INT J TUBERC LUNG DIS 7(5):439–444

Systematic restaining of sputum smears for quality control is useful in Burundi

T. Buzingo,* M. Sanders,* J-P. Masabo,* S. Nyandwi,* A. Van Deun†

*** Programme National contre la Lèpre et la Tuberculose, Bujumbura, Burundi; † Institute of Tropical Medicine, Mycobacteriology, Antwerpen, Belgium**

SUMMARY

SETTING: Routine tuberculosis control services in Burundi.

OBJECTIVE: To determine whether systematic restaining of sputum smears for acid-fast bacilli (AFB) prior to rechecking quality assessment is necessary.

DESIGN: Blinded rechecking of peripheral routine smears, including a second control of discordants, before and after restaining.

RESULTS: Without restaining, 10/825 (1.2%) negative, and 59/189 (31.2%) positive results were declared false. After restaining, there were 34 (4.1%) false negatives and 13 (6.9%) false positives, both highly significant changes. Before restaining, quantification of positive smears was usually considered too high, while after restaining 41 out of 42 positives were found to have too low readings.

CONCLUSIONS: Despite mild climatic conditions in Burundi, restaining all slides before rechecking revealed an unrecognised, widespread problem of false negatives, rather than false positives. This indicated the need for critical re-appraisal of the standard procedure using cold staining, rather than re-training, as would have been inferred from results without restaining. Systematic restaining of all slides prior to rechecking may be more widely needed in National Tuberculosis Programmes to cover all possible serious causes of error. Cold staining should be avoided in field programmes since its performance is easily affected by frequently encountered adverse factors.

KEY WORDS: tuberculosis; smear microscopy; quality control; stains and staining

SPUTUM SMEAR microscopy for acid-fast bacilli (AFB) is the cornerstone of tuberculosis (TB) case-finding. Quality control by rechecking of peripheral slides at a higher level is considered an essential part of a well-functioning TB laboratory network.[1] In 1998, the International Union Against Tuberculosis and Lung Disease (IUATLD) guide 'The Public Health Services national tuberculosis reference laboratory and the national laboratory network' recommended several modifications to the classical rechecking system, based on recent publications.[2,3] One of the newer recommendations is that all slides, positive and negative, should be systematically restained before performing the first rechecking reading, to avoid erroneous controls due to faded or insufficient staining.[4] However, some experts have strongly opposed this recommendation, claiming that fading is not a real problem in the field and that systematic restaining leads to an unacceptable increase in workload.[5,6] In Burundi, of a total of 380 health structures, 96 perform sputum smear microscopy using the Tan Thiam Hok cold staining method[7] and centrally prepared stains. The National TB and Leprosy Programme (PNLT) has a long tradition of rechecking these peripheral smears. As a result of some experiences of faded slides resulting in high rates of apparent false-positives, all slides collected for quality control are restained. The question was thus raised as to whether this is justified, and a small operational study was planned.

METHODS

Each of the microscopy centres is routinely supervised bi-monthly. A random sample of 20% of slides of each result is collected and transported to the National TB Reference Laboratory (LNR) for rechecking by four technicians. For the purpose of this study, the routine rechecking procedure was slightly changed: all slides collected over a 9-month period were first read blind without restaining, and slides showing a discordant result with the peripheral reading were then counter-checked (without restaining) by the head of the laboratory to define the final non-restaining result. Discordants were defined as negative versus positive for AFB (any grading), and as quantification differences

Correspondence to: Mark Sanders, Unité Centrale, Programme National Contre la Lèpre et la Tuberculose, Bujumbura BP 2426, Burundi. Tel: (+257) 224 150. Fax: (+257) 218 585. e-mail: pnlt@cbinf.com

Article submitted 8 May 2002. Final version accepted 8 December 2002.

[A version in French of this article is available from the Editorial Office in Paris and from the IUATLD Website www.iuatld.org]

Table 1 Comparison of errors declared for slides considered negative at the microscopy centres, before and after restaining

	Errors after restaining			
Errors before restaining	None *n* (%)	HFN* *n* (%)	LFN† *n* (%)	Total *n* (%)
None	789 (96.8)	20 (2.5)	6 (0.7)	815 (98.8)
HFN	0 (0)	5 (17.9)	0 (0)	5 (0.6)
LFN	2 (40.0)	3 (10.7)	0 (0)	5 (0.6)
Total	791 (95.5)	28 (3.4)	6 (0.7)	825

* HFN error (1+, 2+ or 3+ smear declared negative).
† LFN error (scanty AFB declared negative).
HFN = high false-negative; LFN = low false-negative; AFB = acid-fast bacilli.

of at least two grades according to the IUATLD/World Health Organization (WHO) scale.[8]

Slides were then individually restained on a rack without contact between slides, using the cold routine technique.[7] This method uses only two stains, a concentrated carbolfuchsin (fuchsin 3%) for 5 min without any heating, followed by rinsing and combined destaining-counterstaining for 3 min. The combined solution contains methylene blue 0.6%, sulphuric acid 8.5% and denatured alcohol 33%. The chemicals used were of analytical grade or certified stains.

After restaining, all slides underwent a second cycle of blinded reading, and discordance between peripheral and restained reading results was again counterchecked. Care was taken to use a different technician for the two blinded readings, and to alternate technicians between readings before and after restaining, to exclude inter-examiner variation bias as much as possible.

Finally, all slides with discordant results between counterchecks before and after restaining were reviewed again. For blind screenings, 300 high-power fields were read as specified in the Burundi guidelines for routine AFB microscopy, in accordance with the first edition of the IUATLD Technical Guide.[9] For counterchecks and confirmatory readings, as many fields were read as necessary to reach the most correct result possible.

Due to residual doubts as to the effectiveness of cold restaining in Burundi, all 346 slides from the first months of the study were cleaned in xylene and sent to the Damien Foundation Bangladesh TB control project (DF) after the controls in Burundi had been completed. The slides were divided over about 40 randomly selected microscopy centres, with a first reading without restaining in one centre, and a second reading after xylene cleaning and hot Ziehl-Neelsen (ZN) (1% fuchsin) in a second centre. Both readings were blinded to any results from Burundi as well as to each other. Discordants between the two DF readings were identified and counterchecked at a third centre to obtain the final result.

All data were entered into an Epi Info file (CDC, Atlanta, GA). Errors were classified as high or low false-positive (HFP, LFP), high or low false-negative (HFN, LFN) and quantification errors (QNT), according to criteria based on the IUATLD/WHO scale. The difference between negative and 1+, 2+ or 3+ was considered as a 'high', and negative versus scanty AFB (<1+) as a 'low' error. QNT was declared for at least 2-log differences in quantification only. Statistical analysis was done using Fisher's exact test for comparison of error rates.

RESULTS

A total of 1014 slides (825 negative, 111 positive and 78 with scanty AFB according to peripheral results) were collected from 72 microscopy centres during the study period (details not shown). The remaining centres were either not accessible, or had no slides for quality control. On average 11 negatives (median nine, range 0–55), two positives (median one, range 0–9) and one slide with scanty result (median zero, range 0–7) were rechecked per centre. The delay between original staining and first rechecking varied from 2 to 7 months, but 88% were examined by the LNR within 4 months.

Table 1 shows the comparison of errors declared for negative smears before and after restaining, and Table 2 shows the same for positive smears and those with scanty AFB. The total number of false-negative slides increased from 10 (1.2%) before to 34 (4.1%)

Table 2 Comparison of errors declared for slides considered positive or with scanty AFB at the microscopy centres, before and after restaining

	Errors after restaining				
Errors before restaining	None *n* (%)	HFP* *n* (%)	LFP† *n* (%)	QNT‡ *n* (%)	Total *n* (%)
None	90 (72.0)	0 (0)	2 (1.6)	33 (26.4)	125 (66.1)
HFP	13 (72.2)	3 (16.7)	0 (0)	2 (11.1)	18 (9.5)
LFP	26 (63.4)	0 (0)	8 (19.5)	7 (17.1)	41 (21.7)
QNT	5 (100)	0 (0)	0 (0%)	0 (0)	5 (2.6)
Total	134 (70.9)	3 (1.6)	10 (5.3)	42 (22.2)	189

* HFP error (negative smear declared 1+, 2+ or 3+).
† LFP error (negative smear declared to contain scanty AFB).
‡ QNT: error, at least 2 log difference in grading.
AFB = acid-fast bacilli; HFP = high false-positive; LFP = low false-positive; QNT = quantification.

Table 3 Qualitative remarks of controllers before restaining versus errors detected after restaining

Errors after restaining	Remarks by controllers before restaining					
	None	Pale AFB	Poor destaining	Smear too large	Other	Total
None	643	37	7	144	8	925
HFN	14	5	0	7	0	28
LFN	4	0	0	1	0	6
HFP	3	0	0	0	0	3
LFP	7	0	0	3	0	10
QNT	22	18	0	1	0	42
Total	693	60	7	156	8	1014

AFB = acid-fast bacilli; HFN = high false-negative; LFN = low false-negative; HFP = high false-positive; LFP = low false-positive; QNT = quantification.

after restaining ($P < 0.001$). Corresponding figures for the HFN category were 5 (0.6%) and 28 (3.4%), respectively ($P < 0.0001$). The total number of false-positive slides fell from 59 (31.2%) before to 13 (6.9%) after restaining ($P < 0.000001$). Only three HFP remained from the 18 that were declared as such before restaining ($P < 0.001$). However, QNT errors increased from 5 (2.6%) before to 42 (22.2%) after restaining.

A review of discordants between counterchecks before and after restaining all confirmed the last result (data not shown).

Of the 72 individual microscopy centres (details not shown), 29 were found to have false-positives without restaining. Of these, 22 seemed to have at least one HFP or more than one false-positive result (several LFP, or a combination of HFP and LFP). After restaining, only 10 centres with a false-positive were left, among which there were only three with serious errors (one HFP or HFP and LFP). For false-negative results, before restaining only six centres each showed at least one such error, and only three of them more than one. After restaining, 19 centres were found to have at least one false-negative, all but one of them including HFN. Moreover, 17 centres were found to have made serious QNT errors after restaining, compared to only five before.

Table 3 shows qualitative remarks made by the controllers about the condition of the smears without restaining, compared to errors detected after restaining. It appears that remarks were made for about 30% of the slides, most often concerning an overlarge smear (156 slides). However, Table 3 also shows that no such remarks were made for more than 50% of slides with errors (50/89), and that very few errors were detected in slides for which there was a remark. The only exception was a finding of pale AFB, which was associated with HFN or QNT errors in 23/60 slides. Nevertheless, of 28 HFN errors detected after restaining, only five smears had been recognised as such, and pale AFB had already been remarked on before restaining.

Table 4 shows the comparison between the final recheck results in Burundi and those in Bangladesh for the 42 smears (of the 346 re-examined in both) for which an error at the original microscopy centre was declared in at least one of these countries. Almost all of the errors declared in Burundi were confirmed in Bangladesh. Only one LFN changed to HFN, and one QNT to no error. However, additional errors were declared in Bangladesh: two HFN, three LFN, seven LFP and three QNT.

DISCUSSION

The aim of AFB microscopy quality assurance rechecking should be to identify centres or individuals with unsatisfactory performance due to technical problems or human shortcomings. Serious technical

Table 4 Comparison of numbers of errors declared after a full cycle of rechecking, with cold restaining in Burundi and hot restaining in Bangladesh

Errors declared in Burundi after cold restaining	Errors declared in Bangladesh with hot restaining						
	None	HFN	LFN	HFP	LFP	QNT	Total
None	0	2	3	0	7	3	15
HFN	0	10	0	0	0	0	10
LFN	0	1	1	0	0	0	2
HFP	0	0	0	2	0	0	2
LFP	0	0	0	0	6	0	6
QNT	1	0	0	0	0	6	7
Total	1	13	4	2	13	9	42

AFB = acid-fast bacilli; HFN = high false-negative; LFN = low false-negative; HFP = high false-positive; LFP = low false-positive; QNT = quantification.

problems may be related to stains and staining technique, and these may remain undetectable even to the best controllers if restaining is not performed prior to rereading. For this reason, systematic restaining of all slides before rereading has been recommended.[4]

Our study shows that restaining all slides before rechecking quality control was necessary in the Burundi NTP, since it changed the results and conclusions dramatically. Without restaining, the problem seemed to be an unacceptably high false-positive rate: over 30% of AFB-positive slides were negative for the controllers, with 9.5% HFP in 22/50 centres with positives rechecked. This would indicate a need for retraining, as technicians do not seem to be clear about how to identify AFB. However, only a few of these remained after restaining, and more than one false-positive occurred in only one centre. Admittedly, the numbers rechecked for each centre were often very low, but these findings are nevertheless reassuring. Moreover, restaining saved a considerable number of microscopists from being falsely accused of making false-positive errors, which can result in widespread demotivation and strained relations with the NTP and its reference laboratory. Understandably, as this is part of their skill, microscopists will often take an accusation of making false-positive errors very badly. A reference laboratory should do all in its power to avoid making false accusations, and should restain unconfirmed positives before making a final judgement.

On the other hand, restaining negative smears revealed almost six times more serious false-negative errors than would have been recognised without restaining (28 instead of five). These occurred in 25% of the centres despite the fact that often only a few negative slides had been included for checking. This may indicate a widespread problem with staining and/or reading of smears that went undetected without restaining.

Several elements of our study seem to indicate a basic problem with stains or staining technique. First, there is the high proportion of serious quantification errors seen after but not before restaining. Of 42 QNT, 41 concerned an insufficient number of AFB reported, while three of the five QNT seen before restaining were declared to be too high. The latter, as well as the apparent false-positives and the frequently too pale colour of AFB, both reported in high numbers only before restaining, were probably caused by fading of the fuchsin stain. However, the pronounced trend toward very low quantification that was seen only after restaining is strongly suggestive of inadequate staining in the periphery. In the Burundi NTP, all stains are prepared centrally and are highly concentrated, so that deficient staining technique seems to be the most plausible cause. All peripheral technicians have been trained repeatedly in the technique and they have clear technical guidelines, although these may not always be closely respected.

Cold staining typically results in a weaker red colour. Especially in conjunction with other problems, such as poor condition of microscopes or inadequate time taken for examination, pale AFB would more readily lead to false-negative errors. Cold AFB staining has been discouraged in low-income countries where it seems to be less reliable,[10] possibly because of frequent occurrence of these concomitant factors; however, problems have also been reported in industrialised countries.[11,12] Although the Burundi reference laboratory, which has good microscopes and motivated technicians, could detect many of the peripheral errors after cold restaining, re-readings done in DF after hot ZN indicate that the Tan Thiam Hok cold staining technique used routinely in Burundi may not be satisfactory. Errors declared after cold restaining in the Burundi LNR were confirmed, but the hot staining revealed another five false-negatives (two of them HFN), or an increase of over 40%. Apart from some quantification differences, almost twice as many LFP errors were declared in Bangladesh. This may be explained by the normal limits of a rechecking system (low chance of finding rare AFB), especially in view of the disregard for the original result while identifying discordants in need of counterchecking after hot ZN. In fact, a thorough countercheck was made due to discordance between the first two Bangladesh readings for only a few of these LFP slides. As a reversed sequence of staining was not available for comparison, these results, in concordance with the other findings of this study, are suggestive of the inappropriateness of the staining method adopted by the Burundi NTP. As such, however, they do not constitute hard proof of the superiority of hot staining.

Our findings suggest that even after a few months and in a rather mild climate (average temperature 25°C, with moderate humidity for a tropical country, i.e., no problems of fungus in the microscopes), fading of fuchsin stain may profoundly perturb rechecking of AFB smears. Earlier studies have demonstrated that fading occurs quickly, even after excellent staining, under extreme conditions of temperature and humidity,[2,4] and opponents of routine restaining before rechecking have therefore argued that the additional workload is not justified under milder conditions.[5,6] Moreover, false accusations of false positives might be avoided from the start by restaining only suspected false-positives before the countercheck.

The present study shows that restaining may be indispensable not only to avoid erroneous declarations of false-positives, but also to identify serious staining problems leading to otherwise unrecognisable false-negatives. It seems plausible that the cold technique used in Burundi was at the origin of the widespread problem of false-negatives, while only a

few of the AFB present were regularly visualised, as suggested by the high frequency of strongly increased grading after restaining. It also seems logical that these few, poorly stained AFB would fade rapidly, resulting in a seemingly negative smear, even in a mild climate.

Opponents of restaining have furthermore pointed out that this makes appraisal of smear and staining quality impossible. Our results indicate that such qualitative remarks may be less helpful than restaining, as they correlated poorly with the errors detected. Pale AFB was the only remark reasonably frequently associated with final findings of HFN and QNT; nevertheless, AFB were totally invisible in the great majority of false-negatives before restaining. Other qualitative remarks had a very poor positive predictive value, as they almost never correlated with an error, and no remarks were made for most of the smears with errors.

The results of this study using restaining thus indicate that a potentially inappropriate staining method was adopted by the Burundi NTP, and it was recognised that a change to the recommended hot ZN staining may be necessary. However, adoption of a better technique does not automatically exclude all errors due to poor staining. The chemicals purchased by NTPs are not always of certified quality, preparation of stains may be deficient (especially when decentralised to the periphery), quality control is the exception rather than the rule, and stains may deteriorate. Technical guidelines on staining may be available, but this does not necessarily mean that they are closely followed in all centres. The only way to ensure that the most serious errors of this type, i.e., those leading to invisible AFB, are not completely overlooked, is to restain all slides prior to rechecking using a certified stain and suitable technique. The need for restaining would obviously depend on the organisation within the NTP with respect to the quality of chemicals used for stains, their preparation, quality control, storage and distribution, as well as on-site supervision (and not only regular training programmes), to ensure that the correct techniques are used and technicians are motivated. If all these conditions are met, systematic restaining before rereading may indeed be a waste of time. If not, it would be wise to conduct a study similar to this before rejecting the need for restaining; even then the results may be valid for a short time only, and systematic restaining may be the only way to continuously cover all possible sources of error. A shift in priorities from big numbers to technical precision may simply be the best option to ensure a highly efficient rechecking system in most high prevalence countries.

CONCLUSIONS

Restaining all AFB smears prior to rechecking for quality control purposes may be more widely necessary, mild climates included, to avoid totally invalid conclusions and incorrect accusations of false-positive results. It may also be the only way to recognise fundamental problems of stains or staining technique, resulting in high rates of false-negatives. This will unavoidably result in increased workload. Our results, obtained with often very small samples for each centre, suggest that a more laborious rechecking technique is preferable to rechecking high numbers using a technique that leaves room for misinterpretation. Each NTP should decide for itself whether restaining is necessary, taking into account factors such as the quality of stains purchased, centralised or decentralised stain preparation and staining technique. It seems likely that a small study will often be required.

Cold staining techniques should be avoided in NTPs with problems such as poor microscopes, inadequately trained or supervised technicians, poor quality reagents, or poor stain preparation technique, including lack of quality assurance. They invariably result in fewer and weakly stained AFB, which may lead to severe loss of sensitivity of the technique. This would be disastrous for the correct examination of paucibacillary specimens, for instance for diagnosis in human immunodeficiency virus positives or in follow-up specimens (i.e., recognition of failures).

Acknowledgements

We are grateful to Dr Md A H Salim and his team at the Damien Foundation Bangladesh for their willingness to undertake the control readings using hot staining, and to Dr Etienne Declercq, Damien Foundation Brussels, for his sustained support of operational trials.

References

1 Rieder H L, Chonde TM, Myking H, et al. The Public Health Service national tuberculosis reference laboratory and the national laboratory network. 1st ed. Paris: IUATLD, 1998.
2 Van Deun A. Rapid fading of carbolfuchsin stained AFB under extreme conditions of temperature and humidity. Int J Tuberc Lung Dis 1997; 1: 384–385.
3 Van Deun A, Portaels F. Limitations and requirements for quality control of sputum smear microscopy for acid-fast bacilli. Int J Tuberc Lung Dis 1998; 2: 756–765.
4 Van Deun A, Roorda F A, Chambugonj N, Hye Md A, Hossain Md. A. Reproducibility of sputum smear examination for acid-fast bacilli: practical problems met during cross-checking. Int J Tuberc Lung Dis 1999; 3: 823–829.
5 de Kantor I N, Laszlo A, Vazquez L, et al. More on periphery to centre quality control of sputum smear microscopy and 'rapid fading' of Ziehl-Neelsen staining. Int J Tuberc Lung Dis 2001; 5: 387–389 (letter).
6 de Kantor I N, Laszlo A, Vazquez L, et al. Periphery to centre quality control of sputum smear microscopy and 'rapid fading' of Ziehl-Neelsen staining. Int J Tuberc Lung Dis 2000; 4: 887–888 (letter).
7 Tan Thiam Hok. A simple and rapid cold staining method for acid-fast bacilli. Am Rev Respir Dis 1962; 85: 753–754.
8 Enarson D A, Rieder H L, Arnadottir T, Trébucq A. Management of tuberculosis: a guide for low income countries. 5th ed. Paris: IUATLD, 2000.
9 International Union Against Tuberculosis and Lung Disease.

Technical guide for sputum examination for tuberculosis by direct microscopy. Bull Int Union Tuberc Lung Dis 1978; (Suppl 2): 4–16.
10 Sticht-Groh V, Bretzel G. Staining for acid-fast bacilli: hot or cold? Tubercle Lung Dis 1994; 75: 318.
11 Allen J L. A modified Ziehl-Neelsen stain for mycobacteria. Med Lab Sciences 1992; 49: 99–102.
12 Somoskovi A, Hotaling J E, Fitzgerald M, et al. Lessons from a proficiency testing event for acid-fast microscopy. Chest 2001; 120: 250–257.

RÉSUMÉ

CONTEXTE : Les services de routine de lutte contre la tuberculose au Burundi.
OBJECTIF : Déterminer dans quelle mesure la recoloration systématique des frottis d'expectoration pour recherche des bacilles acido-résistants (AFB) est nécessaire avant la relecture du contrôle de qualité.
SCHÉMA : Relecture à l'aveugle avant et après recoloration des frottis périphériques de routine, suivie d'un deuxième contrôle en cas de discordance.
RÉSULTATS : Sans la recoloration, ont été déclarés faux 10/825 frottis négatifs (1,2%) et 59/189 frottis positifs (31,2%). Après recoloration, on a trouvé 34 faux négatifs (4,1%) et 13 faux positifs (6,9%), deux changements hautement significatifs. Avant recoloration la quantification des frottis positifs montrait des valeurs trop élevées, tandis qu'après recoloration 41 des 42 positifs avaient des valeurs trop faibles.
CONCLUSIONS : Malgré les conditions climatiques tempérées du Burundi, la recoloration de toutes les lames avant relecture ont révélé un problème répandu et non connu de faux négatifs, au lieu d'un problème de faux positifs. Ceci a montré la nécessité d'une ré-appréciation critique des procédures standard utilisant les colorations à froid, plutôt que d'une ré-entraînement, comme on aurait pu le conclure à partir des résultats sans recoloration. Une recoloration systématique de toutes les lames préalablement au contrôle peut être plus largement indispensable dans des programmes nationaux pour couvrir l'ensemble des causes sérieuses d'erreur. La coloration à froid devrait être évitée dans les programmes sur le terrain, puisque sa performance peut être facilement altérée par des facteurs défavorables fréquemment rencontrés.

RESUMEN

MARCO DE REFERENCIA : Servicios rutinarios de control de la tuberculosis de Burundi.
OBJETIVO : Determinar si la contratinción sistemática de los frotis de esputo en búsqueda de bacilos ácido-alcohol resistentes es necesaria antes de la evaluación de control de calidad.
DISEÑO : Control en ciego de frotis de los laboratorios periféricos, con un segundo control en caso de discordancia, antes y después de contratinción.
RESULTADOS : Sin contratinción, 10/825 (1,2%) frotis negativos y 59/189 (31,2%) frotis positivos fueron declarados falsos. Después de contratinción, se encontraron 34 (4,1%) falsos negativos y 13 (6,9%) falsos positivos ; ambos cambios fueron considerados altamente significativos. Antes de la contratinción, la cuantificación de frotis positivos mostró valores demasiado elevados, y después de la contratinción 41 de 42 frotis positivos tenían valores demasiado bajos.
CONCLUSIÓN : A pesar de las condiciones climáticas temperadas en Burundi, la contratinción de todas las láminas antes de la lectura de control mostró un problema esparcido y desconocido de falsos positivos en lugar de uno de falsos negativos. Necesita una re-apreciación crítica del procedimiento estándar que utiliza la tinción en frío, más bien que un re-entrenamiento del personal, como se hubiera podido inferir a partir de los resultados sin contratinción. La contratinción sistemática de todas las láminas antes de la relectura de control puede ser más ampliamente necesaria en los programas nacionales contra la tuberculosis para cubrir todas las causas serias de error. La tinción en frío debiera ser evitada en los programas de terreno, puesto que su rendimiento es fácilmente afectado por factores adversos frecuentemente encontrados.