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RESEARCH ARTICLE

Is the recent emergence of mephedrone injecting in the United Kingdom associated with elevated risk behaviours and blood borne virus infection?

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The recent, and rapid, emergence of injection of the short-acting stimulant mephedrone (4-methylmethcathione) has resulted in concerns about increased infection risks among people who inject drugs (PWID). Data from the bio-behavioural surveillance of PWID in the United Kingdom were analysed to examine the impact of mephedrone injection on infections among PWID. During the year preceding the survey, 8.0% of PWID (163/2,047) had injected mephedrone. In multivariable analyses, those injecting mephedrone were younger, less likely to have injected opiates, and more likely to have injected cocaine or amphetamines, used needle/syringe programmes or sexual health clinics, been recruited in Wales and Northern Ireland or shared needles/syringes. There were no differences in sexual risks. Those injecting mephedrone more often had hepatitis C antibodies (adjusted odds ratio (AOR) = 1.51; 95% confidence interval (CI): 1.08-2.12), human immunodeficiency virus (AOR=5.43; 95% CI: 1.90-15.5) and overdosed (AOR=1.70; 95% Cl: 1.12-2.57). There were no differences in the frequency of injecting site infections or prevalence of hepatitis B. The elevated levels of risk and infections are a concern considering its recent emergence. Mephedrone injection may currently be focused among higher-risk or more vulnerable groups. Targeted responses are needed to prevent an increase in harm.

Introduction

Over the past decade, the emergence of the use of 'new psychoactive substances' has caused major concerns in many countries [1,2]. New psychoactive substances encompass a range of synthetic substances, including synthetic cannabinoids, cathinones, piperazines, tryptamines and phenethylamines, that are not controlled by two United Nations Conventions (the 1961

Convention on Narcotic Drugs and the 1971 Convention on Psychotropic Substances), but which may pose a public health threat that is comparable to that of the substances listed in these conventions [3]. The use of synthetic cathinones, and especially drugs marketed as mephedrone have caused particular concern in a number of countries including the United Kingdom (UK) [3-6].

Mephedrone is the common name for 4-methylmethcathione, it is a relatively short-acting stimulant with reported effects similar to amphetamine and MDMA [5,7]. It can be administered in a variety of ways, including snorting, ingestion and injection, and compulsive re-dosing over a period of many hours has been reported, due to rapid comedown when snorted or induced tolerance when injected repeatedly [5,8]. The use of drugs marketed as 'mephedrone' under street names such as 'drone', 'm-cat' and 'meow meow', have increased since its use was first reported around 2007. The subsequent emergence of the injection of synthetic cathinones, including mephedrone, has caused particular concerns in Europe [9], with the injection of these drugs having been associated with increases in human immunodeficiency virus (HIV) infections and risk behaviours among people who inject drugs (PWID) in several central European countries [10,11]. The use, and in particular the injection, of mephedrone by some populations of men who have sex with men (MSM), particularly during sex, has also recently been reported in Europe and elsewhere [12-14], often in settings where unsafe sex and sharing of injecting equipment occur [12,13].

In the UK, the use of mephedrone was first noted in 2008 [15,16], leading to 4-methylmethcathione being

FIGURE

Variations in the extent of mephedrone injecting, by age, time in years since first injection, region of recruitment, and sexual activity, United Kingdom, 2013 (n = 2,047)







B. Years since first injected (n=1,989)





D. Sexual activity (n=2,047)





controlled under the Misuse of Drugs Act in 2010 [17]. The injection of this drug is a more recent practice that was first reported in the UK in 2012; it occurred among people who had switched from snorting as well as among people who had previously injected other drugs including opioids and stimulants [18]. Of particular concern is the compulsion to re-dose when using mephedrone, increasing the frequency of injecting from two or three times daily to 15–20 times, raising the risk of injecting site damage and of infection through poor injection hygiene and the reuse and sharing of injecting equipment [19].

In response to the emergence of mephedrone injecting and the associated concerns about the risks, mephedrone was added to the list of drugs specifically asked about in the UK's national bio-behavioural surveillance system of infections and risks among PWID in 2013. In this paper, we used data from this large national survey to (i) assess the current extent of mephedrone injecting in the UK, (ii) examine the factors associated with mephedrone injecting and (iii) describe the frequency of a range of health harms among those injecting mephedrone.

Methods

PWID have been recruited into a voluntary unlinked anonymous monitoring system in the UK since 1990; methodological details of this series of annual crosssectional surveys have been published previously [20,21]. Briefly, agencies providing services to PWID (e.g. needle and syringe programmes (NSPs) and providers of addiction services such as opiate substitution therapy (OST)) at sentinel locations (n = 67 in 2013) throughout the UK except Scotland, invite clients who have ever injected psychoactive drugs to participate in the survey each year. The sentinel sites are selected so as to reflect both the geographical distribution and range of services offered to PWID. Those who consent to participate provide a biological sample, currently a dried blood spot (DBS), and self-complete a brief questionnaire focused on the injection of psychoactive drugs. In 2013, the answer categories to the question asking about the drugs injected during the preceding year was revised to include a new response category: 'mephedrone (m-cat)'. The survey has multi-site ethics approval.

The DBS specimens were tested for antibodies to HIV (anti-HIV), the hepatitis B core antigen (anti-HBc) and hepatitis C virus (anti-HCV). The anti-HIV test was an in-house IgG capture enzyme-linked immunosorbent assay (GACELISA) with similar performance to the HIV 1+2 GACELISA (Abbott Murex Diagnostics Ltd, Dartford, UK). Reactive specimens underwent further testing according to an algorithm that included a second ELISA and Western blot [22]. Anti-HCV testing employed a previously validated commercial enzyme-immunoassay (Ortho HCV 3.0 SAVe, Ortho Diagnostics, New Jersey) [23]. For hepatitis C, a previously described algorithm using antibody avidity testing was applied to the survey

samples to identify probable recent hepatitis C infections, i.e. samples with weak antibody avidity<40% in the presence of HCV RNA [24]. For anti-HBc, an in-house IgG class-specific antibody capture enzyme immunoassay (EIA) was used.

For those who had injected during the preceding year, bivariate associations (p < 0.05) between the outcome variable, i.e. having injected mephedrone, and covariates (demographics, injecting practices, drugs injected, sexual practice and use of health services) were examined using Pearson's chi-squared test. Where possible associations were found (p < 0.10), these were further examined via logistic regression using the forward stepwise procedure to select variables for inclusion in the model, with selection based on the likelihood ratio test (p < 0.05). All analyses were undertaken using SPSS 19.

Associations between mephedrone injecting and a range of health harms were explored by examining the frequency of mephedrone injecting among those with and without harms (anti-HIV, anti-HBc, anti-HCV, reported recent symptoms of injection site infections or injuries, and reported recent overdose). Data were adjusted for age, sex and region as these factors are known to be associated with these harms [20,21,25,26].

Results

Sample characteristics

During 2013, the survey recruited 2,047 individuals who had injected psychoactive drugs during the preceding year. Almost half (47%; n = 953) were aged 35 years or older (mean age: 36 years, median: 35 years), 26% (n = 522) were women and 5% (n = 107) had been born outside of the UK. Almost one fifth, 18% (n = 369), reported that they had been homeless during the preceding year and almost three quarters, 72% (n = 1,471), reported that they had ever been imprisoned.

The majority, 85% (n = 1,733), reported using an NSP service during the preceding year and 69% (n = 1,418) were currently in receipt of a maintenance drug regime such as OST or on detoxification. During the year preceding the survey, 10% (n = 204) had visited a sexual health (genito-urinary medicine) clinic, 20% (n = 411) a walk-in (minor injury/primary care) clinic, 30% (n = 617) an Emergency Department and 65% (n = 1,331) a general practitioner. Overall, 75% (n = 1,531) had ever had a voluntary confidential test for HIV, 81% (n = 1,667) for hepatitis C, and 72% (n = 1,471) had received at least one dose of hepatitis B vaccine.

Drugs injected and injecting risks

The most commonly injected drug during the year preceding the survey was heroin (92%; n = 1,879). Twofifths reported that they had injected crack cocaine (43%; n = 885), almost three-tenths had injected amphetamines (29%; n = 591) and just over one-tenth had injected powder cocaine (12%; n = 245). Injecting

TABLE 1

Factors associated with injecting mephedrone during the preceding year among people who inject drugs, United Kingdom, 2013 (n = 2,047)

Characteristic ^a	Injected mephedrone in the preceding year?										
	Yes		Tetel	р	Odds	0/-01	Adjusted				
	%		lotal	value	ratio	95% CI	odds ratio	95% CI			
All		8.0	163	2,047	NA						
Demographic characteristics											
Age	<25 years	19	28	144							
	25–34 years	9.3	75	809		NA					
	35–44 years	6.5	53	811	(0.001						
	≥45 years	2.5	7	283							
	Per year increase in age					0.94	0.92-0.96	0.95	0.92-0.97		
	<5 years	12	41	356		1.00	Ref				
	5-9 years	10	36	349		0.88	0.55-1.42		b		
Number of years since first	10–14 years	8.5	35	413	0.003	0.71	0.44-1.14				
injected	15–19 years	5.4	23	429		0.44	0.26-0.74				
	≥20 years	6.1	27	442		0.50	0.30-0.83				
	Not known	1.7	1	58		0.13	0.02-1.00				
Region/Country	Midlands and Eastern England	7.2	46	640	(0.001	1.00	Ref	1.00	Ref		
	London and Southern England	4.2	24	567		0.57	0.34-0.95	0.60	0.35-1.02		
	Northern England	7.5	46	613		1.05	0.69-1.60	0.91	0.58-1.43		
	Wales and Northern Ireland	21	47	227		3.37	2.17-5.23	3.06	1.91-4.89		
	Not last year/never	7.4	125	1,678	(-	1.00	Ref		b		
Homeless preceding year	Yes last year	10	38	369	0.067	1.43	0.97-2.09				
Anal or vaginal sex during preceding year	Men who had sex with men	8.2	7	85		1.64					
	Men who had sex with women only	9.7	90	931		1.95	1.29–2.96				
	Women who had sex with men and/or women c	8.2	34	415	0.017	1.63	0.99-2.68	b			
	Not had sex (men and women)	5.2	32	616		1.00	Ref				
Injecting practice during the	preceding year										
Injected heroin	No	21	35	168	<0.001	1.00	Ref	1.00	Ref		
	Yes	6.8	128	1,879		0.28	0.18-0.42	0.35	0.22-0.56		
Injected amphetamine	No	4.7	68	1,456	<0.001	1.00	Ref	1.00	Ref		
(speed)	Yes	16	95	591		3.91	2.82-5.43	2.42	1.68-3.50		
Injected cospine	No	6.8	123	1,802	<0.001	1.00	Ref	1.00	Ref		
Injected cocaine	Yes	16	40	245		2.66	1.81-3.91	2.36	1.53-3.63		
Used needles or syringes previously used by someone else	No	6.8	117	1,729	<0.001	1.00	Ref	1.00	Ref		
	Yes	14	46	318		2.33	1.62-3.35	1.95	1.31-2.92		
Health services usage											
Used needle and syringe programme preceding year	Not last year /Never	4.8	15	314	0.023	1.00	Ref	1.00	Ref		
	Last year	8.5	148	1,733		1.86	1.08-3.21	1.89	1.04-3.42		
Prescribed opiate substitution therapy	Previously/Never	10	63	629	0.016	1.00	Ref	b			
	Currently	7.0	99	1,418	0.010	0.67	0.48-0.93				
Used sexual health (genito-urinary medicine) clinic preceding year	No	6.9	114	1,649		1.00	Ref	1.00	Ref		
	Yes	17	35	204	<0.001	2.79	1.85-4.20	2.10	1.32-3.35		
	Not known	7.2	14	194		1.05	0.59–1.86	0.99	0.54-1.82		

CI: confidence interval; NA: not applicable; Ref: reference value.

^a No associations with: sex; being born in the United Kingdom, ever being imprisoned, injecting crack during the preceding 12 months, using a walk-in (minor injury/primary care) clinic during the preceding 12 months, using an emergency department during the preceding 12 months, visiting a general practitioner during the preceding 12 months, ever having had a voluntary confidential test for human immunodeficiency virus, ever having had a voluntary confidential test for hepatitis C, and uptake of vaccine against hepatitis B.

^b Entered in multivariate analyses but not in the final model.

^c The number of women reporting sex with women was small (< 50) and they are thus not reported separately.

mephedrone during the preceding year was reported by 8% (n = 163) of participants. Overall, 41% (n = 847) of the participants reported injecting only one of these five drugs during the preceding year and 19% (n = 391) reported injecting three or more of them. Those reporting that they had injected mephedrone were more likely to report injecting three or more of the other four drugs (63% (n = 102) vs 15% (n = 289); p<0.001). Of those who reported injecting mephedrone, 13% (n = 21) had also injected all of the other four drugs (i.e. heroin, crack cocaine, amphetamines and powder cocaine); 8% (n = 13) had not injected any of these four other drugs.

Those injecting mephedrone were younger (mean age: 32 years, median: 31 years vs mean age: 36 years, median: 35 years; p<0.001), had been injecting for fewer years (mean duration: 11 years, median: 10.5 years vs mean duration: 14 years, median: 13 years; p=0.001), and were more likely to be living in Wales or Northern Ireland (p<0.001, the level of use was very similar in both of these areas) (Figure). Overall, 16% (n = 318) of all of the participants reported that they had knowingly receptively shared needles or syringes (i.e. injected with needles or syringes that had previously been used by someone else) during the preceding year. Reporting sharing was more common among those injecting mephedrone than those not (28%; n = 46 vs 14%; n = 272; p<0.001). Similarly, those injecting mephedrone were more likely to report having ever receptively shared a needle or syringe (59%; n = 96 vs 46%; n = 864; p=0.001).

Sexual risk and condom use

The majority of all survey participants were sexually active, with just over two-thirds (70%; n = 1,431) reporting that they had had anal or vaginal sex in the preceding year; 5.6% (n = 85) of the men reported sex with other men. Heterosexual men were more likely to report injecting mephedrone than MSM or women (p=0.017, Figure). Of those sexually active, 35% (n = 503) reported having two or more sexual partners during the preceding year overall; 53% (n = 45) of the MSM had two or more partners, 37% (n = 348) of heterosexual men, and 27% (n = 110) of the women (who either had sex with men and/or women, there were<50 women reporting female partners). Of those with two or more partners, 17% (n = 84) reported always using condoms; 13% (n = 6) of the MSM, 17% (n = 59) of heterosexual men, and 17% (n = 19) of the women. Mephedrone injection was not associated with the extent of condom use among those with two or more sexual partners.

Factors associated with mephedrone injecting

The bivariate and multivariable associations are shown in Table 1. In the multivariable analysis, mephedrone injecting during the preceding year was associated with younger age. It was more common in Wales and Northern Ireland, among those who had injected amphetamine or powder cocaine, those who had shared needles or syringes, those using NSPs or sexual health (genito-urinary medicine) clinics. It was less common among those who had injected heroin.

Health harms and mephedrone injecting

Testing of the DBS samples collected in the survey found that overall, 1.1% (n = 23) of the participants had anti-HIV, 15% (n = 311) anti-HBc, and 50% (n = 1,027) anti-HCV. Having had an abscess, sore or open wound at an injection site during the preceding year was reported by 25% (n = 502) of the participants, and an overdose during the preceding year was reported by 14% (n = 277). After adjustment, injecting mephedrone was found to be more common among those with anti-HIV or anti-HCV, and among those reporting an overdose during the preceding year (Table 2).

Those who reported that they had ever had a voluntary confidential test for HIV or hepatitis C were also asked about the result of their last test. These data were used to assess the proportion of those with anti-HIV and anti-HCV who were aware of their infections. Of those anti-HCV-positive, it was possible to assess awareness for 87% (n = 898); of these, 46% (n = 417) were aware of their infection and awareness was similar among those injecting mephedrone and those not (43% vs 47%, p=0.393). Among those with HIV, it was possible to assess awareness for 87% (n = 20); of these, 95% (n = 19) were aware of their infection and again there was no difference in awareness between those injecting and those not injecting mephedrone (100% vs 94% respectively, p=1.000, Fisher's exact test).

A laboratory testing algorithm was applied to the samples collected in the survey to identify probable recent infections with HCV [24], i.e. those with weak anti-HCV avidity in the presence of HCV RNA. This algorithm identified 28 probable recent infections among those participants who had been at risk of hepatitis C infection (n=1,048); thus overall, 2.7% of those who had been at risk had recently become infected with HCV. There was no difference in the extent of these probable recent infections between those who reported injecting mephedrone and those who did not (2.5% vs 2.7% respectively, p=0.936).

Discussion

Considering the recent, and rapid, emergence of the injection of mephedrone, the elevated levels of risk and harm found in our study among those who had injected mephedrone are a concern. Within two years of mephedrone injection first being reported in the UK it was being injected by one in 12 PWID. Worryingly, those with HIV were more than five times as likely to report mephedrone injecting, and mephedrone injecting was also more common among those with antibodies to HCV and those who had recently overdosed. Although there were no differences in sexual risk, injecting risks were significantly higher among those injecting mephedrone.

TABLE 2

Health harms and extent of mephedrone injecting among people who inject drugs, United Kingdom, 2013 (n = 2,047)

Injected mephedrone during the preceding year?	Had harm		n	p value	Odds ratio	(95% CI)	Adjusted odds ratio	(95% CI)ª			
Had an abscess, sore or open wound during preceding year											
Not injected mephedrone	24%	461	1,884	- P ((1.00	Ref	1.00	Ref			
Injected mephedrone	25%	41	163	0.846	1.04	0.72-1.50	1.10	0.75-1.62			
Had antibodies to hepatitis C											
Not injected mephedrone	50%	941	1,884	0 (01	1.00	Ref	1.00	Ref			
Injected mephedrone	53%	86	163	0.491	1.12	0.81-1.54	1.51	1.08-2.12			
Had antibodies to hepatitis B core antigen											
Not injected mephedrone	16%	298	1,884	0.007	1.00	Ref	1.00	Ref			
Injected mephedrone	8.0%	13	163		0.46	0.26- 0.82	0.73	0.40-1.33			
Had antibodies to HIV											
Not injected mephedrone	1.0%	18	1,884		1.00	Ref	1.00	Ref			
Injected mephedrone	3.1%	5	163	0.014	3.28	1.20- 8.95	5.43	1.90-15.5			
Had an overdose during preceding year											
Not injected mephedrone	13%	243	1,884		1.00	Ref	1.00	Ref			
Injected mephedrone	21%	34	163	0.004	1.78	1.19-2.66	1.70	1.12-2.57			

CI: confidence interval; HIV: human immunodeficiency virus; Ref: reference value.

^a Adjusted for age, sex and region/country as these factors are known to be associated with the outcomes.

Our findings suggest the spread of mephedrone injecting within the UK has been fairly rapid since this was first reported in 2012. The rapid emergence of the injection of synthetic cathinones, often as substitute for or in addition to other drugs, has also been reported in several central European countries [9-11]. However, the extent of mephedrone injecting varied markedly across the UK, from around one in 25 in London and the south of England, through around one in 14 elsewhere in England, to one in five in both Wales and Northern Ireland. This indicates that the emergence of mephedrone injecting in both Wales and Northern Ireland has been more extensive and rapid than in England. The reasons for these geographical differences are unknown and further research is required to explore this.

Mephedrone injecting was more common among those who reported injecting other stimulants (amphetamine and powder cocaine) and was less common among those injecting heroin. This is perhaps to be expected given that mephedrone is also a stimulant. This finding suggests that the emergence of mephedrone injecting might, in part at least, be driven by issues such as drug availability, price and/or drug purity, leading to drug substitution among existing populations of people who inject stimulants [27]. As opiate injecting has most probably declined in the UK [28], particularly in England [29], and is now focused in an ageing cohort [30], the emergence of mephedrone injecting may also be part of a generational shift towards the injection of stimulants [31]. Those who had injected mephedrone were overall younger and had been injecting for a shorter time than those who had only injected other drugs. Considering this, and that a small number of those sampled reported injecting only mephedrone, it is possible that a new group of PWID who inject mephedrone, either alone or in conjunction with other drugs, might be emerging [18]. These findings thus indicate that currently mephedrone injecting is mostly occurring among existing populations of PWID, but they also suggest the emergence of a new group of younger PWID with potentially higher risks.

Those reporting mephedrone injection were twice as likely to report sharing injecting equipment, indicating that they are a high risk group. This is supported by the higher HIV and hepatitis C prevalence and overdoses being more common among those injecting mephedrone. The data on the proportions aware of their infection with HIV or hepatitis C indicate that awareness does not vary between those injecting and those not injecting mephedrone, which suggests that there might be no difference in the recency of these infections (recent infections are probably less likely to have been diagnosed than longer standing ones). This is corroborated by our data on probable recent HCV infections, which indicate that there is no difference in the incidence of HCV infection between those injecting mephedrone and those not injecting mephedrone. Our findings thus suggest that mephedrone injection in the UK is currently mainly concentrated among groups of PWID that already have elevated levels of risk, infection and harm.

Considering the higher levels of injecting risk behaviours and infections among those injecting mephedrone, our findings indicate that the emergence of mephedrone injection in the UK has the potential to increase the transmission of infections among PWID, particularly if its use is sustained or becomes more widespread. The rapid emergence of the injection of synthetic cathinones has already been implicated in increases in viral hepatitis and HIV transmission in a number of other European countries [10,11,32].

Mephedrone injection was not associated with increased sexual risk in our study, although it was more commonly reported among those who were sexually active and younger. However, overall levels of unsafe sexual practice were high. Mephedrone use and injecting has been associated with sexual risk in some populations, specifically subgroups of MSM where it has been linked to high risk behaviours and infections [12,13,33,34], and mephedrone use has also been reported to have positive effects on libido [35]. Those injecting mephedrone in our study were more likely to have used a sexual health service, suggesting that mephedrone injection may be related to increased sexual health needs that may not have been detected by the limited data on sexual behaviour collected in our study, and further investigation is required.

The findings presented here suggest that interventions are needed to address mephedrone injection. Responses should first look at ways to improve injection practice and hygiene, as well as promoting awareness among PWID of the risks and harms that are associated with injecting mephedrone [9,18,19]. However, to date, the UKs response to the injection of psychoactive drugs has had a strong focus on the traditional predominant drug, heroin, with an emphasis on a combination of high coverage NSPs and easy to access OST, which have been shown to be effective for reducing infections [36]. Although stimulant injection is not a new phenomenon in the UK, this has predominantly been in the form of crack cocaine injection alongside heroin (both need to be dissolved in acidic solutions), whereas the injection of amphetamines has been comparatively rare but may have increased in recent years [31]. With the emergence of mephedrone injection, responses will need to adapt to the increased use of water-soluble drugs and make greater use of treatments that are appropriate for users of stimulant drugs [37]. There may also be a need to explore the provision of these services in non-traditional settings, such as community-based outreach services and sexual health clinics.

This study has a number of potential limitations. Firstly, the illicit and marginalised nature of injecting drug use makes the recruitment of a representative sample problematic. To maximise representativeness, this survey used an accepted approach for surveillance surveys involving recruitment at multiple sites through targeted services for PWID as a sampling frame [38,39]. In the UK, there is extensive provision of such targeted services, and the uptake and use of these is high, with very few of the PWID recruited through communitybased studies found not to be in contact with these services [40]. For emerging drugs such as mephedrone, there may be new groups of users or populations where new patterns of injecting have emerged, such as some sub-groups of MSM. These groups may be less likely to be in contact with services or have different patterns of service use. This may possibly lead to such users being under-represented. Secondly, the behavioural data used here are based on self-reports, the accuracy of which may be subject to recall bias; however, the reliability of self-reported risk behaviours among PWID has been shown in other studies [41,42]. Considering these issues, the findings presented here should be generalised with caution.

Conclusion

Although the associations found here need further investigation, they suggest that the injection of mephedrone may be focused among younger and higher-risk groups of PWID, who may be particularly vulnerable to harm. Most of those injecting mephedrone were also using other drugs; however, a number were not. These findings, together with the younger age of those injecting mephedrone, suggest that new groups of PWID may also be emerging. Services in contact with PWID, including NSPs and sexual health clinics, will need to be alert to these elevated infection risks and the harm reduction needs of those injecting mephedrone. In the UK, the level of reported needle and syringe sharing among PWID is currently stable and lower than it was a decade ago, while the overall prevalences of HIV and hepatitis C among PWID have changed little in recent years; targeted responses, such as risk reduction interventions for those injecting mephedrone, are therefore needed to prevent an increase in the transmission of infections among PWID. Considering the increasing range of new psychoactive substances [43], vigilance should also be maintained for possible emergence other injected substances.

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Conflict of interest

None declared.

Authors' contributions

All authors contributed to preparing the manuscript, with VH coordinating. VH and FN manage the implementation of the

survey, with KC, JS and LJ assisting. JP oversees the laboratory testing. Analyses were undertaken by VH.

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