DNA transfer during laundering may yield complete genetic profiles

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**A R T I C L E   I N F O**

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**A B S T R A C T**

In a number of child sexual abuse cases, the alleged perpetrator is a member of the nuclear family. In those cases, there is a possibility that the suspect’s DNA was innocently deposited onto the child’s clothing without acts of sexual assault ever occurring, for example via secondary transfer within the washing machine. To assess the quantity and quality of DNA that may be transferred among clothing during laundering, we conducted three series of experiments. First, we evaluated the level of spermatozoa that may be transferred by washing pristine pairs of underwear with bed sheets containing a varying number of ejaculates. Secondly, we explored whether current genetic methods may also detect the transfer of DNA from vaginal secretions during a machine wash. Finally, we analyzed the background levels of DNA on children’s underwear collected from control families where sexual abuse never occurred. For both spermatozoa and vaginal secretions, we revealed that sufficient amounts of DNA may transfer onto laundered clothing to yield complete genetic profiles. Furthermore, DNA from relatives living within the same household was found in most cuttings taken from control children’s underwear. Based on these findings, we present a framework for the handling and interpretation of intrafamilial sexual abuse cases. These suggestions should help determine whether DNA was deposited directly onto a fabric or merely transferred during a wash.

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**1. Introduction**

Shortly after a sexual assault event, medical professionals will gather forensic evidence using sexual assault kits, which are then sent to forensic laboratories for DNA analysis, and have proven invaluable for identifying and/or eliminating suspects. Although examinations are most successful when samples are collected within 24 h of an event, in some cases evidence may still be recovered up to seven days later [1–5]. In situations where children are sexually abused, the events are frequently revealed several days or weeks later, especially when intrafamilial sexual abuse had occurred [6]. Although sophisticated technology may still allow DNA evidence to be retrieved from a child’s body, the increased elapse of time between the event and when medical help was sought will nonetheless decrease the chances of identifying the perpetrator [7,8].

When physical evidence may no longer exist directly on the victim’s body, or when ejaculation did not occur in or on the body, alternate sources of evidence may be investigated to retrieve the perpetrator’s DNA. In child sexual abuse cases in particular, DNA evidence is often found on clothing and bedding rather than from internal bodily samples [1,2,4,7–9]. Samples of clothing yielding complete male genetic profiles within the sperm fraction may likely be interpreted as evidence of sexual assault, especially when found on the child’s undergarments. The increased sensitivity of today’s genetic analyses however, may consequently raise doubts as to the significance of these detected genetic profiles, especially in intrafamilial cases where DNA may be innocently deposited onto a victim’s clothing via numerous means within a shared living space. For example, Kafarowski et al. [10] demonstrated that a single pair of semen-stained panties could physically transfer spermatozoa to all the items it was laundered with. However, an in-depth examination at the possibility of obtaining genetic profiles from items of clothing after being washed among semen-stained items is still lacking.

In Québec (Canada), the Laboratoire de sciences judiciaires et de médecine légale, an accredited provincial forensic laboratory, received 1609 sexual assault cases in 2013–2014, of which 147 involved sexual abuse on children (defined as 10 years or younger).
In 46% of these cases, a member from the nuclear family (i.e., father, stepfather, brother or half-brother) was regarded as a suspect. Considering these numbers and the compelling evidence of spermatozoa transfer in the wash first reported by Kafarowski et al. [10], we thus assessed the amount of DNA that may be deposited onto a child’s underwear during the wash without acts of sexual assault ever occurring. To do so, we designed a series of tests divided into three major sections. We first assessed the level of spermatozoa transfer that occurred during laundering by washing pristine pairs of underwear with bed sheets stained with a varying numbers of ejaculates. Secondly, we explored whether current genetic methods may also detect the transfer of epithelial DNA in the wash, deposited by vaginal secretions. Finally, we analyzed children’s underwear collected from control families where no sexual abuse ever occurred.

These experiments are essential for understanding the background level of DNA that may be found on children’s clothing, and have strong implications towards the interpretation of DNA results gathered from cases of intrafamilial sexual abuse.

2. Methods

The bed sheets used in this study were brand new, white, flat, twin bed sheets made of 100% cotton or a 60% cotton/40% polyester blend (Table 1). Pristine underwear were purchased as 100% white, cotton, adult-sized panties. All items were washed and dried twice prior to conducting the experiments to help soften the fabrics. To confirm the origin of all the DNA profiles obtained, buccal swabs were collected from male and female donors, and from all members within the control families.

2.1. Spermatozoa transfer in laundry

In the first series of experiments, we used methods similar to that of Kafarowski et al. [10]; however spermatozoa transfer among items was assessed through DNA quantification and the resulting genetic profiles rather than through visual quantification of spermatozoa using microscopy. We recruited four male volunteers to deposit two, six or ten ejaculates onto seven different bed sheets via masturbation, oral sex or sexual intercourse (Table 1), and waiting at least 48 h between each ejaculate. Once the last ejaculate was deposited and dried for a minimum of 48 h, the bed sheet was then viewed with the Mini-CrimeScope® MCS-400 (SPEX Forensics) under a CSS wavelength, and all semen stains were counted and outlined with a felt-tipped marker. We then washed each individual sheet with five pairs of pristine panties in a top load washer under commonly-used laundering conditions to simulate normal familial scenarios: warm water (30 °C), a popular phosphate-free detergent (i.e., Sunlight®) and a cold water rinse. Items were then placed in the drier for 60 min under medium heat. The washer’s drum was immediately sampled following the four experiments involving six ejaculates on a bed sheet (A6, B6, C6, and D6) to help determine the quantity of sperm that may persist on a drum following a wash. Two separate swabs were each passed over the entire drum in a spiralling motion, starting from the top of the drum and swabbing progressively lower until reaching its base (or approximately ten rotations). After each individual experiment, a pristine bed sheet was washed to remove any spermatozoa that may have persisted on the washer’s interior drum.

For each of the seven bed sheet experiments, a 1 cm² cutting from the front of one randomly selected pair of underwear was tested for the presence of seminal fluid using the Brentamine Fast Blue B salt test (AP, Sigma-Aldrich) and the ABACard™ p30 (PSA, Abacus). For DNA analysis, six swabs (40 cm² per sample using sterile swabs moistened with nuclease-free water) and seven cuttings (6.25 cm² per sample, a size similar to casework samples collected in our laboratory) were collected from all five pairs of panties for each separate bed sheet test (Fig. 1). Furthermore, to run comparisons with Kafarowski et al. [10], five smaller cuttings (1 cm × 1 cm) were taken from one pair of underwear washed with the blended bed sheet containing ten ejaculates (A10), and sperm cells were counted using the Sperm Hy-Liter™ kit (Independent Forensics). The following modifications were made to the manufacturer’s protocol: (i) Cells were pelleted and resuspended in 100–300 μL PBS buffer (0.01 M, pH 7.0), and 20 μL of the solution was applied to an 11 mm diameter sample window slide, and (ii) 10 × DTT was used for sample preparation. We then examined the slides under a Carl Zeiss Axio Imager M2 microscope using 4',6-diamidino-2-phenylindole (DAPI) and fluorescein isothiocyanate (FITC) filters, as well as phase contrast. Images were captured using the Axio CamMRc camera and the AxioVision Rel software (Carl Zeiss Canada).

All samples underwent the following genetic analyses: differential DNA extractions were carried out using the DNA IQ™ magnetic beads system (Promega) and eluted with 50 μl of elution buffer. Epithelial and spermatozoa fractions were then quantified using the 7500 Real-Time PCR System (Applied Biosystems) and the Quantifiler® DuO DNA Quantification Kit (Applied Biosystems). Amplifications were carried out only when DNA concentrations reached >0.008 ng/μl (or total DNA in 50 μl ≥ 0.4 ng) within the epithelial fraction, and >0.001 ng/μl (or total DNA in 50 μl ≥ 0.05 ng) within the spermatozoa fraction. To obtain DNA profiles, a total of 15 autosomal STR genetic loci were amplified in 15 μl using the AmpFLSTR® Identifier™ Plus PCR Amplification Kit (Applied Biosystems) on a 96-well GeneAmp® PCR System 9700 (Applied Biosystems) for 28 cycles. Amplification products were separated by capillary electrophoresis on an ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems) and data was analyzed.

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Table 1

<table>
<thead>
<tr>
<th>Bed sheet ID</th>
<th>Donor</th>
<th>No.</th>
<th>Fabric</th>
<th>Drying time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>A</td>
<td>2</td>
<td>100% cotton</td>
<td>2 days</td>
</tr>
<tr>
<td>A6</td>
<td>A</td>
<td>6</td>
<td>100% cotton</td>
<td>2 days</td>
</tr>
<tr>
<td>B6</td>
<td>B</td>
<td>6</td>
<td>100% cotton</td>
<td>2 days</td>
</tr>
<tr>
<td>C6</td>
<td>C</td>
<td>6</td>
<td>100% cotton</td>
<td>2 days</td>
</tr>
<tr>
<td>D6</td>
<td>D</td>
<td>6</td>
<td>100% cotton</td>
<td>2 days</td>
</tr>
<tr>
<td>A10</td>
<td>A</td>
<td>10</td>
<td>60% cotton/40% polyester</td>
<td>5 months</td>
</tr>
<tr>
<td>B10</td>
<td>B</td>
<td>10</td>
<td>100% cotton</td>
<td>2 days</td>
</tr>
</tbody>
</table>

Fig. 1. Location of the cuttings and swab samples collected from each pair of underwear to assess DNA transfer. A–G represent cuttings (2.5 cm × 2.5 cm) and H–M represent swabbed areas (40 cm²).
using the ABI PRISM® GeneMapper® ID software v.3.2 (Applied Biosystems). The analytical and stochastic thresholds used were 50 RFU and 200 RFU, respectively.

2.2. DNA transfer from vaginal secretions

Considering the large number of epithelial cells shed within vaginal secretions, we tested the degree of vaginal DNA that may be transferred during laundering. We recruited two female volunteers (i.e. one pre-menopausal and one post-menopausal) to wear five pairs of 100% cotton panties over five days (i.e. each pair of underwear was worn for one day). It was requested that the women should not menstruate during the course of the experiment, and that no sexual intercourse had occurred for at least five days prior to wearing the test panties (and was avoided during their use as well) to avoid the detection of male DNA via postcoital fluid. The five pairs of worn panties from each volunteer were then washed with their own set of five pairs of pristine underwear under normal conditions (as previously described), along with a clean bed sheet to mimic a regular load of laundry. All ten pristine pairs of panties were subsequently sampled by swabbing and cutting (Fig. 1) and submitted for standard DNA extractions using the DNA IQ™ magnetic beads system. One sample from each pair of underwear that yielded the highest quantity of DNA was selected to undergo DNA amplification, as previously described. A two-tailed, unpaired t-test was then used to verify whether one volunteer contributed a greater amount of epithelial DNA to the other items it was laundered with (i.e. pre- vs. post-menopausal woman). Statistical analyses were performed using GraphPad Prism 5.0.

2.3. Background DNA in children's underwear

To help determine the amount of background DNA that may be found on children's clothing, we collected female children's underwear from 11 control families (i.e. staff and family friends) with no history of intrafamilial sexual abuse. Of these 11 families, males in three were vasectomized, therefore had no possibility of spermatozoa being present in the laundry (Table 2). The underwear were required to have been worn by the child for a period of at least six months, regularly washed with the rest of the family's laundry under normal conditions and submitted to the lab already washed and dried. We sampled all of the children's underwear via swabbing and cutting (Fig. 1) performed genetic analyses as previously described. Results yielding DNA mixtures were compared to the DNA profiles of each family member, and the degree at which each member contributed to the mixture was evaluated. To test for the presence of seminal fluid, 1 cm² cuttings were collected from underwear that yielded quantities of male DNA ≥ 5 ng. For each of those underwear, seven cuttings were tested using the Brentamine Fast Blue B salt test and three were tested using the ABAcard® p30. We also collected three 1 cm² cuttings for the visual detection of spermatozoa using the Sperm Hy-Liter™ kit, as previously described.

3. Results

3.1. Spermatozoa transfer in the laundry

Seven bed sheets containing a varying number of ejaculates were each washed with five pristine pairs of underwear, therefore 35 total pairs of underwear were used to assess the level of sperm transfer that may have occurred during laundering. Seminal fluid tests (AP and PSA) proved negative on all seven randomly chosen pairs of pristine underwear that had been washed with soiled bed sheets. All 30 swab samples collected from the five pairs of underwear washed with the cotton/polyester bed sheet containing ten ejaculates (A10) did not yield DNA that met the amplification threshold in either the epithelial or sperm fraction. Therefore, swabbing was omitted for underwear washed with the six remaining bed sheets.

3.1.1. Cuttings – sperm fraction

All of the pristine underwear used in the bed sheet experiments yielded a total of 245 cuttings and ranged between 0 and 5.9 ng of male DNA within the sperm fraction. Underwear washed with two or six ejaculates yielded the lowest DNA quantities ( <0.8 ng, Fig. 2). The results obtained from pristine underwear washed with ten ejaculates differed between A10 and B10. In the A10 test, the quantity of male DNA reached as much as 5.9 ng, while the maximum quantity observed in the B10 test was a mere 1.2 ng. Similarly, all cuttings from the five pristine pairs of underwear washed with the A10 bed sheet yielded complete genetic profiles (n = 35 cuttings), while those from the underwear washed with the B10 bed sheet yielded 0–28 alleles (Fig. 2). The laundry experiment with the bed sheets containing two ejaculates (A2) yielded a maximum of 4 alleles. In the four experiments involving bed sheets containing six ejaculates, most cuttings yielded negative results (largely seen in A6 and D6), and others yielded partial genetic profiles that reached up to 17 alleles (B6 and C6).

In four of the five small cuttings collected from a pair of underwear washed with the A10 bed sheet, the Sperm Hy-Liter™ kit revealed from 8 to 22 spermatozoa per slide. The fifth cutting did not reveal the presence of any spermatozoa.

3.1.2. Washer’s interior drum

Swabs collected from the washer’s interior drum after each of the four experimental loads containing six ejaculates yielded 0.4–2.4 ng within the sperm fraction, and amplified between 3 and 18 alleles (results not shown).

**Table 2**

Information for each pair of female children's underwear collected from 11 different families, all used to assess background DNA. M = mother; F = father; S = sister; B = brother. Multiple data within the same row, and separated by a comma, represent underwear analysed from different female children within the same family.

<table>
<thead>
<tr>
<th>Family</th>
<th>Child's age</th>
<th>Pairs of underwear donated</th>
<th>Duration worn (years)</th>
<th>Cohabitation</th>
<th>Vasectomized father</th>
<th>Type of washer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>2</td>
<td>1.5</td>
<td>M, F, B</td>
<td>No</td>
<td>Top load</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>M, F, S</td>
<td>No</td>
<td>Top load</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>2</td>
<td>≥0.5</td>
<td>M, F, B</td>
<td>No</td>
<td>Top load</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>M, F, B</td>
<td>No</td>
<td>Top load</td>
</tr>
<tr>
<td>E</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>M, F, B</td>
<td>No</td>
<td>Top load</td>
</tr>
<tr>
<td>F</td>
<td>6, 9, 11</td>
<td>2, 2, 2</td>
<td>1, 1, 0.5</td>
<td>M, F, S, S</td>
<td>Yes</td>
<td>Top load</td>
</tr>
<tr>
<td>G</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>M, F, B</td>
<td>No</td>
<td>Top load</td>
</tr>
<tr>
<td>H</td>
<td>11</td>
<td>1</td>
<td>≥0.5</td>
<td>M, F, B</td>
<td>Yes</td>
<td>Top load</td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>M, F</td>
<td>No</td>
<td>Front load</td>
</tr>
<tr>
<td>J</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>M, F, B</td>
<td>Yes</td>
<td>Top load</td>
</tr>
<tr>
<td>K</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>M, F, B, S</td>
<td>No</td>
<td>Front load</td>
</tr>
</tbody>
</table>
3.1.3. Cuttings – epithelial fraction

Results within the epithelial fractions varied: the sperm donor’s genetic profile was indiscernible, partial or almost complete (up to 27 alleles, results not shown). Because some ejaculates were deposited onto the bed sheets during sexual relations (i.e. oral sex or coitus interruptus), the female partner’s DNA was also detected in some cuttings, either alone or in combination with the male profile, and contributed up to 29 alleles (results not shown).

3.2. DNA transfer from vaginal secretions

All 60 swab samples collected from the ten pairs of pristine panties washed with underwear worn by the two female donors did not yield DNA quantities that met the minimum amplification threshold. The 70 cuttings sampled however, yielded DNA quantities that ranged from 0.2 to 3.2 ng, with the post-menopausal woman’s contribution located on the lower end of this range (Fig. 3). T-tests confirmed that the pre-menopausal woman contributed a greater amount of DNA than the post-menopausal woman, and once normality tests confirmed that the data followed a normal distribution, a subsequent parametric two-tailed t-test also proved highly significant (p < 0.0001).

For each pair of pristine underwear, the cutting that yielded the largest quantity of DNA was submitted for amplification. Of these ten samples, complete genetic profiles were obtained from two of them, and both corresponded to the pre-menopausal woman’s profile. Partial genetic profiles from both female donors (i.e. 11–26 alleles) were detected within the eight remaining samples (results not shown), further supporting the transfer of DNA from vaginal secretions among clothing during laundering.

3.3. Background DNA in children’s underwear

A total of 24 pairs of female children’s underwear were donated by 11 different families, ranging from one to six pairs per family (Table 2). Seminal fluid tests performed on the three pairs of underwear, where at least one sample reached 5 ng of male DNA (C1, C2, D1), were all negative (AP n = 21, PSA n = 9). All 144 swabs did not yield DNA quantities that met the minimum amplification threshold in either the sperm or epithelial fractions.

3.3.1. Cuttings – epithelial fraction

A total of 168 cuttings were submitted for genetic analysis, of which 24% did not yield DNA that met the amplification threshold, and 11% did not yield an interpretable profile. An additional 13% yielded the child’s profile only, all of which originated from the crotch area of the underwear. The remaining 52% (or 87 cuttings) yielded interpretable mixtures of DNA corresponding to multiple family members (Fig. 4). The quantity of male DNA obtained varied greatly and reached up to 6.7 ng (Fig. 5). In the eight non-vasectomized families (A–E, G, I, and K) where there was a possibility of spermatozoa being present in the wash, DNA corresponding to the father was found in 40 of the cuttings (38%). In the families with vasectomized males (F, H and J), only one cutting (1.6%) from Family F yielded a minute amount of DNA (i.e. 5% of the total DNA mixture) that corresponded to the father. All but one of the 11 families included siblings. In the eight families where a brother was present, only one family (G) portrayed a strong brotherly DNA contribution of up to 60%. In two of the three families where at least one sister was present within the family nucleus (B and F), a correspondence with their DNA within the epithelial fraction was observed in most of the cuttings in the epithelial fraction. DNA corresponding to the mother was detected in 51% of the cuttings and contributed from 5 to 90% of the total genetic mixture.

3.3.2. Cuttings – sperm fraction

Of the 168 total cuttings, 96% did not yield male genetic profiles within the sperm fraction. The remaining 4% (i.e. six cuttings)
yielded partial genetic profiles corresponding to the father, and ranged from 7 to 17 alleles (i.e. DNA quantities from 0.1–1 ng). These samples originated from the two pairs of underwear donated by family D (i.e. 3 cuttings per underwear). Microscopic analysis of spermatozoa revealed 9, 10 and 12 sperm cells on the 3 cuttings analyzed from one of these pairs of underwear, however no sperm cells were observed on any cuttings sampled from the other two pairs (C1, C2).

4. Discussion

The objective of this study was to assess the quantity of DNA and the quality of DNA profiles found among laundered items of clothing due to secondary transfer during a machine wash. We first evaluated the propensity of sperm cells to transfer during a machine wash. Although studies have shown that spermatozoa may remain within a clothing’s fibers after a wash [10–16], we showed that a portion of sperm that are disengaged from the clothing may not exit with the water either. DNA from spermatozoa persisted on the washing machine’s interior drum after each of the six semen-stained bed sheet experiments. Furthermore, we demonstrated that a portion of disengaged sperm cells may also transfer onto other items of clothing it is laundered with, as first observed by Kafarowski et al. [10]. The current study has, for the first time, established that sperm transferred in the wash can generate interpretable profiles. We have further shown that DNA from epithelial cells in vaginal secretions may also transfer during the wash and yield interpretable genetic profiles. Moreover, our studies with control families showed that DNA corresponding to other household members was found in most children’s underwear as well.

4.1. Spermatozoa transfer

Kafarowski et al. [10] counted up to eight sperm cells in each sample (1 cm²) excised from pristine items of clothing that were washed with a single pair of semen-stained female underwear. The initial semen stain was deposited via post-coital vaginal drainage following intercourse, yielding a fairly low volume of initial ejaculate, especially considering that a portion of the ejaculate may have remained within the vaginal cavity. In our laundry experiment where ten times the amount of semen was used (A10) but only about a tenth of the pellet was examined, a maximum of 22 sperm cells were observed from the 1 cm² cuttings, a result consistent with Kafarowski et al.’s [10] findings.

Cuttings from underwear washed with the A10 bed sheet yielded significantly more DNA than those washed with B10. Although it could be hypothesized that donor A may have had a greater sperm count than donor B, results from other laundry experiments involving both donors (e.g. A6 vs. B6) did not differ as considerably. We can thus assume that sperm count differences between donors A and B were not significant enough to impact the level of sperm transfer. An additional parameter to thus consider is the type of fabric of each sheet: A10 was a cotton/polyester blended bed sheet and B10 was made of 100% cotton. The blended sheet may have thus released more sperm during laundering.
Spermatozoa have been shown to persist on cotton clothing after being immersed in water for 144 h [17], and sperm are more persistent on laundered cotton than on laundered nylon, a synthetic fiber closely related to polyester [12]. Although comparison tests between polyester and cotton fabrics are lacking in the literature, it may be hypothesized that cotton fibers retain more spermatozoa, and therefore provide less opportunities for transfer to occur.

Every swab sample failed to yield DNA concentrations that met the minimum amplification threshold, however a majority of the sampled cuttings produced sufficient quantities of DNA for amplification. These results strongly suggest that any spermatozoa transferred during laundering were absorbed and/or trapped deeper into the fabrics of pristine items of clothing rather than deposited onto their surfaces. When providing opinions regarding the manner of deposition, swab samples yielding significant quantities of DNA in the sperm fraction would be indicative of semen deposition directly onto the child’s undergarment or via post-coital drainage, as opposed to alternative mechanisms such as transfer during laundering.

4.2. DNA transfer from vaginal secretions

Spermatozoa are fairly robust, thus requiring a stronger lysis step during differential DNA extractions. Epithelial cells on the other hand, are more delicate and require a mild lysis step only, which may have deemed them irrelevant for prior research concerning transfer during laundering. Although secondary transfer of DNA from blood has been reported in the wash [18], our study is the first to demonstrate a clear transfer of DNA from vaginal epithelial cells among clothing during laundering. DNA from vaginal secretions not only transferred from worn underwear to pristine items of clothing, but could yield complete (i.e. pre-menopausal woman) or partial (i.e. pre- and post-menopausal women) genetic profiles as well. The volunteer undergoing menopause had transferred a significantly less amount of DNA among clothing during laundering than the pre-menopausal woman. Normal vaginal discharge is largely composed of squamous epithelial cells in a serous transudate mixed with material originating from sweat, sebaceous and Bartholin’s glands [19]. A decline in vaginal lubrication is commonly observed in post-menopausal women, thus leading to less vaginal discharge [20], which was reflected in the DNA quantification results of our post-menopausal donor. However, because of our limited sample size (i.e. one pre-menopausal and one post-menopausal woman), we cannot dismiss that the observed differences may be due to between-individual variability rather than differing menopausal stages. Epithelial DNA transfer is important to consider when analyzing samples that originate from households containing one or more female members, as it may significantly contribute to the DNA mixtures often observed in the wearer’s profile.

4.3. Background DNA in children’s underwear

Epithelial DNA corresponding to family members residing within the same household was found on control children’s underwear, and depicted as mixtures of variable proportions. All 144 swabs did not yield DNA that met the amplification threshold, while most of the cuttings yielded interpretable DNA results similar to our laundering experiments. This suggests that the DNA mixtures observed in the children’s underwear occurred due to secondary transfer of DNA during laundering. DNA corresponding to the mothers’ profiles had a strong presence in many of the excised samples, which we hypothesize may be attributed to the high quantity of epithelial cells that are shed through normal vaginal secretions and transferred during laundering. Ten of the eleven families were composed of two or more siblings, and sibling profiles contributed to DNA mixtures in three of these families. In family F, the sister was close to the age of puberty and may thus have been more likely to contribute a greater amount of vaginal secretions. The other two families consisted of a sister aged three years old (family B) and a 10-year-old brother (family G). Although puberty is not an explanatory factor in these cases, saliva, nasal secretions and/or blood may have contributed sufficient epithelial DNA for transfer during laundering to occur, as these biological fluids are rich in DNA. The mere shedding of epithelial skin cells may also have been the source of this detectable DNA. DNA corresponding to the fathers’ profiles was found in multiple families as well, and in some cases was observed to make up 30% of the genetic mixture. Although saliva, blood and/or epithelial skin cells may have been a source for the father’s DNA appearing in the cuttings, mixtures where the father contributed a high proportion of epithelial DNA may have had an alternate source as well. We hypothesize that residual spermatozoa deposited onto fabrics or items of clothing other than the child’s (e.g. bed sheets or the father/mother’s underwear), yet laundered together, may have contributed to the DNA found in the epithelial fraction. The machine-washing process creates a fair amount of agitation, thus potentially weakening the spermatozoa’s cellular integrity and structure. The subsequent encounter of a mild lysis during differential extraction may be enough to further compromise its structure, and thus allow the DNA from weakened sperm cells to be incorporated into the epithelial fraction of a differential extraction rather than into the sperm fraction. The presence of high quantities of male DNA in families where the father was not vasectomized compared to families where the father was, helps support this hypothesis.

A particularly striking observation in one of the control children’s underwear was the visual confirmation of sperm cells. This emphasizes that the mere presence of one or few spermatozoa on a child’s pair of underwear cannot confirm acts of intrafamilial abuse, and stresses the importance of gathering all available evidence before drawing any final conclusions [10,13, see Proposed framework for examination and interpretation below].

4.4. Additional considerations

Certain parameters not tested in this study may affect sperm transfer during laundering as well. In all of the experiments, 100% cotton underwear were used to represent children’s underwear. Cotton blended fabrics are also often used in children’s underwear, which may influence the degree at which spermatozoa can be retained onto items of clothing during laundering. Moreover, we did not test the effects that bleach may have on sperm transfer. Andrews and Coquoz [21] found that blood, saliva and semen stains washed with a detergent composed of 16% bleaching agents could still yield sufficient quantities of DNA for further genetic analyses. Although DNA may still be gleaned after full volumes of ejaculates have been exposed to bleach, it remains unknown whether the considerably fewer number of sperm cells that transfer onto clothing during laundering would not be damaged by the bleaching agents and still yield interpretable genetic profiles. We used warm water (30°C) in all of our experiments and did not assess the effect of varying water temperatures on spermatozoa transfer. DNA from sperm cells however, has been found to persist after a machine wash in water temperatures of 60°C [12]. Although different parameters may affect spermatozoa transfer during laundering, a preliminary study by Brayley-Morris et al. [15] found similar quantities of DNA in clothing regardless if different washing machines, detergents or DNA extraction methods (i.e. differential vs. regular extractions) were used. The possibility for spermatozoa...
to transfer during laundring is thus possible under a wide range of conditions.

4.5. Proposed framework for examination and interpretation

Our study shows that spermatozoa and DNA from vaginal secretions may transfer among items of clothing during laundring and yield complete genetic profiles. We also found DNA corresponding to other household members in most children's underwear. Based on these findings, we propose some recommendations for the handling and interpretation of intrafamilial sexual abuse cases, where the distinction between DNA that was directly deposited onto fabrics, and DNA that was transferred during laundring, is crucial.

4.5.1. Sampling

In all three experiments (i.e. spermatozoa transfer, DNA transfer from vaginal secretions and background DNA in children's underwear), every swab sample (n = 234) failed to yield quantities of DNA that could meet the minimum amplification threshold. This strongly suggests that swabbing cannot collect spermatozoa and epithelial DNA that was transferred during laundring. Therefore, clothing in intrafamilial cases of sexual abuse should routinely be swabbed, at least in a first step. Alternatively, two samples could be collected simultaneously from the same area: one by swabbing and one by cutting. If swab samples were to yield significant quantities of male DNA, the probability that this DNA was deposited directly onto the item of clothing would be quite high. On the other hand, if swab samples are weak or negative, an additional analysis of cut samples may then be required.

4.5.2. Serological testing

AP and PSA testing in underwear washed with two, six or ten ejaculates were all negative. These serological tests were also negative in control children's underwear, even when a cutting produced a partial genetic profile within the sperm fraction. Positive presumptive testing with an associated male profile in the sperm fraction can thus generally be interpreted as an indication that semen was deposited directly onto the fabric rather than transferred during the wash.

4.5.3. Microscopic visualization of spermatozoa and DNA quantities

Although we used conditions favourable for spermatozoa transfer in the wash, (i.e. 10 ejaculates and mild laundring conditions: warm water, no bleach), no more than 22 sperm cells were observed on microscopic slides. Also, the male DNA quantities obtained were relatively modest in the epithelial and sperm fraction for both the controlled spermatozoa transfer experiment (≤5.9 ng) and the background DNA in children's underwear (≤6.7 ng). If significantly higher numbers of sperm cells or DNA quantities are obtained for cuttings of similar size, one may likely exclude secondary transfer in the wash as an explanation for the observed results. Although we employed as many realistic parameters as possible, the actual numbers that we observed should be used with some caution as they are linked to the specific parameters and conditions used in this study. Comparisons to casework results should thus take into account the wide range of possible variability (both known and unknown) that may be encountered in any given case scenario.

4.5.4. Control samples

If results remain inconclusive and settling on the possibility of secondary transfer in the wash is vital to the case, it may thus be necessary to collect control samples on the same piece of clothing to better assess the background level of DNA on that particular item. Considering that the father's profile (presumably from transfer in the wash) was visible in only 1 out of 7 cuttings in some of the children's underwear from our control families, it may be necessary to analyze as much as 10 control samples.

This framework should help forensic biologists interpret difficult cases of intrafamilial sexual abuse. However, it is important to note that reaching a definite conclusion on whether the DNA was directly deposited onto the child’s clothing, or merely transferred in the wash, may still remain a challenge in some situations. A third option to consider is the possibility of secondary transfer of DNA onto an item of clothing that came into contact with a semen-stained object prior to laundring. Although secondary transfer scenarios and conditions have been tested for blood and saliva [22], experiments with semen are still lacking.

5. Conclusion

In recent years, sexual violations towards children have increased across North America [23,24], generating greater challenges for forensic biologists. It is thus imperative that analysts have sufficient background knowledge on the quantity and quality of DNA that may be transferred onto children's clothing during laundring, and detected afterwards through genetic analyses. We hope that our findings will provide such insight and help shed light on the parameters required to distinguish DNA that was applied directly on a fabric, or transferred innocently during laundring.

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References


