# Dental health and oral mutans streptococci in 2–4-year-old Estonian children

## JANA OLAK<sup>1</sup>, REET MÄNDAR<sup>2</sup>, SARA KARJALAINEN<sup>3</sup>, EVA SÖDERLING<sup>3</sup> & MARE SAAG<sup>1</sup>

<sup>1</sup>Department of Stomatology and <sup>2</sup>Department of Microbiology, University of Tartu, Tartu, Estonia, and <sup>3</sup>Institute of Dentistry, University of Turku, Turku, Finland

International Journal of Paediatric Dentistry 2007; 17:92–97

**Objective.** The aim of this study was to assess the dental health and presence of mutans streptococci (MS) in 2–4-year-old Estonian children.

**Methods.** The dental health of 472 2–4-year-old children was examined using a mirror and a penlight in 14 daycare centres representing seven communities around Estonia. The mean ( $\pm$  SD) age of the children was 41.4  $\pm$  4.1 months (n = 222). Plaque samples of 222 children were employed to determine the presence of MS using the Dentocult® SM Strip mutans test.

**Results.** Caries was diagnosed in 42% of the children, and the average ( $\pm$  SD) dmft index was 1.6  $\pm$  2.5, ranging from 1.1  $\pm$  1.2 in Tartu to 2.4  $\pm$  3.1 in Võru. The proportion of caries-free children decreased from 82% in the younger to 63% in the older group (*P* = 0.001). Among the tested subjects, 58% were colonized with MS, and those with caries were colonized more often than children with no visible caries (80% and 51%, respectively; *P* = 0.001). **Conclusions.** The prevalence of dental caries in Estonian 2–4-year-olds is higher than in the Nordic countries, but similar to other Baltic nations. Colonization by MS was associated with dental caries.

## Introduction

Dental caries is one of the most common chronic diseases of early childhood. Its prevalence varies considerably, however, even in developed Western countries. Among 3-year-old children, this has been reported to be 1% in Italy<sup>1</sup>, 8% in Finland<sup>2</sup> and 56% in Poland<sup>3</sup>. High caries prevalence rates ranging between 46% and 65% have also been reported for Chinese, Brazilian and American 3-year-olds<sup>4-6</sup>. According to World Health Organization (WHO) data, caries prevalence among Swedish 3-year-olds was 5% in 2002<sup>1</sup>. The prevalence of caries increases with age. According to Grindefjord et al., 11.3% of children in Stockholm area had carious teeth at the age of 2.5 years, while one year later, caries was detected in 36.7% of children<sup>7</sup>. In Latvia, Estonia's neighbour, the prevalence of caries in 3-year-olds is 37%<sup>1</sup>. Among a low socioeconomic multicultural population of immigrants of Malmö in Sweden, dental caries was detected in 85% of 238 children who were examined<sup>8</sup>.

Correspondence to:

Jana Olak, Department of Stomatology, University of Tartu, Raekoja plats 6, Tartu 51003, Estonia. E-mail: Jana.Olak@kliinikum.ee In Estonia, the prevalence has been studied only in older age groups, from the age of 5 years onwards<sup>9</sup>, but no information is available on the dental health of 2–4-year-old children.

Dental caries in very young children may progress as rampant caries in primary teeth, called early childhood caries (ECC), or nursing caries, nursing bottle caries, baby bottle caries and baby bottle tooth decay<sup>10</sup>. According to the Clinical Guideline of the American Academy of Pediatric Dentistry, ECC is 'the presence of 1 or more decayed, missing or filled surfaces' in any primary tooth in a child at 71 months of age or younger. In children younger than 3 years of age, any sign of smooth-surface caries is indicative of severe early childhood caries. From ages of 3 through 5 years, one or more cavitated, missing or filled smooth surfaces in primary maxillary anterior teeth, or a decayed, missing or filled surface with a score of 4 (age 3 years), 5 (age 4 years) or 6 (age 5 years) constitutes severe early childhood caries<sup>11</sup>. The prevalence of nursing caries varies between 1% and 12% in developed countries, but in developing countries, the prevalence has been reported to be as high as 70% in the preschool population. The prevalence of ECC in Finland has been estimated to be around  $1\%^{12}$ .

Dental caries is closely associated with mutans streptococci (MS) colonizing the oral cavity. Streptococcus mutans and S. sobrinus in particular are known to be crucial in the initial phase of dental caries. It has been shown that many children acquire MS in early childhood, mostly transmitted from their mothers<sup>13,14</sup>, and that MS levels are closely related to caries prevalence in preschool children regardless of the extent of caries<sup>15</sup>. It is also generally accepted that the earlier the child is colonized with (MS), the greater the likelihood of developing dental caries. The children who harboured S. *mutans* in their plaque at the age of 2 years appeared to be the most caries-active individuals. Their caries index values at the age of 4 years differed significantly from the values of the children who harboured S. mutans later or who remained free of *S. mutans* infection<sup>16</sup>.

The time of MS acquisition in infants is important, since caries risk in children may be dependent on early colonization with MS<sup>16</sup>. The most critical time in these terms takes place between 19 and 31 months, although younger children may also become colonized<sup>13</sup>. Therefore, detection of oral MS in early childhood may predict future caries risk.

Caries development may be partly compensated by other factors, such as good oral hygiene and a noncariogenic diet. Diet and oral hygiene may interact so that, in the presence of 'good habits' and a cariogenic diet, the development of caries may be controlled<sup>17,18</sup>. In this study, the authors aimed to study the relationship between MS and dental caries. They did not investigate oral hygiene habits and diet.

The aim of this study was to evaluate the dental health and the presence of MS colonization in 2–4-year-old Estonian children since such information is needed in order to develop targeted caries prevention programmes.

## Subjects and methods

## Study population

Children aged between 2 and 4 years were recruited from communal daycare centres. Fourteen daycare centres throughout the country were selected to represent the age cohort of 2–4-year-olds: four centres were chosen to represent the capital city of Tallinn (450 000 inhabitants), two to represent the larger towns of Tartu, Pärnu and Jõhvi, and one to represent the smaller communities of Viljandi, Valga and Võru. Altogether, 472 children (234 boys and 238 girls) were examined. The ages of the children ranged between 30 and 54 months, with an average of  $41.4 \pm 4.1$  months (median = 42 months).

A separate group of 222 children (106 girls and 116 boys) from Tartu (total population = 100 000) was studied to estimate early colonization of oral MS in relation to children's dental health. These were children from eight childcare centres in different parts of Tartu. Their ages ranged between 24 and 47 months, with a mean of  $33.6 \pm 6.3$  months (median = 33 months).

According to the latest national data from 2005, the fluoride content of drinking water is 0.35 mg L<sup>-1</sup> in Võru, 0.25 in Valga, 0.40 in Tallinn, 0.47 in Jõhvi, 0.70 in Tartu and 1.07 mg L<sup>-1</sup> in Pärnu<sup>19</sup>. According to a separate interview, 86% of the 2–4-year-old children in Estonia use fluoridated toothpaste regularly, but only 2% have been exposed to professional fluoride applications or have used fluoride tablets (J. Olak, unpublished data).

The Ethics Committee of Human Research at the University of Tartu, Tartu, Estonia, has approved the research protocol of the study. Informed consent was obtained from the parents of all children. Participation was voluntary.

## Clinical examination

Examinations were conducted according to the WHO criteria<sup>20</sup> at the daycare centres using a mirror and a penlight. For lighting, a Halogen 76600 PenLight (Welch Allyn, Skaneatles, NY, USA) was used. Lesions were recorded as present when a carious cavity was clearly seen upon visual inspection of the child sitting upright. According to WHO directions from 1997, the stages of caries that precede cavitation, as well as other conditions similar to the early stages of caries, are excluded because they cannot be reliably diagnosed, and white or chalky spots should be coded as sound. Nevertheless, on the other hand, WHO directions state that a caries should be recorded as present when a lesion in a pit or fissure, or on a smooth surface, has an unmistakable cavity, undermined enamel, or a detectably softened floor or wall.

According to these directions, a tooth with any kind of caries on any tooth surface was diagnosed as decayed. In addition, the presence of ECC was recorded. The criterion for establishing a diagnosis of the ECC was dental caries affecting at least two or more maxillary primary incisors<sup>10</sup>. No X-rays were used. The same investigator (J.O.) performed all examinations. The reproducibility of the investigator (J.O.) was 0.89, determined by duplicate examinations of 25, 2–4-year-old children.

#### Plaque samples for mutans streptococci

Plaque samples were collected with dental floss from interproximal spaces of the maxillary central incisors and the lower molars<sup>21</sup>. In cases where the second molar had not yet erupted, the interdental space between the canine and the first mandibular molar was used. The plaque covered floss was immediately rubbed against the prepared site of the Dentocult® SM Strip mutans test strip (Orion Diagnostica, Espoo, Finland). The strips were immediately immersed into the liquid broth medium supplied by the manufacturer and incubated for 48 h at 37 °C. For suppression of other bacteria, a bacitracin disc was added to the broth medium, according to the manufacturer's instructions.

After incubation, the strips were examined and classified using dichotomized categorization as follows: samples with no colonies were classified as MS = 0, and test strips with one or several colonies were classified as MS > 0.

#### Statistical analysis

Fisher's exact test was used to compare caries prevalence proportions between boys and girls, to compare colonization percentages between children with and without caries, and to compare dmft scores between communities. The chi-square test was used for estimating differences in caries prevalence between the two age groups, and differences in prevalence of MS with respect to caries and age. The differences were considered significant if these had *P*-values of < 0.05.

#### Results

Caries was diagnosed in 42% of the children (44% of boys and 34% of girls) (P = 0.05). The dmft scores ranged from  $1.1 \pm 1.2$  in Tartu to  $2.4 \pm 3.1$  in Võru, and the mean for the whole cohort was  $1.6 \pm 2.5$ . The proportion of children with caries ranged from 30% in Viljandi to 56% in Võru and Valga, while the proportion of children with ECC among all the examined children ranged from 14% in Tartu to 31% in Võru, as shown in Table 1. The authors found a significant difference between the highest and lowest ECC scores of Võru and Tartu (P = 0.01). Neither extreme value differed significantly from the average obtained for the whole sample, however (Table 1). To evaluate the level of MS colonization in 2-4-year-old Estonian children, a separate study of 222 children from Tartu was carried out. Among this group of children, caries was diagnosed in 56 individuals (25%), and the proportion of caries-free children decreased from 82% in the younger age group (24–35-month-olds) to

| Table 1  | Dental | health | of  | 2_4-v | ear-old | children | in | selected | communities o | f Estonia |
|----------|--------|--------|-----|-------|---------|----------|----|----------|---------------|-----------|
| Table 1. | Dentai | nearti | UI. | Z-4-y | cai-oiu | ciniuren |    | selected | communities o | LStoma.   |

| Town     | dmft = 0 (%) | dmft > 0 (%) | dmft (mean ± SD) | ECC (%) | Children ( <i>n</i> ) |
|----------|--------------|--------------|------------------|---------|-----------------------|
| Viljandi | 69.7         | 30.3         | $1.2 \pm 2.4$    | 18.2    | 66                    |
| Pärnu    | 67.3         | 32.7         | $1.2 \pm 2.1$    | 14.0    | 55                    |
| Tartu    | 65.7         | 34.3         | $1.1 \pm 1.2$    | 13.8    | 99                    |
| Jõhvi    | 60.6         | 39.4         | 1.7 ± 2.9        | 16.9    | 71                    |
| Tallinn  | 58.1         | 41.9         | $1.8 \pm 2.9$    | 22.7    | 117                   |
| Valga    | 43.8         | 56.2         | $2.1 \pm 2.6$    | 15.6    | 32                    |
| Võru     | 43.8         | 56.2         | $2.4 \pm 3.1$    | 31.2    | 32                    |
| Mean     | 58.4         | 41.6         | $1.6 \pm 2.5$    | 18.9    | 472                   |

(dmft) decayed, missing and filled teeth; and (ECC) early childhood caries.

|                    | dmft = 0 |    | dmft > 0 |    | Total |     |
|--------------------|----------|----|----------|----|-------|-----|
| Age range (months) | n        | %  | n        | %  | n     | %   |
| 24–35              | 113      | 82 | 25       | 18 | 138   | 100 |
| 36–47              | 53       | 63 | 31       | 37 | 84    | 100 |
| Total              | 166      | 75 | 56       | 25 | 222   | 100 |

(dmft) decayed, missing and filled teeth. \*P = 0.005, chi-square test.

Table 3. Colonization of mutans streptococci (MS) according to dental health.

|              | dmf | t = 0 | dmf | ft > 0 | Total |     |
|--------------|-----|-------|-----|--------|-------|-----|
| Colonization | n   | %     | n   | %      | n     | %   |
| MS = 0       | 82  | 49    | 11  | 20     | 93    | 42  |
| MS > 0       | 84  | 51    | 45  | 80     | 129   | 58  |
| Total        | 166 | 100   | 56  | 100    | 222   | 100 |

(dmft) decayed, missing and filled teeth. \*P = 0.001, Fisher's exact test.

63% in the older age group (36–47-month-olds, P = 0.005) (Table 2).

From 222 children examined, a total of 129 (58%) of the 2–4-year-old children were colonized by MS. Mutans streptococci growth was slightly more frequent among the younger age group than among the older age group, but the difference between groups was not significant (P = 0.1); therefore, the whole cohort was analysed together. Quite expectedly, colonization was detected more frequently (80%) among children with caries than among children with caries than among children with no caries (51%) (P = 0.001, Table 3).

#### Discussion

This study was carried out to evaluate the oral health of 2–4-year-old children in Estonia since no such information has been available until now. At the cavitation level, the lack of a radiological examination has a minimal effect on the occurrence of caries, whereas it has some effect on the detection of the number of lesions in those who already have clinically detectible caries<sup>22</sup>. In the WHO directions, radiography for detection of approximal caries is not recommended because of the impracticability of using the equipment in all situations<sup>20</sup>. Finally, according to the Ethics Committee at Tartu

University, radiographs are not allowed for epidemiological or prophylactic study. For this reason, there exists a possibility of overlooking approximal caries lesions in this study.

Dental floss was used for plaque sampling. According to Wennerholm *et al.*, more surfaces were found to be positive for MS when samples were taken with a toothpick than with a carver, needle or floss. Sampling of the approximal surfaces showed no difference between the toothpick and the floss method. Samples taken with floss showed a tendency to indicate higher levels of MS than samples collected with toothpicks. The highest level of MS is on occlusal and approximal surfaces<sup>23</sup>. For this study, it was important to ascertain if the child harboured MS or not.

Sixty-seven per cent of Estonian preschool children go to communal daycare centres. For this study, 14 daycare centres in different parts of Estonia were chosen according to WHO directives: four daycare centres from the capital city, two from larger towns and one from different regions, with a minimum 25 patients from each<sup>20</sup>. Selection of daycare centres was random. The principle that the daycare centres included in this study should be situated in different parts of the town was applied.

Although the same investigator performed all the examinations, there were no significant differences in the availability of dental services, the mean values of this age cohort in different communities varied. The variation may be associated with different fluoride levels in the drinking water, since higher caries prevalence was found in Southern Estonia, Valga and Võru, where the concentration is below  $0.5 \text{ mg L}^{-119}$ . Similarly, in areas such as in Pärnu and Tartu, where the fluoride concentration is around  $1 \text{ mg } \text{L}^{-1}$ , the caries prevalence is clearly lower than in the fluoride-deficient areas. These findings suggest that caries data from only one community are not representative nationally, and therefore, as in the case of a small and uniform country like Estonia, caries prevalence may vary. In the authors' opinion, the dietary and dental hygiene habits throughout Estonia are quite similar, but this has not yet been studied.

The finding that caries was slightly more frequent in boys than in girls is in line with earlier studies on Estonian schoolchildren<sup>24</sup>.

Early childhood caries has been defined as dental caries affecting at least two of the four maxillary primary incisors, with the presence of one or several decayed tooth surfaces in children younger than 5 years of age<sup>10</sup>. This type of caries is generally connected with poor dietary habits, and inappropriate breast- and/ or bottle-feeding. The major reservoir from which infants acquire MS is their mother's saliva<sup>11,13</sup>. Children with ECC need special attention when designing prevention programmes for families with small children. The consequences of ECC include a higher risk of new carious lesions in both the primary and permanent dentitions, and increased treatment cost and time<sup>11</sup>.

In young children, early MS colonization, plaque accumulation, diet, immigrant background, socioeconomic factors and the mother's education have been shown to be strongly associated with caries development<sup>25</sup>. According to Alaluusua and Renkonen, the presence of MS is a significant indicator of early caries attack<sup>16</sup>. Furthermore, the role of dietary habits and oral hygiene in the development of caries lesions cannot be excluded<sup>26–28</sup>.

These findings are in line with other studies showing an association between colonization of MS and caries. The prevalence of MS colonization has been shown to increase from 30% in 1.5-year-olds to 42% in 3-year-olds<sup>29</sup>. In this study, more than half of the 2–4-year-old children were found to be carriers of MS. Future studies are needed to reveal whether the early MS colonization of children, demonstrated here can be suppressed by preventing transmission of maternal MS. Feeding habits and dental hygiene in association with dental caries need to be evaluated in Estonia in the future.

Caries prevalence rates among 3-year-olds in the Nordic countries range between 7% and 28%<sup>1,2,7,8</sup>. The prevalence rates are clearly higher in the Baltic countries, the figures varying from 37% to 84%<sup>1,30</sup>. The dental healthcare system and dental caries prevention programmes have been very efficient in the Nordic countries. In Estonia, several projects for prevention of dental caries have been financed by the Estonian Health Insurance Fund. A programme for systematic prevention of dental caries in very young children is currently being elaborated. Furthermore, the teeth of young children in Estonia seem to deteriorate rapidly with age, since the vast majority of 7-year-old Estonian children have been reported to have caries<sup>9</sup>.

#### What this paper adds

• For the first time the dental health and presence of mutans streptococci (MS) in 2–4-year-old Estonian children was assessed.

#### Why this paper is important to paediatric dentists

- The current study provides data for comparison of dental health in small children.
- These data are needed to develop targeted caries prevention programmes for small children in Estonia.
- Early colonization with MS is associated with development of dental caries in small children.

#### Conclusions

The authors conclude that the prevalence of dental caries in 2–4-year-old Estonian children is higher than that in Nordic countries, but similar to other Baltic nations, and that early colonization with MS is associated with caries development.

#### Acknowledgements

This study was supported by: Grant No. 5293 from the Estonian Science Foundation; Orion Diagnostica, Espoo, Finland; and the Estonian Ministry of Education, Target Financing No. TARMB 0418.

#### References

- 1 World Health Organization. *WHO Oral Health Country/ Area Profile Programme*. [WWW document.] URL http://www.whocollab.od.mah.se/index.html
- 2 Karjalainen S, Söderling E, Sewón L, Lapinleimu H, Simell O. A prospective study on sucrose consumption, visible plaque and caries in children from 3 to 6 years of age. *Community Dent Oral Epidemiol* 2001; **29**: 136–142.
- 3 Szatko F, Wierzbicka M, Dybizbanska E, Struzycka I, Iwanicka-Frankowska E. Oral health of Polish threeyear-olds and mothers' oral health – related knowledge. *Community Dent Health* 2004; **21**: 175–180.
- 4 Li Y, Wang W, Caufield PW. The fidelity of mutans streptococci transmission and caries status correlation with breast-feeding experience among Chinese families. *Caries Res*, 2000; **34**: 123–132.
- 5 Rodrigues CS, Sheiham A. The relationship between dietary guidelines, sugar intake and caries in primary

teeth in low income Brazilian 3-year-olds: a longitudinal study. *Int J Paediatr Dent* 2000; **10**: 47–55.

- 6 Milgrom P, Riedy CA, Weinstein P, Tanner ACR, Manibusan L, Bruss J. Dental caries and its relationship to bacterial infection, hypoplasia, diet, and oral hygiene in 6- to 36-month-old children *Community Dent Oral Epidemiol* 2000; **28**: 295–306.
- 7 Grindefjord M, Dahllöf G, Modeer T. Caries development in children from 2.5 to 3.5 years of age: a longitudinal study. *Caries Res* 1995; **29**: 449–454.
- 8 Wennhall I, Matsson L, Schröder U, Twetman S. Caries prevalence in 3-year-old children living in a low socio-economic multicultural urban area in Southern Sweden. *Swed Dent J* 2002; **26**: 167–172.
- 9 Dragheim E, Petersen PE, Kalo I, Saag M. Dental caries in schoolchildren of an Estonian and a Danish municipality. *Int J Paediatr Dent* 2000; 10: 271–277.
- 10 Peressini S, Leake JL, Mayhall JT, Maar M, Trudeau R. Prevalence of early childhood caries among First Nations children, District of Manitoulin, Ontario. *Int J Paediatr Dent* 2004; 14: 101–110.
- American Academy of Pediatric Dentistry. Policy on early childhood caries (ECC). *Pediatr Dent* 2005–2006; 27: 31–33.
- 12 Milnes AR. Description and epidemiology of nursing caries. *J Public Health Dent* 1996; **56**: 38–50.
- 13 Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants. Evidence for a discrete window of infectivity. *J Dent Res* 1993; 72: 37–45.
- 14 Klein MI, Florio FM, Pereira AC, Höfling JF, Goncavales RB. Longitudinal study of transmission, diversity, and stability of *Streptococcus mutans* and *Streptococcus sobrinus* genotypes in Brazilian nursery children. J *Clin Microbiol* 2004; **42**: 4620–4626.
- 15 Seki M, Yamashita Y. Decreasing caries prevalence in Japanese preschool children is accompanied with a reduction in mutans streptococci infection. *Int Dent J* 2005; **55**: 100–104.
- 16 Alaluusua S, Rekonen O-V. Streptococcus mutans establishment and dental caries experience in children from 2 to 4 years old. Scand J Dent Res 1983; 91: 453–457.
- 17 Harris R, Nicoll AD, Adair PM, Pine CM. Risk factors for dental caries in young children: a systematic review of the literature. *Community Dent Health* 2004; 21: 71–85.
- 18 Wendt L-K, Carlsson E, Hallosten A-L, Birkhed D.

Early dental caries risk assessment and prevention in pre-school children: evaluation of a new strategy for dental care in a field study. *Acta Odontol Scand* 2001; **59**: 261–266.

- 19 Indermitte E, Saava A, Saag M, Russak S. Drinking Water level in different regions of Estonia – its importance in relation to dental caries and fluorosis. Tartu University Press, 2005. (In Estonian).
- 20 World Health Organization. *Oral Health Surveys. Basic Methods*, 4th edn. Geneva: World Health Organization, 1997.
- 21 Pienihäkkinen K, Jokela J. Clinical outcomes of riskbased caries prevention in preschool-aged children. *Community Dent Oral Epidemiol* 2002; **30**: 143–150.
- 22 Roeters FJ, Verdonschot EH, Bronkhorst EM, van't Hof MA. Prediction of the need for bitewing radiography in detecting caries in the primary dentition. *Community Dent Oral Epidemiol* 1994; **22**: 456–460.
- 23 Lindquist B, Emilson CG. Distribution and prevalence of Mutans Streptococci in the human dentition. *J Dent Res* 1990; **69**: 1160–1166.
- 24 Kõll-Klais P, Mändar R, Leibur E, Kjaeldgaard M. High levels of salivary lactobacilli in Estonian schoolchildren. *Eur J Paediatr Dent* 2004; **5**: 107–109.
- 25 Grindefjord M, Dahllöf G, Nilsson B, Modéer T. Stepwise prediction of dental caries in children up to 3.5 years of age. *Caries Res* 1996; **30**: 256–266.
- 26 Ramos-Gomez FJ, Weintraub JA, Gansky SA, Hoover CI, Featherstone JD. Bacterial, behavioural and environmental factors associated with early childhood caries. *J Clinl Paediatr Dent* 2002; **26**: 165–173.
- 27 Maciel SM, Marcenes W, Sheiham A. The relationship between sweetness preference, levels of salivary mutans streptococci and caries experience in Brazilian pre-school children. *Int J Paediatr Dent* 2001; **11**: 123–130.
- 28 Mattos-Graner RO, Correa MS, Latorre MR, Peres RC, Mayer MP. Mutans streptococci oral colonization in 12–30-month-old Brazilian children over a one-year follow-up period. *J Public Health Dent* 2001; **61**: 161–167.
- 29 Thorild I, Lindau-Jonson B, Twetman S. Prevalence of salivary *Streptococcus mutans* in mothers and their preschool children. *Int J Paediatr Dent* 2002; **12**: 2–7.
- 30 Symposium Report. The prevalence of dental caries in Europe 1990–1995. *Caries Res* 1996; **30**: 237–255.

Copyright of International Journal of Paediatric Dentistry is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.