| General anaesthesia | 2 ( 2·6) | 7 ( 9·1) |
| Poor maternal weight gain | 4 ( 5·2) | 10 (13·0) |
| 'Influenza' reported | 12 (15·6) | 3 ( 3·9) |
| MODE OF DELIVERY | | |
| Uncomplicated | 55 (71·4) | 53 (68·8) |
| Forceps | 16 (20·8) | 15 (19·5) |
| Caesarean section | 6 ( 7·8) | 9 (11·7) |
| CONGENITAL ABNORMALITIES | | |
| None | 70 (90·9) | 75 (97·4) |
| Heart murmurs | 2 ( 2·6) | 0 |
| Deformities of lower limbs | 3 ( 3·9) | 0 |
| Miscellaneous | 2 ( 2·6) | 2 ( 2·6) |
| NUMBER OF SINGLETONS | 75 | 76 |
| SEX OF SINGLETONS | | |
| Male | 51 (68·0) | 33 (43·4) |
| Female | 24 (32·0) | 43 (56·6)† |
| NEONATAL JAUNDICE IN SINGLETONS | | |
| None recorded | 43 (57·3) | 47 (61·8) |
| Mild | 22 (29·3) | 22 (28·9) |
| Severe (serum bilirubin >200 $\mu$mol/l) | 10 (13·3) | 7* ( 9·2) |
| MEAN BIRTH WEIGHT OF SINGLETONS (kg) | 3·30 | 3·30 |
| MEAN SKULL CIRCUMFERENCE OF SINGLETONS (cm)** | 34·3 | 34·2 |
| MEAN LENGTH OF SINGLETONS (cm)†† | 50·7 | 51·1 |

† Statistically significant difference ($\chi^2$ = 9·24 0·001 <P <0·01).
* One baby had an ABO incompatibility with positive direct Coombs test.
** Figures recorded for only 50 cases and 37 controls.
†† Figures recorded for only 31 cases and 24 controls.

women experiencing influenza infection. However, all these differences were based on small numbers and none of them reached statistical significance. It was interesting that the history of recent 'influenza' was recorded in the notes of only 12 women; three controls also experienced 'influenza-like' illnesses.

There was a slight excess of congenital abnormalities in the cases because two babies had heart lesions (ventricular septal defect; systolic murmur not characterised due to lack of parental cooperation) and three had minor orthopaedic abnormalities (bilateral eversion of feet; left metatarsus varus; upturned fifth toes). The mothers of the babies with heart lesions experienced influenza between 16–40 and 11–38 weeks gestation respectively. The mothers of the other three babies were infected between weeks 25–40, 10–40, and 12–25 respectively. Two babies born to controls had minor congenital abnormalities (umbilical hernia; birthmark). These numbers were too small for statistical evaluation.

The mean birth weights of the cases and controls were identical, and an equal number of low-birthweight babies was born to each group. The mean skull circumferences and mean body lengths were also very similar although these figures had not been recorded for all babies. There was a slight excess of unexplained severe neonatal jaundice in the cases.

The only statistically significant difference between the cases and the controls was that a large excess of male children was born to the women who had influenza and more female children were born to the controls.

## Discussion

Several studies have associated influenza during pregnancy with many adverse effects, but it is difficult to imagine how a local infection of the maternal respiratory tract could damage the fetus. The demonstration that influenza viruses can, under certain circumstances, cross the placenta[7,8] provided one possible explanation. The increased consumption of drugs[2] during an influenza outbreak has provided another.

In this study we demonstrated that the majority of women of childbearing age are susceptible to influenza infection and that a surprisingly large number of women were infected during three winters when only comparatively small influenza epidemics occurred. Thus, even if these viruses only rarely damage the fetus, the total number of pregnancies at risk is very high.

We were unable to confirm the suggestion[4] that women who have influenza during pregnancy might produce babies with lower birth weights. The only statistically significant difference between the cases and the controls was that women who had influenza infections subsequently delivered more male babies and that women not infected with these viruses delivered more female babies. Although this difference was statistically highly significant, a biological explanation for these results was not apparent. It is known that the way in which a woman responds to infection with hepatitis B virus can affect the sex of her children[9] if the infection is established before conception. However, the influenza infections reported here occurred long after conception, so a similar mechanism cannot be invoked to explain our results. It is possible to speculate that maternal influenza infection could be more 'toxic' to female fetuses and thereby cause a differential abortion rate, but we have no results which could support this rather unusual suggestion. We do suggest, however, that future studies of influenza infection during pregnancy should particularly look for evidence of an altered sex ratio. An altered sex ratio was not detected in an earlier study by Coffey and Jessop,[1] but their influenza cases were not confirmed by laboratory tests.

In this study influenza infections were diagnosed serologically. It was therefore of interest that only 12 (15·6%) of the 77 women who experienced infection had this fact mentioned in their medical notes. Many women probably had asymptomatic or very mild infections, some may not have bothered to mention their symptoms to the clinic staff or, finally, the attending doctor may not have considered this information important enough to be recorded in the notes. However, this information was recorded for three control women despite the fact that they did not have serological evidence of influenza infection. This illustrates the fact that the clinical diagnosis of infection with influenza viruses is difficult, especially in non-pandemic years. Studies of pregnant women which have relied upon clinical diagnoses only may therefore have underestimated any adverse effects of influenza by failing to detect all cases and by including women not infected with influenza viruses. Conversely, the adverse effects noted might have been produced by another virus causing a febrile illness[10] and this intriguing explanation could provide one reason for our inability to confirm the adverse fetal effects attributed to influenza; it could also explain why an association[5] between maternal 'influenza' and childhood leukaemias has been confirmed by some workers[11] but not by others.[12]

Influenza infection during pregnancy has also been suggested as a cause of congenital abnormalities.[1,2] There was a slight excess of congenital abnormalities in our cases but the significance of this observation is uncertain because the infections occurred during the

second or third trimesters. The teratogenicity of influenza viruses could be investigated systematically only by detecting influenza infections in the first trimester of pregnancy. The taking of serial nasal swabs for virus culture is not practicable and it is not possible at present to diagnose first trimester infections serologically because women do not attend for antenatal care early enough to allow paired sera to be taken. Such an investigation will only become possible when a new pandemic strain appears, for it could then be reliably assumed that antibodies detected in booking sera against the new strain had been produced recently. It is therefore interesting to note that, when this approach was used during the 1957 pandemic,[3] congenital abnormalities could be statistically associated with influenza even when the infection occurred after the first trimester. One explanation that has been given[8] for this association is that a woman bearing a fetus destined to be born with a congenital abnormality may have an increased susceptibility to influenza infection. In view of the cases reported here, this is a concept which might profitably be pursued further.

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