TRSTMH

Focused Assessment with Sonography for Urinary Schistosomiasis (FASUS) – pilot evaluation of a simple point-of-care ultrasound protocol and short training program for detecting urinary tract morbidity in highly endemic settings

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Full Title:	Focused Assessment with Sonography for Urinary Schistosomiasis (FASUS) – pilot evaluation of a simple point-of-care ultrasound protocol and short training program for detecting urinary tract morbidity in highly endemic settings
Abstract:	Background Urogenital schistosomiasis (UGS) causes inflammation and fibrosis of the urinary tract. In resource-limited settings, affordable tools for morbidity assessment in clinical care are needed. Point-of-care ultrasound has not yet been validated for UGS-related pathology. Methods We developed a protocol for Focused Assessment with Sonography for Urinary Schistosomiasis (FASUS), assessing pathology of the bladder wall, ureters and kidneys. Following standardized training, two clinicians performed FASUS on children and adults with haematuria in Lambaréné, Gabon. Recorded ultrasound clips were remotely reviewed by two ultrasound experts as diagnostic reference. Results In 2015 and 2016, scans were performed in 118 patients. Image quality was sufficient in 90% of bladder views and more than 97% of kidney views. UGS-compatible pathology was detected in 51/118 (43%) by the operator and in 46/107 (43%) by the experts among baseline scans of sufficient quality. Inter-rater agreement between operators and experts was very good ($\kappa > 0.8$) for hydronephrosis and good ($\kappa > 0.6$) for bladder wall thickening. Conclusions FASUS is a promising clinical, point-of-care tool for detecting USG-related renal tract morbidity in symptomatic patients. Based on larger validation studies, appropriate diagnostic and therapeutic algorithms for the use of FASUS should be established.
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Abstract

Background: Urogenital schistosomiasis (UGS) causes inflammation and fibrosis of the urinary tract. In resource-limited settings, ~~novel diagnostic~~affordable tools ~~are needed~~ for morbidity assessment in clinical care are needed. Point-of-care ultrasound has not yet been ~~investigated on the detection of~~validated for UGS-related pathology.

Methods: We developed a protocol for Focused Assessment with Sonography for Urinary Schistosomiasis (FASUS), assessing pathology of the bladder wall, ureters and kidneys. Following standardized training, two clinicians performed FASUS on children and adults with haematuria in Lambaréné, Gabon. Recorded ultrasound clips were remotely reviewed by two ultrasound experts as diagnostic reference.

Results: In 2015 and 2016, ~~FASUS~~ scans were performed in 118 patients. Image quality was sufficient in 90% of bladder views and more than 97% of kidney views. UGS-compatible pathology was detected in 51/118 (43%) by the operator and in 46/107 (43%) by the experts among baseline scans of sufficient quality. Inter-rater agreement between operators and experts was very good (κ > 0.8) for hydronephrosis and good (κ > 0.6) for bladder wall thickening.

Conclusions: FASUS is a promising ~~bedside~~ clinical, point-of-care ~~diagnostic~~ tool ~~with good diagnostic accuracy~~for detecting USG-related renal tract morbidity in symptomatic patients ~~in this pilot assessment~~. Based on ~~data from~~larger validation studies ~~larger cohorts examined with FASUS~~, appropriate diagnostic and therapeutic algorithms for the use of FASUS should be established.

Keywords: Schistosoma Haematobium, Hydronephrosis, Diagnostic Imaging, Point-of-Care Testing, Tropical Medicine, Gabon

Introduction

Urogenital schistosomiasis (UGS), a waterborne disabling parasitic neglected tropical disease, affects an estimated 112 million people, with more than 400 million people at risk.[1, 2] Chronic infection and its sequelae cause substantial morbidity reported mainly, but not exclusively, in school-aged children and young adults.[3, 4] UGS-related pathology comprises bladder wall thickening, distal ureteric wall thickening, and in an advanced stage also upper urinary tract obstruction and renal failure,[5] as well as squamous cell carcinoma of the bladder.[6] If treated early in the course of disease, a certain proportion of the pathological changes in the urinary tract are reversible.[3] In patients with irreversible damage, options for treatment will depend on the type of damage and available treatment.

In most high and moderate-prevalence areas the only available diagnostic tools are detection of haematuria by urine dipstick and direct parasitological examination of the urine. These tools, as well as novel methods such as detection of specific antibodies, antigens or genetic material,[7-9] can confirm infection but detect neither anatomical changes due to the infection nor sequelae after treatment.

In prevalence surveys, uUltrasound has long been established as is a n well-documented, effective imaging tool to assess urinary tract morbidity in UGS and to monitor treatment response, with results comparable to more invasive methods such as cystoscopy.[10] Being a test for morbidity and not active infection, ultrasound does not replace but complement parasitological tests.

In 1996 the World Health Organization (WHO) published a guideline standardised protocol for the ultrasonographic assessment of schistosomiasis-related morbidity, commonly known as the Niamey protocol.,[11] This protocol resulted from an expert meeting collating available knowledge and experience on previously published standardized protocolsHowever, this protocol is specifically designed developed for application in large-scale control programs and not .for diagnosis in the clinical setting. Suggested evaluations of thisIt landmark document for ultrasound in schistosomiasis and requireincludes a ing detailed and relatively complex grading system, which is less relevant to clinical application. relatively advanced ultrasound skills due to its complexity and recommendations for improvement have been formulated[12]. Contrary to widespread experience of using ultrasound in prevalence surveysTo date, ultrasound it has not been widely used or validated in for routine patient care in schistosomiasis endemic areas. In addition, prevalence surveys mostly rely on operators with professional ultrasound training. In many clinical settings, this expertise is lacking.

Point-of-care ultrasound (POCUS)OCUS has been successfully implemented for various other indications in tropical medicine, e.g. HIV-associated tuberculosis and echinococcosis.[13]

Field Code Changed

The aim of our study was to pilot a POCUS protocol for detection of UGS-related renal tract morbidity in symptomatic patients, to evaluate practical feasibility and evaluate the performance of operators with limited training, using low cost equipment.

Now that point-of-care ultrasound (POCUS) is expanding rapidly in resource constrained settings, a simplified and more pragmatic protocol to identify relevant pathology may be of value to establish the clinical diagnosis, assess urinary tract damage, guide appropriate management and monitor treatment response.[12]

Being a test for morbidity and not active infection, such a protocol will not replace but complement parasitological tests. POCUS has been successfully implemented for various other indications in tropical medicine, e.g. HIV-associated tuberculosis and echinococcosis.[13]

The aim of our study was to develop pilot a Point of care ultrasound (POCUSPOCUS) protocol for detection of UGS-related renal tract morbidity in symptomatic patients, y and to evaluate practicalits feasibility and evaluate the performance of operators withaccuracy after limited training, using low cost equipment of operators using low-cost equipment, .

Methods

This study was conducted at the Centre de Recherches Médicales de Lambaréné (CERMEL) located in the semi-rural setting of Lambaréné, Gabon, where UGS is highly prevalent.[14-19] It was part of a larger study, which additionally assessed epidemiological aspects as well as immunological and metabolomic aspects of UGS; these will be reported separately. In summary, this study comprised the following parts:Procedures relevant for the pilot assessment of our POCUS protocol were A) development of a POCUS scan n ultrasound protocol for point-of-care detection of UGS-related pathology; B) training of two operators on this protocol; C) evaluation of the diagnostic accuracy of this protocol in patients with UGS withPOCUS operators, using remote expert image review as reference. D) Comparing ultrasound findings with parasitology test results. A portable low-cost ultrasound device (MINDRAY Digital Ultrasonic Diagnostic Imaging System model DP-10) with a curved array transducer (MINDRAY model 35C50EB) was used for training as well as for study procedures.

Protocol development

A simple and easy-to-learn protocol was designed by the study team, selecting clinically relevant criteria adapted from the Niamey protocol, which . This WHO guideline provides instructionsstandards for the evaluation of the urinary bladder (shape, bladder wall thickening, irregularities and polyps), ureters (ureteral dilation), renal pelvis (degree of hydronephrosis), and additional evaluations (bladder volume, residual urine after voiding, fibrosis of the renal pelvis); final evaluations includes multiple measurements for the calculation of a final severity score." [11] Based on the nomenclature of previous POCUS techniques[13] we called named our protocol:it "Focused Assessment with Sonography for Urinary Schistosomiasis" (FASUS). The emphasis in our selection of criteria was on identification of bladder wall pathology as well as renal pelvis and ureter dilatation, assumedwas on image acquisition and interpretation, to be feasible for operators without previouswith little or no previous ultrasound experience, but adequate to make a diagnosis of likely UGS-related pathology; more sophisticated procedures such as measurement of bladder volume were omitted, as well as complex severity grading scores.. . The initial protocol comprised a transverse scan of the bladder and a longitudinal and transverse scan of both kidneys. During the course of the study a longitudinal bladder scan was added, given the potential added value in detection of bladder dome pathology. Abnormalities compatible with UGS were defined as any bladder wall thickening ≥ 5mm, as in the Niamey criteria, or bladder wall calcification, regardless of kidney and ureter findings. be d to be littlerelevance Abnormalities of the kidneys and ureters without bladder pathology were not considered compatible with UGS, as this is a highly uncommon finding and etiologies other than UGS are more likely.. A description of FASUS probe positions in FASUS is presented in *Figure 1*. The scan planes and documented variables are presented in *Table 1*. Examples of target pathologies are presented in *Figure 2*.

Training

Two operators, a clinician (JR) and a medical student (AV) with little and no previous ultrasound experience respectively, were trained on site. The training took around 20 hours overall and included a theoretical part (study of ultrasound literature[20] and study of the FASUS protocol) followed by a practical part comprising five test scans in healthy volunteers per operator. Still images and video clips recorded during the five test scans were independently and remotely reviewed by two experts, a paediatric infectious diseases clinician with four

years (SB) and a clinical radiologist with over 20 years of ultrasound experience (EJ). Detailed feedback on image quality and visualization of target structures was provided, along with practical advice for improvement of technique. ~~AV was additionally trained by JR on site.~~

Prospective evaluation of the protocol

Participants of all ages were recruited by convenience sampling in areas of Lambaréné identified as hotspots for UGS in previous studies.[16, 17] Inclusion criteria w~~as~~ere ~~history of,~~previous or ongoing macro-haematuria, exclusion criterion was known disease of the urinary tract related to causes other than UGS. Convenience sampling was considered appropriate, as this was not intended as a prevalence survey, but as a cohort of patients with clinical symptoms. After an initial clinical assessment, FASUS and urine collection for parasitology were performed. Following praziquantel treatment, active follow up visits were attempted after one and three months.

Clinical and laboratory procedures

Clinical assessment consisted of a questionnaire on present or previous symptoms of UGS, known bladder or kidney disease, previous praziquantel treatment, duration of fresh water contact and pregnancy. Three urine samples provided between 10 a.m. and 2 p.m. on three consecutive days were requested. Semi-quantitative urine dipstick testing as well as urine filtration (10 ml urine) for microscopy for presence of *S. haematobium* eggs were performed (Dipstick: Combur 10 Test®, Roche Diagnostics Ltd, Risch, Switzerland; Millipore filter: Nuclepore Track-Etch Membrane Filtration Products, Whatman plc, Maidstone, UK; microscope: Eclipse E200MV R, Nikon, Tokyo, Japan). A positive result was defined as any detection of eggs in the filtered urine. Heavy intensity infection was defined as ≥ 50 eggs/10 ml urine.[21] In addition, 10ml of urine were centrifuged for five minutes at 710 x g and after discarding of the supernatant, 500µl of urine was stored at -20°C for *Schistosoma* genus PCR. In case of negative microscopy, urine real-time PCR for *Schistosoma*-specific DNA was performed at CERMEL as reported previously.[22]

Treatment and follow up

Patients with confirmed urogenital schistosomiasis by at least one urine sample or by FASUS expert interpretation were treated with a single dose of praziquantel (40mg/kg). If non-UGS-related pathology was found in the FASUS exam, the participant was referred for further management.

Follow up (FU) visits took place one (M1) and three (M3) months after treatment. Procedures performed during follow up comprised a clinical investigation, urine analysis by dipstick, and microscopy. Patients with FASUS findings suggestive of UGS at enrolment underwent a follow up scan, reviewed and documented similar to enrolment, but using a slightly shorter report form. In case of persistent signs of urinary tract pathology at M3, retreatment with praziquantel was provided. If advanced UGS-related pathology was not responsive to treatment, the participant was referred to urologic care in the capital for further management.

Focused assessment with sonography for urinary schistosomiasis (FASUS)

FASUS scans were performed and evaluated by one of the two operators (*Table 1*). FASUS videoclips and images were stored and remotely reviewed and independently interpreted by two ultrasound experts (SB and EJ). This reference test was chosen because cystoscopic evaluation of the bladder as gold standard would

not have been medically nor ethically justified, and a locally performed expert reference scan was not feasible in the absence of experts.

A portable low-cost ultrasound device (MINDRAY Digital Ultrasonic Diagnostic Imaging System model DP-10) with a curved array transducer (MINDRAY model 35C50EB) was used for training as well as for study procedures

. Participants underwent FASUS examination either at their homes or at CERMEL following standardized scanning conditions (same ultrasound equipment, patients in supine position, d~~reclined on examination couch or bed, room d~~arkened room, operator seated on the patient's right side). FASUS at enrolment was performed by operator one (JR) and all follow up scans by operator two (AV). ~~A darkened room and S~~sufficient bladder filling, defined as complete distention of the bladder, was~~ere~~ attempted as a precondition to the exam. Anonymized patient data and ultrasound findings were documented on a clinical record form. Images were stored as digital video clips (AVI format) on the ultrasound device; if pathology was suspected, additional still images (JPEG format) were stored. Anonymized data were transferred to an external hard drive, compressed using the open source video transcoder "HandBrake" (https://handbrake.fr) and bundled into zip files using the program "7zip" (http://www.7-zip.org), in order to reduce file size for upload. Zip files were uploaded to the secure teleradiology platform "Collegium Telemedicus" (https://collegiumtelemedicus.org) together with basic clinical data (age, sex, current complaints, history).

All images were independently reviewed by the two experts, blinded to the operators' findings and laboratory data. Review criteria included the diagnostic quality of the scan (overall image quality, depth, gain, focus, anatomical orientation, sufficient bladder filling) as well as the presence of pathology as presented in **Error! Reference source not found.**. The experts documented their findings in an anonymized digital PDF report form. Any discrepant reading between the reviewers was resolved by consensus. For pragmatic purposes, discrepancies in the severity grading of bladder wall pathology were not resolved and only the expert findings of the radiologist EJ are presented here.

Analysis

Data was entered into OpenClinica (OpenClinica® version 3.0.4, Boston, USA). Data analysis was performed using Microsoft Excel (Microsoft company, Redmond, WA, USA).

Results

Participants

Between December 2015 and June 2016, 119 participants were enrolled. One patient withdrew consent after enrolment. The cohort comprised 27 (23%) pre-school age children (PSAC, age < 6 years), 56 (47%) school-age children (SAC, 6-15 years) and 35 (30%) adults (> 15 years); 56/118 (47%) were female. Microscopic haematuria on dipstick was present in 103/118 (87%) patients. Urine microscopy was positive for *S. haematobium* in 105/118 (89%) patients; 69/118 (58%) patients were heavily infected. *S. haematobium* PCR was negative in all 13 patients with negative microscopy. M1 follow up was done for a total of 70/118 (59%) patients, M3 follow up was done for a total of 82/118 (69%) patients. Age and gender distribution of the follow up cohort was comparable to the entire study population.

Image quality

Of a total of 224 FASUS scans, 178 (79%) were performed at the patients' residence and 46 (21%) at CERMEL. The proportion of views considered of sufficient quality for diagnostic interpretation is presented in *Table 2*. Quality of bladder views was sufficient in 22/27 (81%) PSAC, 54/56 (96%) SAC and 31/35 (89%) adults. Image depth, gain and focus were rated as good in 99%, 98% and 98% of all scans, respectively. The bladder was entirely displayed in 95% of the recorded sweeps. Bladder filling was judged sufficient in 86%; in 7 out of 11 bladder scans with insufficient quality, the bladder was underfilled. Anatomical orientation of the kidney scans was good in 92% with the recorded sweep displaying the renal pelvis in 99%.

Ultrasound findings

Pathological features compatible with UGS among baseline scans of sufficient quality were documented in 51/118 (43%) of cases by the operator and in 46/107 (43%) by the experts, respectively. Frequencies of UGS-related pathologies at baseline by operator and expert are presented in *Figure 3*. Detection rates of bladder wall pathology, kidney pelvis and proximal ureter dilatation were similar between operator and experts; , while distal ureter dilatation or thickening was detected 3 times as often folds more by the experts than by the operator. Frequencies of UGS-related pathologies at baseline as assessed by the experts in relation to parasitology results are presented in *Figure 4*. With increasing intensity of infection, bladder and upper urinary tract pathology was more frequently detected.

Taking expert findings as the gold standard, the operators missed features compatible with UGS in 21/202 (10%) cases and documentedoverdiagnosed false positivefeatures compatible with UGS FASUS in 9/202 (4%). Sensitivities and specificities of operator FASUS against the reference standard of a remote expert review are presented in *Table 3*.

The highest agreement between operator and expert was seen in renal pelvis dilatation, followed by bladder wall thickening ≥ 5mm and proximal ureter dilation. For distal ureter pathology agreement was lowest for both operators; inter-rater agreement expressed as Cohen's Kappa is presented in *Table 3*.

The development of image quality, diagnostic accuracy and inter-rater agreement with increasing number of scans is presented in *Figure 5* for each operator.

Among 85 cases assessed by both transverse and longitudinal bladder scan, pathology compatible with UGS was detected in 35 (41%) by transverse scan alone compared to 38 (45%) by a combination of both scans.

In five cases, renal pelvis and/or ureter dilatation was present without bladder wall thickening ≥ 5mm. One of these was pregnant, in three others bladder wall irregularities were present. All five were positive for *S. haematobium* in urine microscopy.

Pathology not related to UGS was detected in 1/118 (1%) patients by the operators and in 9/118 (8%) patients by the experts and comprised free fluid in the pelvis (n = 3), non-specific kidney lesions (n = 2), pregnancy-related hydronephrosis, a urachal remnant, non-specific uterine lesions and non-specific strands in the bladder ~~(one case each)~~.

During follow up, pathology compatible with UGS was present in 15/46 (33%) cases at M1 and in 16/49 (33%) cases at M3 among scans with sufficient quality. Hydronephrosis was present in 3/51 (6%) and 3/54 (6%) at M1 and M3, respectively. As criteria for follow up were not consistent, these rates cannot be used to measure treatment success.

Discussion

This pilot ~~diagnostic accuracy~~ study was the first to evaluate a POCUS protocol for the detection of UGS-related pathology of the urinary tract in symptomatic patients living in a high-endemic area. It showed that POCUS operators with limited training can detect such pathology with high ~~diagnostic~~ accuracy, compared to expert ultrasound operators; inter-rater ~~-~~agreement between operator and expert was good for the detection of bladder wall pathology and very good for hydronephrosis. Image quality was sufficient in more than 88% of all scans.

Protocol design

In our protocol, pathology compatible with UGS was defined as bladder wall thickening ≥ 5mm. This cut-off was chosen, based on existing Niamey criteria. ~~–~~However, in many cases bladder wall irregularities < 5mm were detected, ~~suggesting~~ indicating that less advanced pathology, ~~also~~ compatible with UGS, can also be detected with current equipment. and short focused training.~~-~~ This finding may be of value, as earlier detection of morbidity may influence management decisions. ~~–~~Differentiation of bladder wall irregularities from a normal bladder wall remains challenging as illustrated by *Figure 2* and reported by other studies.[12] Therefore, a threshold of 5 mm appears sensible to limit false positive cases. To keep the protocol ~~as simple as possible~~as pragmatic as possible for use in the clinical setting, a classification of pathology severity, combining bladder and upper urinary tract pathology, as applied in other studies,[5, 23] was not used. However, as upper urinary tract pathology with concurrent bladder wall irregularities less than~~<~~ 5mm is also likely to be due to UGS (though classified as “not compatible with UGS” in our study), this approach needs to be ~~reconsidered~~reconsidered in larger validation studies.~~-~~

As expected, the operators detected fewer abnormalities not related to UGS. The aim of POCUS is to answer a very specific clinical question, in this case: "are there findings compatible with UGS", and not to provide a comprehensive ultrasound examination or to exclude the presence of other pathology.

While the Niamey Protocol[11] comprises only a transverse view of the bladder, we added a longitudinal bladder view assuming a higher sensitivity for detection of pathology especially at the bladder dome. With three additional cases of bladder pathology detected by the additional longitudinal view, ~~a gain in sensitivity was observed~~we recommend including this view in further evaluations of this protocol. ~~Further studies with larger sample sizes are required to externally validate our results. A possible future approach could be that when a transverse scan does not show any pathology, a longitudinal scan should be performed in addition.~~

Training

During training and ~~study~~ data collection, the team was repeatedly approached by local health care staff willing to learn the method, showing the general interest in the achievement of ultrasound skills among local clinicians.

Remote training support and image assessment proved effective to attain good image quality, but as it comprised only scans in healthy volunteers, identification of pathology could be learnt in theory only. An e-learning module with quiz-like character could resolve this deficit. Although remote feedback was given for study patient scans as well, it was staggered and therefore time-delayed. The optimal number of training scans for FASUS needs to be determined; a broad spectrum of training requirements for point-of-care

ultrasound protocols, ranging from a few hours to several days, has been proposed depending on sonographic complexity and previous ultrasound knowledge.[24-26]

Image quality

Overall image quality was very good with more than 90% of bladder walls and more than 97% of kidneys and proximal ureters being assessable by the expert. Display of distal ureters was slightly more challenging. The high expert ratings on gain, depths and focus settings show the effectiveness of the chosen remote training model.

Image quality was best among school-children, due to excellent display of deep anatomic structures related to smaller size. Inferior quality among pre-school children and some adults can be explained by limited compliance and additional body fat, respectively.

Interestingly, only two kidney scans and no bladder scans were rated as “unable to assess” by operator one, and only two bladders and one kidney scan by operator two, showing that the operators attempted to make assessments, despite limitations in image quality. This is a potential source of error and future training should emphasize this risk.

As reported in other studies,[12] sufficient bladder filling is crucial for the quality of bladder scans. In the absence of a definition or measurement for sufficient bladder filling, in our study insufficient bladder filling was judged by eyeballing bladder content and wall. For future application of FASUS, a clear definition of sufficient or insufficient bladder filling would be valuable; however, including measurements of bladder volumes for age-related normal ranges would considerably increase complexity ~~of the so far simple protocol~~. ~~Definitely, the~~Learning the critical point of insufficient bladder filling needs to be part of FASUS training~~s~~.

~~Diagnostic~~ Aaccuracy ~~of~~ of pilot operators, compared to experts:

Overall, the ~~diagnostic~~ accuracy of FASUS, performed by our pilot operators, was good for the detection of bladder wall thickening ≥ 5mm and very good for the detection of hydronephrosis. Accuracy for the detection of ureter pathology was lower, with 8/10 and 5/6 cases of distal ureter pathology missed by the two operators, respectively. As all cases of distal ureter pathology occurred together with bladder wall pathology, the additional diagnostic value of distal ureter assessment needs to be questioned. Considering its difficulty, it should rather be omitted from the protocol.

While image quality and sensitivity ~~,~~ improved for both operators over the course of the study, there was a slight decline in specificity. Although conclusions need to be drawn with caution due to the small sample size, this might point at a shift from underestimation to overestimation of pathology with increasing number of scans.

In another study on the learning curve of the~~,~~ more complicated~~,~~ Niamey protocol for prevalence surveys~~,~~ by Bonnard et al., sensitivity and specificity of the learner in comparison to the teacher had both reached 100% after 5 days and 91 examinations.[27] However, the teacher directly supervised the learner, in contrast to the staggered remote feedback over the period of around three months per operator in our study. Furthermore, the authors left unclear whether the learning clinician had previous ultrasound experience. Two~~Few~~ other studies on remotely reviewed POCUS in tropical medicine describing the detection of extra-pulmonary TB provided a ~~diagnostic , accuracy~~ sensitivity and specificity of the operator compared to the expert that was

comparable to our study.[28, 29] However, unlike with our approach, exams were transmitted and assessed remotely in real-time and not via saved video clips, limiting comparison.

As urinary tract pathology increased with increasing intensity of infection in this as well as other studies,[30] an algorithm that combines parasitological with ultrasonographic findings, especially in patients with heavy intensity of infection, could maximize diagnostic accuracy.

Overall, the learning of this POCUS technique via remote review and feedback appears to be feasible and leads to good ~~diagnostic~~ accuracy that can be maintained over longer periods.

Of note, ~~As recruitment was done via convenience sampling,~~ as the intention of this study was not to identify infection rates, the reported rate of UGS-related pathology among the study population does not reflect that in the general population in Gabon.

Management of pathological findings

As sonographic detection of UGS-related pathology has mostly been used in the evaluation of large-scale control programs, no algorithms for individual patient management based on ultrasound are available. FASUS could help in the identification of at-risk patients in need of regular follow up and re-treatment, in areas where regular preventive treatment is not indicated or insufficiently implemented. Patients with advanced pathology such as therapy resistant hydronephrosis or suspicious bladder lesions could be referred for further diagnostics and management, where available.

Limitations

As a pilot study, the protocol underwent changes during the course of the study, thus parts of the data are incomplete. ~~As recruitment was done via convenience sampling, the rate of UGS-related pathology among the study population does not reflect that in the general population in Gabon.~~

Our reference test of a remote expert review is inferior to a locally performed expert reference scan, but was the best and only feasible option in the absence of local expertise. With only quality-reduced CINE clips and still images as a basis for the interpretation, pathology might have been missed by the experts or normal findings and artefacts mistaken for pathology. However, this telemedicine-based training and image review design provides a feasible model for ~~pilot studies of novel indications for~~ POCUS training in resource-limited settings, where no alternative options exist.

Conclusions

FASUS is a promising bedside diagnostic tool ~~with good diagnostic accuracy in this pilot assessment~~for detection of UGS- related morbidity in symptomatic patients living in high-endemic areas. Simple protocols and short training appear adequate and feasible, despite the common limitations present in a low resource setting, such as novice clinician trainees, low-budget ultrasound devices, and examinations performed in the field. If further validation studies confirm the utility of FASUS, internationally agreeable standards for training and competency ~~duration, contents and intensity~~ should be set. Based on data from larger cohorts ~~examined with FASUS in future studies~~, appropriate diagnostic and therapeutic algorithms for its use in routine patient care should be established.

Authors' statements

Authors' contributions: JR, AB, TH, EJ and SB conceived the study and JR, AB, TH, AAA, MPG, MR, EJ and SB designed the study protocol; JR and AV carried out the ultrasonographic assessments; EJ and SB performed the digital review of ultrasound images. AV, NGT and PM carried out the laboratory tests; JR, AV and RO performed the data analysis. JR, AB, TH, EJ and SB drafted the manuscript; all authors critically revised the manuscript and all authors contributed and approved of the final manuscript.

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Competing interests: None declared.

Ethical approval: This study was approved by the scientific review committee and the institutional ethics committee of CERMEL. Written informed consent was obtained from all patients (or legal guardians in case of children) prior to study enrolment.

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Figure legends

Figure 1: FASUS probe positions

1 and 2: Transverse and longitudinal suprapubic pelvis scan.

3 and 4: Longitudinal and transverse right kidney scan.

5 and 6: Longitudinal and transverse left kidney scan.

Figure 2: Examples of target pathologies detected by FASUS

Figure 3: Pathology detected by FASUS at baseline by operator and expert

NA: not available.

Figure 4: Pathology detected by FASUS at baseline (expert review) in relation to parasitology

[a] Urine microscopy and PCR.

[b] Renal pelvis dilatation > 1cm, proximal ureter dilatation or distal ureter dilatation/thickening.

Figure 5: Operator improvement in bladder wall assessment

[a] Image quality of bladder scans sufficient for expert interpretation.

[b] Ultrasound operator findings compared to expert consensus after remote review of recorded clips.

Table 1: FASUS protocol

Scan planes	**Assessed anatomy**	**Pathology variables[a]**
Supra-pubic pelvis		
1) Transverse	Bladder wall Distal ureters	a) Bladder wall irregularities < 5 mm thickness b) Wall thickening 5 – 10 mm c) Maximum thickening, masses or pseudopolyps ≥ 10 mm d) Bladder wall calcification e) Distal ureter dilatation or thickening f) Findings not related to urogenital schistosomiasis
2) Longitudinal[b]	Bladder wall Distal ureters	
Kidneys (right and left)		
1) Longitudinal	Proximal ureter and collecting system	g) Proximal ureter dilatation h) Findings not related to urogenital schistosomiasis
2) Transverse	Renal pelvis	i) Renal pelvis dilatation (> 1cm) • If abnormal, confirmed after bladder voiding j) Findings not related to urogenital schistosomiasis

[a] Any of b), c) or d) was defined as pathology compatible with urogenital schistosomiasis.

[b] Added to the protocol after recruitment of 45 cases because a higher sensitivity in the detection of bladder wall thickening was assumed.

Table 2: Ultrasound image quality sufficient for expert interpretation

		Bladder scan[a]		Right kidney scan		Left kidney scan	
Ultrasound operator[b]	**Total scans**	**Bladder wall**	**Distal ureters**	**Renal pelvis**	**Prox. ureter**	**Renal pelvis**	**Prox. ureter**
Operator 1, n (%)	118[c]	107 (91)	100 (88)	117 (99)	115 (97)	115 (97)	114 (97)
Operator 2, n (%)	106	95 (90)	93 (88)	106 (100)	103 (97)	104 (98)	104 (98)

Prox: Proximal.

[a] Combination of transverse and longitudinal scan, when available.

[b] Baseline scans performed by operator one, follow up scans performed by operator two.

[c] For distal ureter views at baseline: n = 114.

Table 1: Accuracy of newly trained operators, compared to expert ultrasound opinion~~Diagnostic accuracy of FASUS~~

~~Diagnostic~~ a~~A~~ccuracy[a]	Bladder wall thickening ≥ 5mm		Distal ureter dilatation/ thickening		Proximal ureter dilatation		Renal pelvis dilatation > 1cm[b]	
Operator[c]	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Total scans[d]	107	95	87	93	111	102	110	104
Sensitivity (%)	83	68	17	23	57	50	100	83
Specificity (%)	90	95	97	99	100	95	98	100
Observed inter-rater agreement	0.87	0.83	0.86	0.88	0.97	0.94	0.98	0.99
~~**Inter-rater agreement (**~~**Cohen's Kappa**~~**)**~~	0.73 "good"	0.64 "good"	0.19 "poor"	0.31 "fair"	0.71 "good"	0.23 "fair"	0.85 "very good"	0.90 "very good"

[a] Ultrasound operator findings compared to expert consensus after remote review of recorded clips.

[b] If confirmed after bladder voiding, or if severe.

[c] Baseline scans performed by operator one, follow up scans performed by operator two.

[d] Number of scans of sufficient image quality for interpretation by operator and expert consensus.

Figure 1

Click here to access/download;Figure;Figure 1.jpg

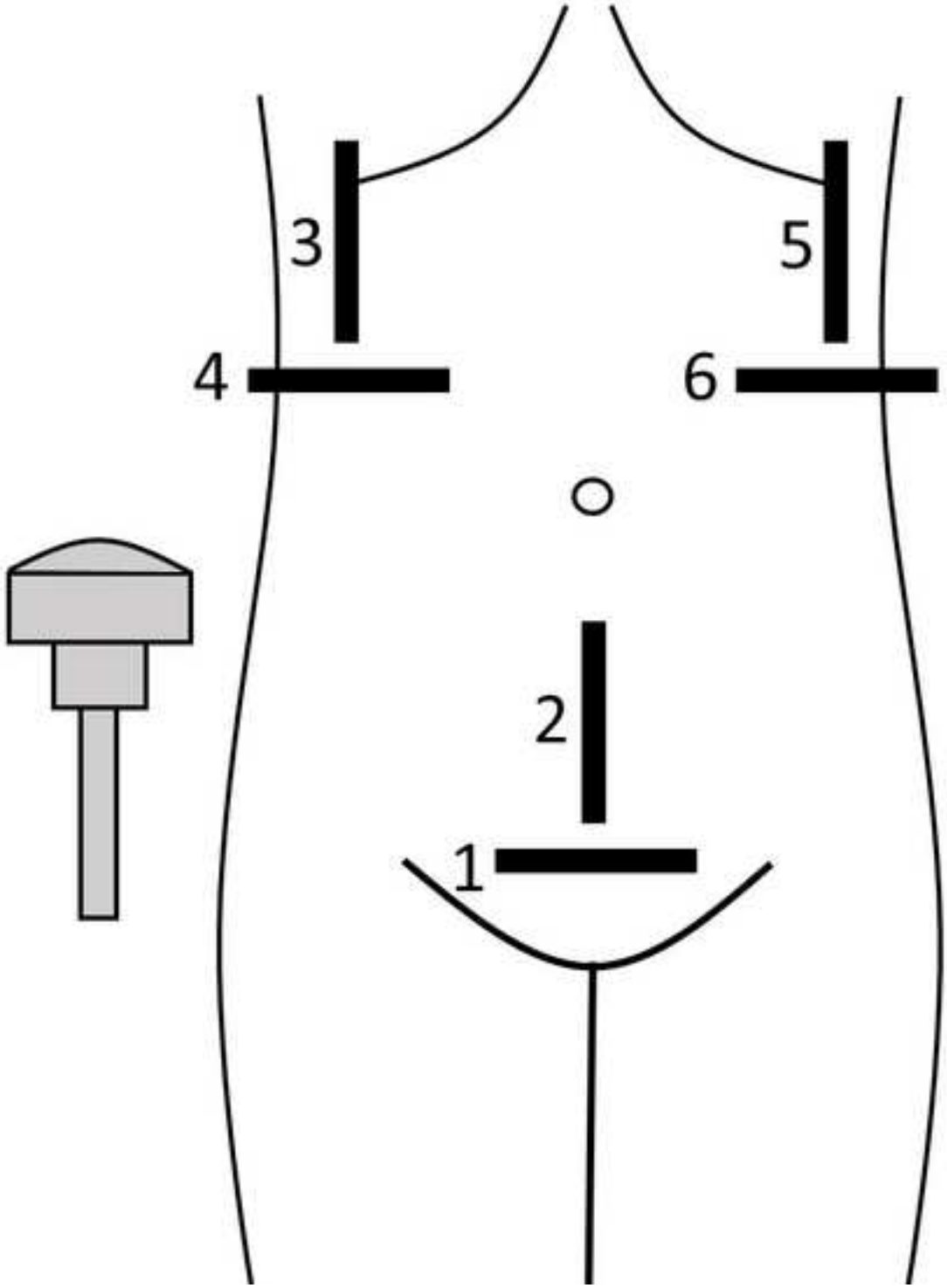
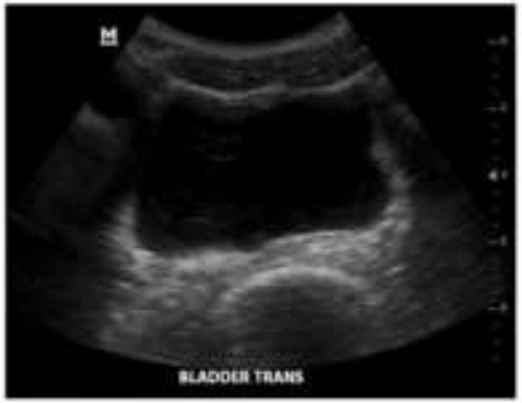
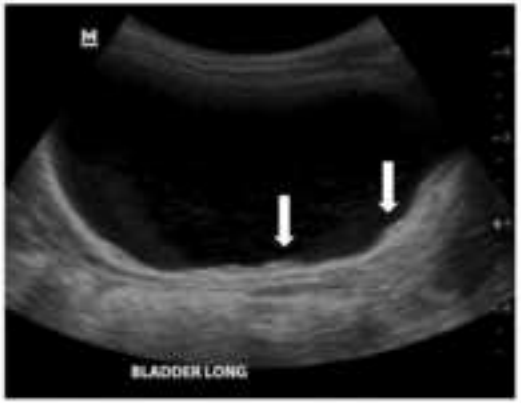


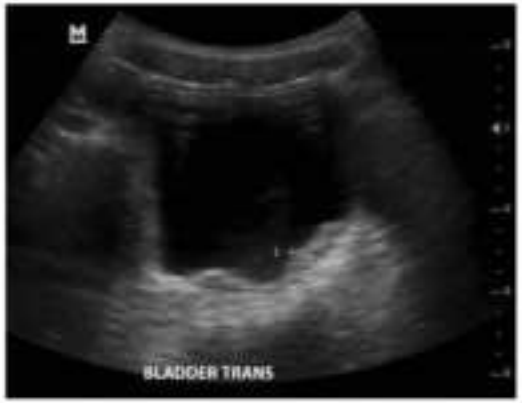
Figure 2



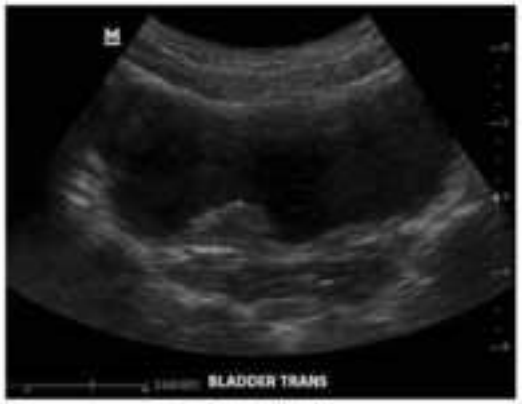
a) Normal bladder



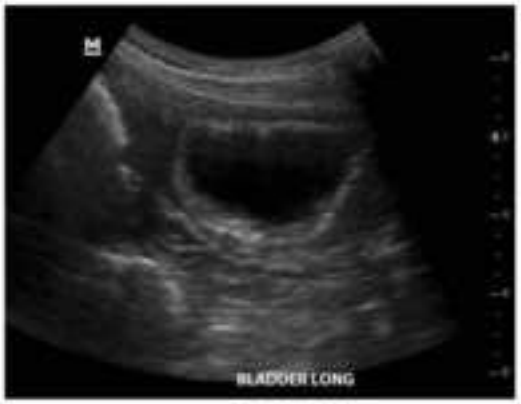
b) Bladder wall irregularities



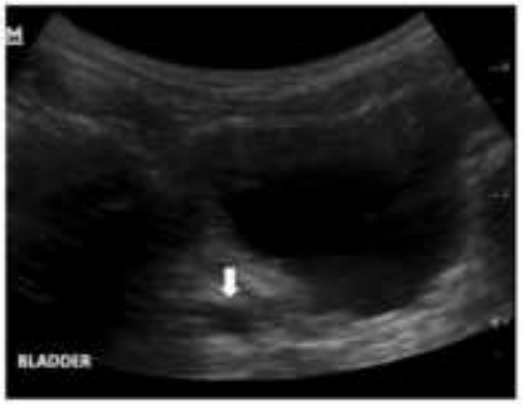
c) Bladder wall thickening 5-10mm



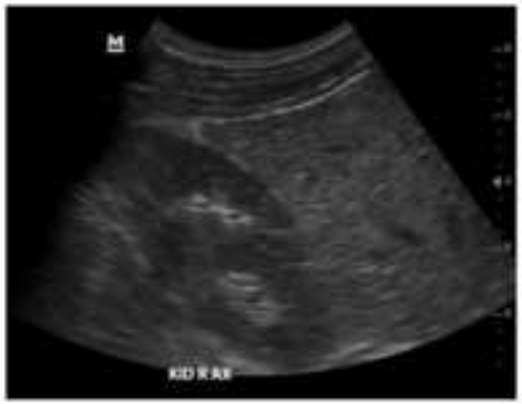
d) Bladder wall thickening >10mm



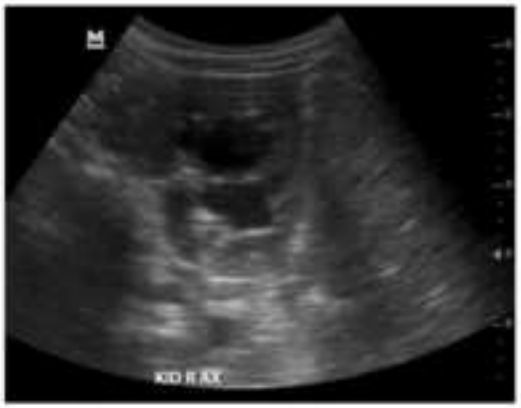
e) Bladder wall calcification



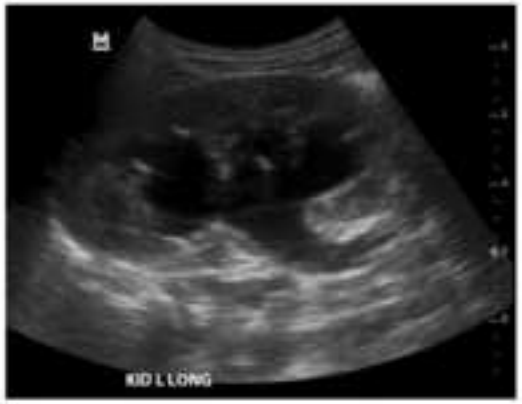
f) Distal ureter dilatation



g) Normal kidney



h) Kidney pelvis dilatation



i) Proximal ureter dilatation

Figure 3 colour

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Figure 3 grayscale

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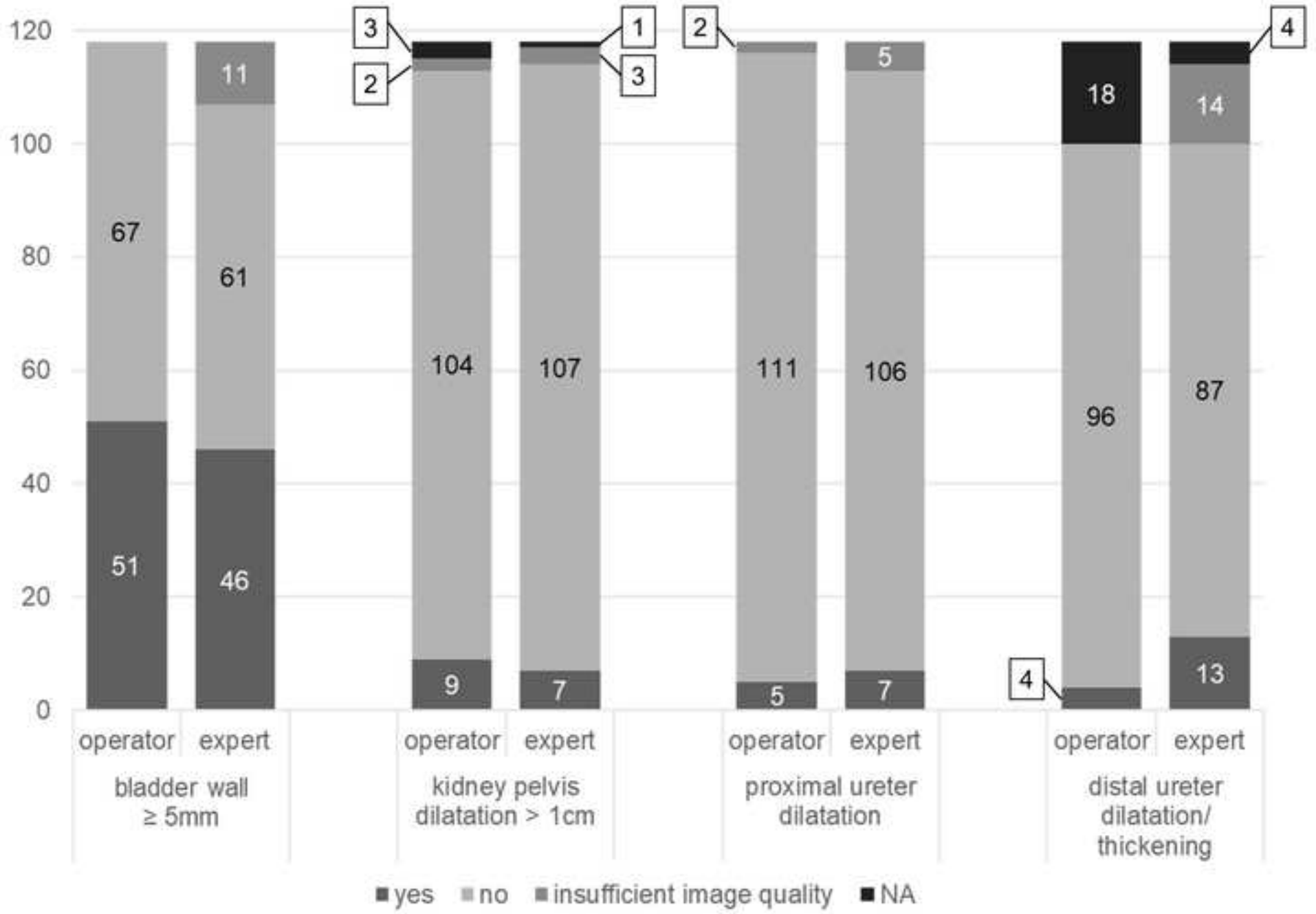

Figure 4 colour

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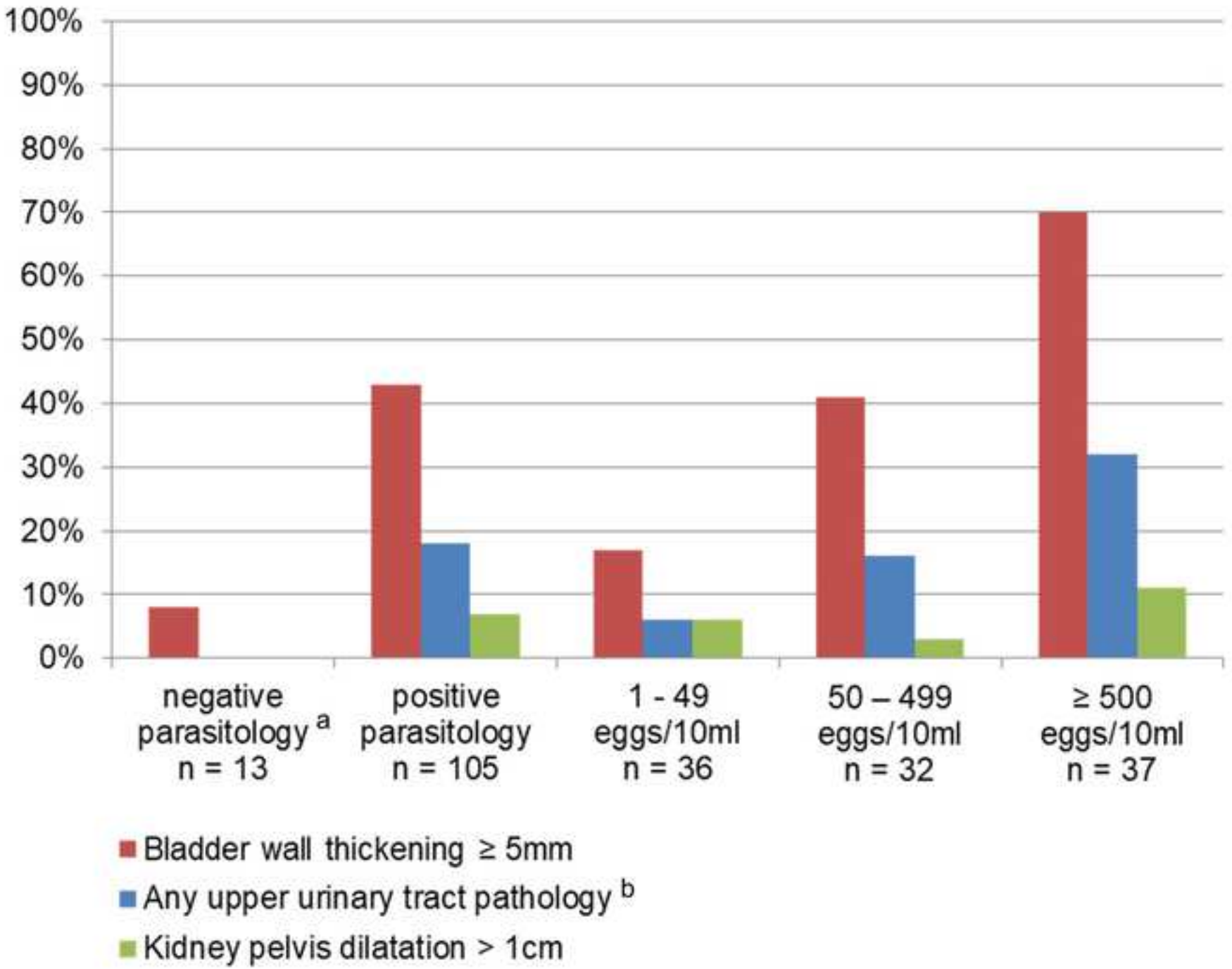

Figure 4 grayscale

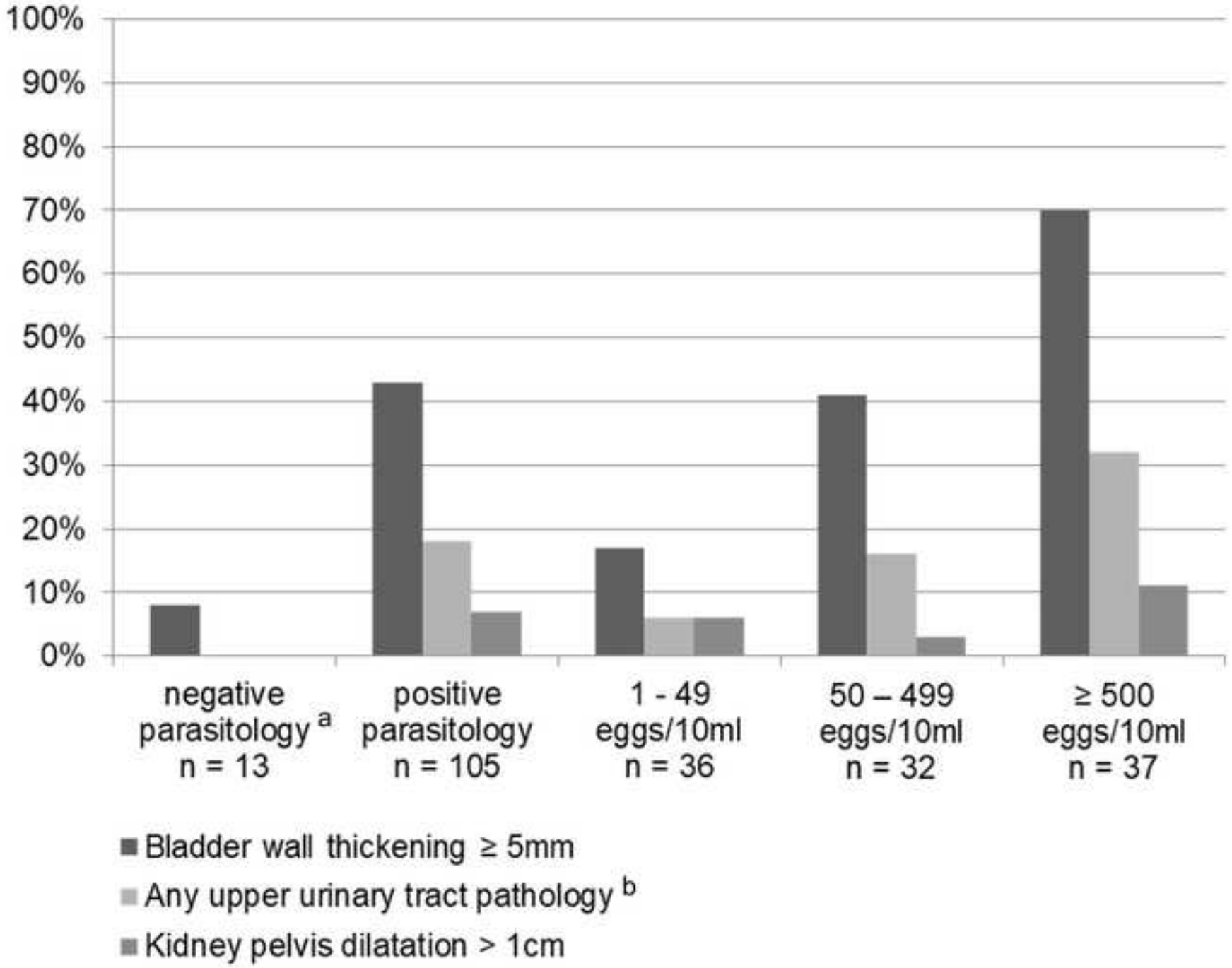

Figure 5 colour

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Figure 5 grayscale

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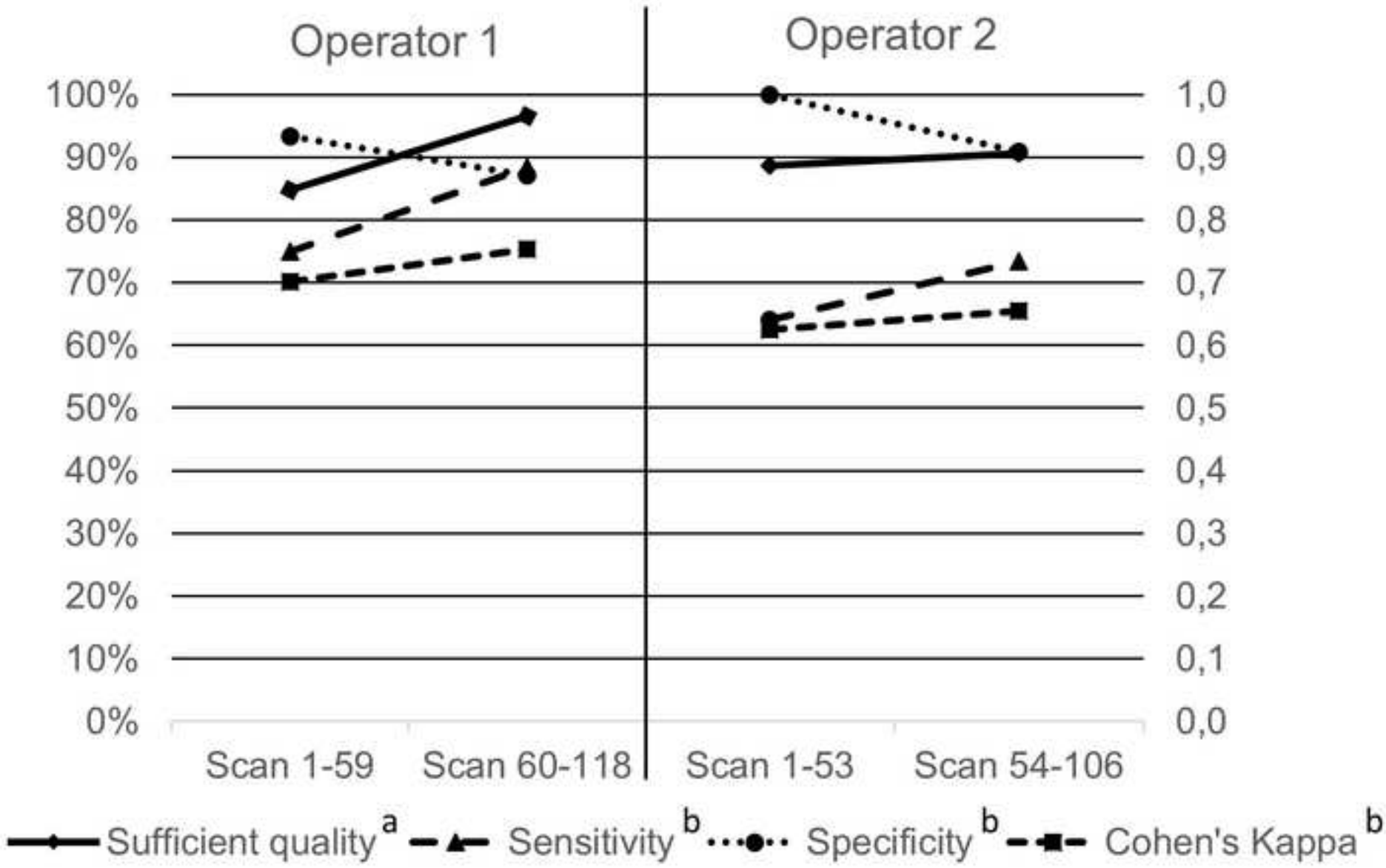

To:

Professor Sir Brian Greenwood
Editor-in-chief
Transactions of the Royal Society of Tropical Medicine and Hygiene

Tübingen, 29-06-2019

Subject: Revision of the article "Focused Assessment with Sonography for Urinary Schistosomiasis (FASUS) – pilot evaluation of a simple point-of-care ultrasound protocol and short training program for detecting urinary tract morbidity in highly endemic settings" (TRSTMH-D-19-00077)

Dear Professor Joshi,

Thank you very much for inviting us to revise our manuscript previously titled *Focused Assessment with Sonography for Urinary Schistosomiasis (FASUS) – diagnostic accuracy of a simple protocol to detect urinary tract morbidity in resource-limited settings*. We thank the reviewers for their constructive comments which we have addressed point-by-point below.

We also appreciate that the reviewer's comments have highlighted the fact that we have not emphasized clearly enough that this is a pilot study to assess a potential role for bedside ultrasound as a clinical tool, not intended for use in epidemiological surveys. In addition to addressing the reviewers' comments, we have therefore altered the introduction and title accordingly.

Thank you very much.

Yours sincerely

Jonathan Remppis (on behalf of all co-authors)

Revision notes

Reviewer 1

General comments

Comment 1

The authors have highlighted some limitations of the study such as the small sample size, representativeness of Gabon population and the gold standard used to assess the diagnosis accuracy. However, they have to address how they controlled such limitations. For e.g., they should have compared the baseline sociodemographic and economic characteristics of the study participants to the general population from the study area (Demographic health survey?). That could help to assess the representativeness of the study population.

Answer:

We agree that if the representativeness of the study population is mentioned as limitation of the study, measures on controlling this limitation should be further addressed in the manuscript. The focus of this manuscript was to pilot the diagnostic performance of the novel ultrasound approach in a clinical setting (ultrasound naïve operator versus ultrasound expert) and not on epidemiological questions. Therefore, by reappraising the section on limitations of our study we think that the respective sentence "As recruitment was done via convenience sampling, the rate of UGS-related pathology among the study population does not reflect that in the general population in Gabon." is not appropriately placed within the limitations section. To avoid misunderstanding on the epidemiologic situation in Gabon, we do however think it is important to make clear to the reader that the rate of findings cannot reflect that in the general population. We therefore moved the respective sentence to the end of the "diagnostic accuracy" section of the discussion.

Changes (page 12, paragraph 6):

The following sentence was removed: "As recruitment was done via convenience sampling, the rate of UGS-related pathology among the study population does not reflect that in the general population in Gabon."

Changes (page 12, paragraph 4):

We added the following sentence "Of note, as the intention of this study was not to identify infection rates, the reported rate of UGS-related pathology among the study population does not reflect that in the general population in Gabon."

Comment 2

By reading the paper, it seems that the authors have more assessed the reliability of the focused assessment with Sonography for urinary schistosomiasis (FASUS) than the diagnosis accuracy. They should be more clear about what kind of assessment they made (reliability, accuracy or both). As well, whatever the assessment, they should give more precision such as positive, negative predictive values for assessing diagnosis accuracy.

Answer:

We thank the reviewer for this important comment. After reappraisal of our study design, we agree that it is not suitable for the assessment of the diagnostic accuracy of FASUS itself. By the selection of our inclusion criteria (previous or ongoing macro-haematuria) and enrolment via convenience sampling, we have instead evaluated whether FASUS enables previously untrained operators to detect UGS-related urinary tract pathology in the clinical setting of a symptomatic patient. As this aspect is of major importance for the correct understanding of our paper, we have modified several paragraphs throughout the manuscript and also changed the title of our study.

For similar reasons, our investigations do not allow a valid calculation of positive and negative predictive values (PPV and NPV) of FASUS, although we agree that their assessment would be very

important in connection with the diagnostic accuracy of FASUS. The PPV and NPV depend on the prevalence of the assessed pathology and are not intrinsic to the test. Because of the preselection of our study population the reported prevalence of pathological finding cannot be compared to the prevalence among the general population. Future studies that perform FASUS on a representative sample of the general population will be suitable for calculation of the PPV and NPV.

Changes (page 1, title):
The title was changed from: "Focused Assessment with Sonography for Urinary Schistosomiasis (FASUS) – diagnostic accuracy of a simple protocol to detect urinary tract morbidity in resource-limited settings" to "Focused Assessment with Sonography for Urinary Schistosomiasis (FASUS) – pilot evaluation of a simple point-of-care ultrasound protocol and short training program for detecting urinary tract morbidity in highly endemic settings."

Changes (page 2, paragraph 4):
In the conclusions section of the abstract we changed the sentence "FASUS is a promising bedside diagnostic tool with good diagnostic accuracy in this pilot assessment." to "FASUS is a promising clinical, point-of-care tool for detecting USG related renal tract morbidity in symptomatic patients."

Changes (page 3, last paragraph):
In the introduction section we changed the sentence "The aim of our study was to develop a POCUS protocol for UGS-related morbidity and to evaluate its feasibility and accuracy after limited training of operators using low-cost equipment" to "The aim of our study was to pilot a POCUS protocol for detection of UGS-related renal tract morbidity in symptomatic patients, to evaluate practical feasibility and evaluate the performance of operators with limited training, using low cost equipment"

Changes (page 4, paragraph 1):
In the methods section we changed the sentence "evaluation of the diagnostic accuracy of this protocol in patients with UGS with remote expert image review as reference" to "evaluation of the diagnostic accuracy of POCUS operators, using remote expert image review as reference".

Changes (page 6, paragraph 2):
We added the following sentence "Convenience sampling was considered appropriate, as this was not intended as a prevalence survey, but as a cohort of patients with clinical symptoms".

Changes (page 10, paragraph 1):
In the discussion section we changed the sentence "This pilot diagnostic accuracy study was the first to evaluate a POCUS protocol for the detection of UGS-related pathology of the urinary tract. It showed that POCUS operators with limited training can detect such pathology with high diagnostic accuracy" to "This pilot study was the first to evaluate a POCUS protocol for the detection of UGS-related pathology of the urinary tract in symptomatic patients living in a high-endemic area. It showed that POCUS operators with limited training can detect such pathology with high accuracy, compared to expert ultrasound operators"

Changes (page 11, paragraph 6):
In the discussion section we changed the subheading "Diagnostic accuracy" to "Accuracy of pilot operators, compared to experts". The subsequent sentence "Overall, the diagnostic accuracy of FASUS was …" was changed to "Overall, the accuracy of FASUS, performed by our pilot operators, was …"

Changes (page 11, last paragraph):
We changed the sentence "In another study on the learning curve of the Niamey protocol by Bonnard et al., sensitivity and specificity had …" to "In another study on the learning curve of the more complicated Niamey protocol for prevalence surveys by Bonnard et al., sensitivity and specificity of the learner in comparison to the teacher had …"

Changes (page 12, paragraph 3):
In the sentence "Overall, the learning of this POCUS technique via remote review and feedback appears to be feasible and leads to good diagnostic accuracy that can be maintained over longer periods" we removed the word "diagnostic".

Changes (page 12, last paragraph):
In the conclusions section we changed the sentence "FASUS is a promising bedside diagnostic tool with good diagnostic accuracy in this pilot assessment" to "FASUS is a promising bedside diagnostic tool for detection of UGS-related morbidity in symptomatic patients living in high endemic areas".

Changes (Table 3):
The title of Table 3 was changed from "Diagnostic accuracy of FASUS" to "Accuracy of newly trained operators, compared to expert ultrasound opinion".
In the upper left cell of the table, we changed "Diagnostic accuracy" into "Accuracy".

Comment 3
The reliability (reproducibility) is often measured by the use of the Kappa coefficient which adjusts the observed agreement for expected chance agreement. The authors should provide in a table the observed agreement and not only the Kappa coefficient. As well, the reliability of a diagnostic test depends on the accuracy and reproducibility of the results. Both the predictive values and the Kappa coefficient are supposed to depend on the prevalence and this should be noticed when the results of different studies are compared.

Answer:
We agree that when reporting on reliability of our test the observed agreement should be reported in addition to the Kappa coefficient to provide sufficient information for the reader. We therefore added the observed agreement between each operator and the experts for the respective pathology variables to our Table 3.
We also agree that Kappa scores cannot be compared between our study and the studies cited as the prevalence of the assessed pathology differed among the study populations. We therefore specified in our paper that only sensitivity and specificity were comparable to our study.

Changes (Table 3):
We added a row to table 3 labelled "Observed inter-rater agreement" that lists the observed agreement factors for each variable. The label of the row below was changed from "Inter-rater agreement (Cohen's Kappa)" to "Cohen's Kappa".

Changes (page 11, last paragraph):
We changed the sentence "Few other studies on remotely reviewed POCUS in tropical medicine describing the detection of extra-pulmonary TB provided a diagnostic accuracy comparable to our study" to "Two other studies on remotely reviewed POCUS in tropical medicine describing the detection of extra-pulmonary TB provided a sensitivity and specificity of the operator compared to the expert that was comparable to our study"

Comment 4
Ethical statement session is missing

Answer:
The ethical statement is given in the authors' statements (page 13): "Ethical approval: This study was approved by the scientific review committee and the institutional ethics committee of CERMEL. Written informed consent was obtained from all patients (or legal guardians in case of children) prior to study enrolment."

Introduction

Comment 5
4th paragraph: Now that point-of-care…….settings…. Please give a reference for this statement.

Answer:
Other applications of POCUS in tropical medicine such as POCUS for HIV-associated tuberculosis and echinococcosis are already listed in in the same paragraph with the review by Bélard et al. as reference (reference nr. 13). We therefore removed the sentence from the manuscript.

Changes (page 3, last paragraph):
We removed the sentences "Now that point-of-care ultrasound (POCUS) is expanding rapidly in resource constrained settings, a simplified and more pragmatic protocol to identify relevant pathology may be of value to establish the clinical diagnosis, assess urinary tract damage, guide appropriate management and monitor treatment response"

Methods

Comment 6, protocol development
Comment 6.1:
Please, provide a reference for the nomenclature of previous POCUS techniques.

Answer:
As the before mentioned and cited review of Bélard et al. Am J Trop Med Hyg, 2016 discusses a number of available POCUS modules (FAST (focused assessment with sonography for trauma), FASH (focused assessment with sonography for HIV-associated tuberculosis), FASE (focused assessment with sonography for echinococcosis)) applying a nomenclature consistent with the abbreviation FASUS, we added this reference to this statement.

Changes (page 5, paragraph 2):
"Based on the nomenclature of previous POCUS techniques[13] we named our protocol "Focused Assessment with Sonography for Urinary Schistosomiasis" (FASUS)."

Comment 6.2:
Describe shortly the Niamey protocol.

Answer and changes (page 5, paragraph 2):
As suggested by the reviewer we shortly summarized the Niamey protocol in the methods section. We therefore changed the sentence "A simple and easy-to-learn protocol was designed by the study team adapted from the Niamey protocol" to "A simple and easy-to-learn protocol was designed by the study team, selecting clinically relevant criteria adapted from the Niamey protocol, which provides standards for the evaluation of the urinary bladder (shape, bladder wall thickening, irregularities and polyps), ureters (ureteral dilation), renal pelvis (degree of hydronephrosis), and additional evaluations (bladder volume, residual urine after voiding, fibrosis of the renal pelvis); final evaluations includes multiple measurements for the calculation of a final severity score.[11]"

Comment 6.3:
Authors should give more details concerning the threshold of bladder wall thickening > 5mm.

Answer:
The threshold of 5 mm originates from the Niamey protocol. We therefore chose the same criteria initially while designing the study. During our study we observed that operators and equipment could

actually detect more subtle pathology. Further considerations on the definition of pathology compatible with UGS are discussed in the discussion section (page 10, paragraph 2).

Changes (page 10, paragraph 2):
We added the sentence "This cut-off was chosen, based on existing Niamey criteria." The following sentence was changed from "However, in many cases bladder wall irregularities < 5mm were detected, suggesting less advanced pathology also compatible with UGS" to "However, in many cases bladder wall irregularities < 5mm were detected, indicating that less advanced pathology, compatible with UGS, can also be detected with current equipment. and short focused training. This finding may be of value, as earlier detection of morbidity may influence management decisions"

Comment 7: Training
Comment 7.1:
The two operators (JR & AV) have a different level of knowledge (medical student & a clinician). Why the authors did not take operators with the same level? How they can guarantee that did not bias the diagnosis accuracy.

Answer:
Within our study setting and frame it was not feasible to acquire two operators of exactly the same level of experience. POCUS techniques are supposed and designed to be operational by operators with different levels of experience and feasible. We therefore think that a bias by the operators' level of clinical experience is unlikely.

Comment 7.2:
The authors highlighted that AV was additionally trained by JR on site. The authors should explain why because this could lead a bias in the accuracy assessment.

Answer and changes (page 6, first paragraph):
Thank you for highlighting this aspect, as the sentence might indeed lead to confusion. As local principal investigator JR was involved in the study design; he trained AV on all study related aspects beyond the performance of FASUS (study flow, taking informed consent, collection of clinical and parasitological data, documentation, upload of records etc.). The specific training of FASUS was the same for JR and AV and performed as described in the methods section. To avoid confusion we therefore removed the sentence "AV was additionally trained by JR on site".

Comment 8: prospective evaluation
The study participants have been followed-up after praziquantel treatment after one and three months. Why 3 months of follow-up? Is it enough for assessing morbidity? If yes, give the reasons.

Answer:
Longitudinal studies (Jukiko et al, JID, 1999; Hatz et al, Am J Trop Med Hyg, 1998) have observed a marked decrease in the occurrence of bladder pathology one month and a further decrease three months after treatment. After three months, no further reduction of pathology was noted and there was an increasing rate of pathology reappearance.

Comment 9
Analysis: Any sample size calculation? If not, any power calculation with the study sample?

Answer:
We performed a sample size calculation during the development of the study protocol setting 95% confidence and 80% power to detect a difference in discordance of 10% below which the new diagnostic test is non-inferior to the reference standard and yielded a sample size of at least 116 patients. This calculation must however be understood as an estimate, since our study is a pilot and mainly intended to inform larger studies, including their sample sizes.

Comment 10: results

Comment 10.1:

The cohort attrition seems to be high; 41% of loss of follow-up at M1 and 31% at M3. The authors have to compare the characteristics of participants who completed the study and those who did not.

Answer and changes (page 8, paragraph 1):

Thank you for indicating this aspect. We added the following sentence to the manuscript:
"Age and gender distribution of the follow up cohort was comparable to the entire study population".

Comment 10.2:

Image quality: Why different place of assessment (Home and hospital)? The authors should have discussed that could not have affected the diagnosis accuracy.

Answer:

The reason why parts of the ultrasound assessments were performed at the patients' residence and parts at the CERMEL was logistical. Following the reviewer's comment we reappraised the likelihood that the place of FASUS may have influenced our data. As scanning conditions were standardized by our study standard operating procedure (i.e. same ultrasound equipment, patients in supine position, darkened room, operator seated on the patient's right side) an impact of the place of FASUS performance on the data appears unlikely to us.

Changes (page 7, paragraph 3):

To clarify this, we added the following to the methods section: "… following standardized scanning conditions (same ultrasound equipment, patients in supine position, darkened room, operator seated on the patient's right side)". We changed the sentence "A darkened room and sufficient bladder filling, defined as complete distention of the bladder, were attempted …" to "Sufficient bladder filling, defined as complete distention of the bladder, was attempted …".

Comment 11: Ultrasound findings

Comment 11.1:

Please, give shorts comments on figure 3 & 4.

Answer and changes (page 8, paragraph 3):

We added the following sentences to the manuscript: "Detection rates of bladder wall pathology, kidney pelvis and proximal ureter dilatation were similar between operator and experts; distal ureter dilatation or thickening was detected 3 times as often by expert than by operator." And: "With increasing intensity of infection, bladder and upper urinary tract pathology was more frequently detected."
In the sentence "Frequencies of UGS-related pathologies at baseline…" we added "…as assessed by the experts…".

Comment 11.2:

Sensitivity and specificity are not enough to assess the diagnosis accuracy. The authors should also assess predictive values

Answer:

We addressed this aspect in our response to your comment 2.

Comment 12: Discussion

Comment 12.1

3rd paragraph: ……..a gain in sensitivity was observed. Please, give more information about it, especially in the result session.

Answer:
More details about the additional value of the longitudinal bladder scan are reported in the results section on page 9 (first paragraph): "Among 85 cases assessed by both transverse and longitudinal bladder scan, pathology compatible with UGS was detected in 35 (41%) by transverse scan alone compared to 38 (45%) by a combination of both scans."

Comment 12.2
The authors should also discuss the feasibility of this kind of assessment in resource-limited settings.

Answer:
We think we have addressed this point at several occasions throughout the discussion and conclusion with the following statements:
"During training and study data collection, the team was repeatedly approached by local health care staff willing to learn the method, showing the general interest in the achievement of ultrasound skills among local clinicians."
"Overall, the learning of this POCUS technique via remote review and feedback appears to be feasible and leads to good accuracy that can be maintained over longer periods."
"However, this telemedicine-based training and image review design provides a feasible model for POCUS training in resource-limited settings, where no alternative options exist."
"Simple protocols and short training appear adequate and feasible, despite the common limitations present in a low resource setting, such as novice clinician trainees, low-budget ultrasound devices, and examinations performed in the field."

Reviewer 2:

Thank you for conducting this study to design a protocol for FASUS.

Comment 1
It would be good to discuss some background on the Niamey protocol and its limitations/need for improvement.

Answer and changes (page 3, paragraph 4):
We thank the reviewer for this comment. We have amended and revised the respective section in the introduction as follows: "In 1996 the World Health Organization (WHO) published a standardised protocol for the ultrasonographic assessment of schistosomiasis-related morbidity, commonly known as the Niamey protocol. However, this protocol is specifically designed for application in large-scale control programs and not for diagnosis in the clinical setting. It includes a detailed and relatively complex grading system, which is less relevant to clinical application. Contrary to widespread experience of using ultrasound in prevalence surveys, it has not been validated for routine patient care in endemic areas. In addition, prevalence surveys mostly rely on operators with professional ultrasound training. In many clinical settings, this expertise is lacking."

Comment 2
Would a study comparing to the full Niamey protocol be worthwhile to do? It would give some insight into how much different the results would be without bladder filling (as the FASUS might also inform how the Niamey protocol can be simplified or improved further)
In my opinion, the gold standard is the Niamey protocol and not expert findings. (page 8/27)

Answer:
We agree that a study comparing FASUS to the full Niamey protocol would be of great value and should be performed. As no expert sonographers were available on-site in our setting, we chose the remote expert review of recorded ultrasound clips as reference. Future studies comparing the two

protocols may indeed inform on how the Niamey protocol can be improved but may also inform on how the FASUS protocol can be improved.

Comment 3
Please also comment on other experts' view to revise the Niamey protocol vis a vis the development of the FASUS protocol.

Answer:
The review of Akpata et al. 2015 (reference number 12 in our manuscript) provides a good insight on experts' views on the Niamey protocol and suggestions for its simplification. We took this review into account during the design of the FASUS protocol and followed some suggested propositions by modifying the scoring of bladder wall and upper urinary tract pathology.

Additional changes:
While revising the manuscript, we noticed several sentences in need of revision, independent from the reviewers' comments. The respective modifications are listed below:

Changes (page 2):
Abstract, background section:
We changed the sentence "In resource-limited settings, novel diagnostic tools are needed for morbidity assessment" to "In resource-limited settings, affordable tools for morbidity assessment in clinical care are needed" and the sentence "Point-of-care ultrasound has not yet been investigated on the detection of UGS-related pathology" to "Point-of-care ultrasound has not yet been validated for UGS-related pathology"

Abstract, methods section:
We changed the sentence "Recorded ultrasound clips were remotely reviewed by two experts as reference" to "Recorded ultrasound clips were remotely reviewed by two ultrasound experts as diagnostic reference".

Abstract, results section:
We removed the word "FASUS" from the sentence "In 2015 and 2016, FASUS scans were performed in 118 patients".

Abstract, conclusions section:
We changed the sentence "Based on data from larger cohorts examined with FASUS, appropriate …" to "Based on larger validation studies, appropriate …"

Changes (page 3, paragraph 1):
We added the sentence "In patients with irreversible damage, options for treatment will depend on the type of damage and available treatment".

Changes (page 3, paragraph 3):
We changed the sentence "Ultrasound is a well-documented, effective imaging tool…" to "In prevalence surveys, ultrasound has long been established as an effective imaging tool…".
We added the sentence "Being a test for morbidity and not active infection, ultrasound does not replace but complement parasitological tests"

Changes (page 4, paragraph 1):
We removed the sentence "Being a test for morbidity and not active infection, such a protocol will not replace but complement parasitological tests"

Changes (page 3, second-to-last paragraph):

We changed the sentence "POCUS has been successfully…" to "Point-of-care ultrasound has been successfully…"

Changes (page 5, paragraph 1):
We added the following sentence "It was part of a larger study, which additionally assessed epidemiological aspects as well as immunological and metabolomic aspects of UGS; these will be reported separately."
We changed the following sentences "In summary, this study comprised the following parts: A) development of an ultrasound protocol for point-of-care detection of UGS-related pathology; B) training of two operators on this protocol; C) evaluation of the diagnostic accuracy of this protocol in patients with UGS with remote expert image review as reference" to "Procedures relevant for the pilot assessment of our POCUS protocol were A) development of a POCUS scan protocol; B) training of two operators; C) evaluation of the diagnostic accuracy of POCUS operators, using remote expert image review as reference. D) Comparing ultrasound findings with parasitology test results"
The following sentence was moved to page 7, first paragraph: "A portable low-cost ultrasound device (MINDRAY Digital Ultrasonic Diagnostic Imaging System model DP-10) with a curved array transducer (MINDRAY model 35C50EB) was used for training as well as for study procedures."

Changes (page 5, paragraph 2):
We changed the sentence "The emphasis was on identification of bladder wall pathology as well as renal pelvis and ureter dilatation, assumed to be feasible for operators without previous ultrasound experience; more sophisticated procedures such as measurement of bladder volume were omitted" to "The emphasis in our selection of criteria was on image acquisition and interpretation, feasible for operators with little or no previous ultrasound experience, but adequate to make a diagnosis of likely UGS-related pathology; more sophisticated procedures such as measurement of bladder volume were omitted, as well as complex severity grading scores"
In the sentence "Abnormalities compatible with UGS were defined as any bladder wall thickening ≥ 5mm…" we added "as in the Niamey criteria".
After the sentence "Abnormalities […] were not considered compatible with UGS" we added "as this is a highly uncommon finding and etiologies other than UGS are more likely".
In the sentence "FASUS probe positions in FASUS is…" we removed "in FASUS".

Changes (page 6, paragraph 2):
We changed the sentence "Inclusion criteria were history of, or …" to "Inclusion criteria was previous or …"

Changes (page 8, paragraph 4):
We changed the sentence "… missed features compatible with UGS in 21/202 (10%) cases and documented false positive FASUS in 9/202 (4%)." To "…missed features compatible with UGS in 21/202 (10%) cases and overdiagnosed features compatible with UGS in 9/202 (4%)."

Changes (page 9, paragraph 3):
In the sentence "Pathology not related to UGS…" we changed "operator" to "operators" and removed "(one case each)".
In the sentence "inter-rater agreement between…" we changed "bladder wall" to "bladder wall pathology".

Changes (page 10, paragraph 2):
We changed the sentence "To keep the protocol as simple as possible a classification of pathology, combining…" to "To keep the protocol as pragmatic as possible for use in the clinical setting, a classification of pathology severity, combining …"
We changed the sentence "However, as upper urinary tract pathology with concurrent bladder wall irregularities < 5mm is likely to be due to UGS (though classified as "not compatible with UGS" in our study), this approach needs to be reconsidered" to "However, as upper urinary tract pathology with

concurrent bladder wall irregularities less than 5mm is also likely to be due to UGS (though classified as "not compatible with UGS" in our study), this approach needs to be reconsidered in larger validation studies"
We added the sentence "As expected, the operators detected fewer abnormalities not related to UGS. The aim of POCUS is to answer a very specific clinical question, in this case: 'are there findings compatible with UGS', and not to provide a comprehensive ultrasound examination or to exclude the presence of other pathology."

Changes (page 10, paragraph 4):
We changed the sentence "…detected by the additional longitudinal view, a gain in sensitivity was observed" to "…detected by the additional longitudinal view, we recommend including this view in further evaluations of this protocol".
We removed the sentence "Further studies with larger sample sizes are required to externally validate our results. A possible future approach could be that when a transverse scan does not show any pathology, a longitudinal scan should be performed in addition".

Changes (page 10, paragraph 5):
We removed "study" from "study data collection".
We added "remote" to "feedback".

Changes (page 11, paragraph 4):
After "…only two kidney scans […] were rated as 'unable to assess' by operator one…" we added the sentence "This is a potential source of error and future training should emphasize this risk".

Changes (page 11, paragraph 5):
After the sentence "…increase complexity" We removed "of the so far simple protocol".
The next sentence was changed from "Definitely, the critical point …" to "Learning the critical point…".
We changed "FASUS trainings" to "FASUS training".

Changes (page 12, paragraph 7):
We changed the sentence "However, this telemedicine-based training and image review design provides a feasible model for pilot studies of novel indications for POCUS in resource-limited settings" to "However, this telemedicine-based training and image review design provides a feasible model for POCUS training in resource-limited settings, where no alternative options exists"

Changes (page 12, last paragraph):
We changed the sentence "internationally agreeable standards for training and duration, contents and intensity should be set" to "internationally agreeable standards for training and competency should be set".
In the next sentence we removed "examined with FASUS in future studies".

Changes (title of Figure 4):
The title of Figure 4 was changed from "Pathology detected by FASUS at baseline in relation to parasitology" to "Pathology detected by FASUS at baseline (expert review) in relation to parasitology"