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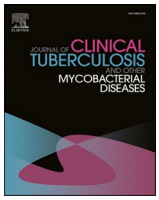

Implementation of an integrated control programme for neglected tropical diseases of the skin in Ghana: The essential role of the laboratory

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ABSTRACT

Introduction: In this study, we report on findings from approaches used, the outcomes and the lessons learnt from the laboratory support provided for integrated control of skin NTDs including Buruli ulcer (BU), and yaws in seven selected districts in Ghana.

Methods: Actions implemented from July 2018 to October 2022 included; training district-level health workers on specimen collection, storage, and transport to laboratories, integrated case searches, continual monitoring and supervision for trained health workers, laboratory confirmation of BU and yaws samples and providing results of the analysed samples to guide decision making. Descriptive analysis of data was performed.

Results: A total of 18,683 (including suspected BU 976; suspected yaws 10,995) individuals were screened for BU and yaws. Of 976 suspected BU cases, 16.8% [median (IQR) age 24 (12.0–37.8) years] were confirmed positive by IS2404 PCR; BU mostly presented as ulcers (78.7%); category I (37.2%) and category II (36%). 480 individuals (4.4%) had DPP positive yaws. Multiplex PCR analysis of 75 selected DPP positive cases identified; 7 DPP positive yaws cases as *Treponema pallidum*, 28 as *Haemophilus ducreyi* and 7 as *Treponema pallidum/Haemophilus ducreyi* coinfection. Laboratory results were sent to the districts within a median (IQR) of 5 (3 – 9) days.

Conclusion: The implementation of integrated diagnostic confirmation for skin NTDs is feasible with provision of timely results within a week. Multiplex diagnostic tools differentiated *Treponema pallidum* and *Haemophilus ducreyi*. There is a need to sustain active case search activities, enhance health worker training, and improve laboratory confirmation of cases as part of the overall strategy for the integrated control of skin neglected tropical diseases.

1. Introduction

Neglected Tropical Diseases (NTDs), common among disadvantaged and marginalized members of society in low- and middle-income countries, are ancient diseases of poverty that affect over 1 billion people around the globe and cause the death of around 200,000 people every year. In addition, NTDs are associated with significant socioeconomic consequences [1].

Skin-related Neglected Tropical Diseases (skin-NTDs) including Buruli ulcer, cutaneous leishmaniasis, leprosy, lymphatic filariasis (lymphoedema and hydrocele), mycetoma, onchocerciasis, scabies, and yaws have significant cutaneous manifestations that are correlated with long-term disfigurement, disability, stigmatization, reduced productivity [2], mental health distress and reduced quality of life of the affected individuals [3]. In Ghana, a West African country where about 42 % of the population live in rural, underserved communities with poor access

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to health care, skin NTDs like Buruli ulcer, yaws and leprosy are endemic [4–6].

Traditionally, there has been a disease-specific (vertical programmatic) approach to the management and control of NTDs in Ghana with each disease having an individual control program that focuses on community-based mass campaigns and clinical case management [7]. Although progress has been made in the fight against skin NTDs, there is a growing call to integrate the management of NTDs to improve outcomes [8]. The 2030 WHO roadmap for the control, eradication and elimination of NTDs which was launched in 2021 calls for an integrated approach to skin NTDs with accelerated programmatic action, intensification of cross-cutting approaches, and changed operating models and culture to facilitate country ownership as the main strategic pillars [9]. An integrated approach to skin NTDs is warranted due to their co-endemicity, similarity in their epidemiology and clinical signs and to facilitate optimal utilisation of limited financial and human resources available in these regions [10,11]. Different innovative methods including the use of mobile health technologies have been employed to enhance the clinical management and epidemiological surveillance of NTDs worldwide [12,13].

Laboratory confirmation of cases is a key component of any successful control programme. For Buruli ulcer (BU), rapid diagnostic tests are unavailable and laboratory confirmation is provided by dedicated reference laboratories in endemic countries [14].

Investment in laboratory research, epidemiology, diagnostic tools, and management strategies for control of skin NTDs is still an important priority [12]. Between July 2018 and October 2022, the ANESVAD Foundation supported the Ghana Health Service to implement an integrated approach for skin NTDs in selected districts of the country. As part of the process, the Kumasi Centre for Collaborative Research into Tropical Medicine (KCCR) was tasked to provide laboratory support for the diagnosis and control of skin NTDs in 7 selected districts in Ghana. In this study, we report on findings from approaches used, the outcomes and the lessons learnt from the support provided for integrated control of skin NTDs in the selected districts.

2. Methods

2.1. Ethical consideration and consent to participate

This study presents the outcome of an implementation activity under the auspices of the Ghana Health Service. Approval was obtained from the Committee on Human Research and Publication Ethics of the School of Medicine and Dentistry of the Kwame Nkrumah University of Science and Technology (approval number: CHRPE/AP/335/19). Further, permission was sought from local authorities and school heads. All participants provided informed consent.

2.2. Study sites

The integrated approach was undertaken in 7 selected districts in Ghana in conjunction with the National Buruli Ulcer and Yaws Control Programme (NBUYCP) and the Leprosy Control Programme of the Ghana Health Service. The selected districts, located in the middle and northern zones of Ghana were; Asante Akim North (ASN), Sekyere Afram Plains (SAP), Upper Denkyira East (UDE), Upper Denkyira West (UDW), Wassa Amenfi East (WAE), Nkoranza South (NKS), Wa Municipal (WM) which transported samples to KCCR.

These districts were carefully selected by the national control programmes because they are endemic for the diseases of interest [15]. All the study districts except Wa Municipal, are located within the tropical rainforest zone which provides the resources for farming and fishing, the main occupations among community members. The districts are mainly rural and most community members have limited access to healthcare.

2.3. Implementation

The implementation steps included preparatory meetings for stakeholders, training of health workers and community-based surveillance volunteers (CBSVs), integrated case searches, laboratory confirmation and reporting, patient management as well as monitoring and evaluation visits. Integrated case searches, diagnosis and management happened concurrently.

2.4. Stakeholder meetings

In attendance at stakeholder meetings were representatives from the Ghana Health Service (including the control programmes), participating laboratories, districts, community leaders and representatives from ANESVAD foundation. In each district, meetings were also held with the district health directorate, local and traditional authorities as well as school administrators to introduce the programme and seek support for the implementation of the project.

2.5. Harmonization of standard operating procedures (SOPs) for laboratories

Standard operating procedures (SOPs) guiding all stages of the integrated program were harmonized. These included SOPs on; sample collection and labelling, documentation, storage, transport, sample accessioning in the laboratory, and laboratory processing. Meetings were held between laboratory personnel from the reference centres that were involved in offering laboratory support for the integrated program in districts located in their respective zones. During the meeting, SOPs were collated, reviewed, and adapted to create harmonized documents to guide the implementation.

2.6. Training

The participants for the trainings, were carefully selected in consultation with the districts and in collaboration with the national programme managers. The categories of staff trained included Medical Doctors, Physician Assistants, Disease Control Officers, General Nurses, Public Health Nurses, Laboratory Technicians, Community Health Nurses, Community Based Surveillance Volunteers (CBSVs) and some selected traditional healers. The content of the training included information on disease epidemiology, clinical recognition and diagnosis, differential diagnosis, sample collection and transport, complications, social consequences of skin NTDs, reporting tools and disease management. The content of the training was adapted depending on the cadre receiving the training. For instance, training for CBSVs and traditional healers focused mainly on clinical recognition and diagnosis to increase their ability to suspect and appropriately link cases to the healthcare system. Similarly, for health workers, there was a focus on clinical diagnosis, sample taking and transport, performing rapid diagnostic test for yaws and management of confirmed cases. On-site training workshops were organized in each district using the harmonized SOPs.

2.7. Integrated active case finding

Active case searches were done independently in each of the seven districts. The active case searches were undertaken following a pre-prepared schedule by the local health authorities that included visits to all villages and basic schools in the districts. The activities started with community entry, sensitization and social mobilisation in all villages a day prior to screening; appropriate local and school authorities such as the village chief, opinion leaders, local administrative leaders and teachers were involved in these exercises. Tools employed included video shows on projectors (mostly done in the evenings because most farmers would have returned home), documentaries, success stories, posters and jingles played at the Community Information Centres (CICs)

on skin NTDs. After this, trained health workers and CBSVs provided clear explanations of the diseases using the WHO posters and booklets on BU, leprosy and yaws.

After the sessions, individuals who provided informed consent had a skin examination performed. All skin examinations were performed by gender appropriate examiners in a well-lit private area in the community centre or school to ensure confidentiality.

2.7.1. Definition of terms

Suspected BU: A suspected case of Buruli ulcer was defined as a person living in or having travelled to an endemic area and presenting with a painless lesion (nodule, plaque, oedema or ulcer) consistent with signs of the disease [16].

Suspected Yaws: A suspected case of yaws was defined as a person presenting with skin lesions consistent with yaws (i.e. skin papilloma, solitary or multiple atraumatic painless skin ulcerations with typical rolled edges) [17].

Yaws-like lesions were defined as atraumatic skin ulcerations without the typical rolled edges of yaws.

2.8. Laboratory confirmation

Individuals with suspected yaws and yaws-like lesions were screened serologically using a rapid point-of-care treponemal test (SD Bioline Syphilis 3.0 RDT kit, Standard Diagnostics Inc., Suwon, South Korea) and confirmed using a rapid non-treponemal and treponemal test (DPP Syphilis Screen and Confirm Assay, Chembio Diagnostic Systems, Medford, NY, USA), according to manufacturer instructions for the detection of antibodies to treponemal and non-treponemal antigens.

We intended to perform multiplex PCR that simultaneously detected *Treponema pallidum* and *Haemophilus ducreyi* on about 25 % of the DPP-positive yaws cases. However, for operational and logistic reasons, this target could not be met, and sample collection for PCR was done from only 75 of the DPP positive cases. Swab samples were taken for both ulcers and moist papillomas, while scab was removed from dry or closed papillomas of patients who were DPP positive. Samples were placed in sterile patient ID pre-labeled cryotubes containing 700 μL cell lysis solution (CLS, Qiagen, Hilden, Germany). Swabs (for ulcerative lesions) or fine needle aspirate samples (for non-ulcerative lesions like nodule, plaque or oedema) were collected from individuals with suspected BU as appropriate.

All clinical samples were stored at fridge temperature in the health facilities or district health directorates. Subsequently, samples in cryotubes were placed in biohazard bags and transported by courier at room temperature (25 – 32 degrees Celsius) to the Kumasi Centre for Collaborative Research into Tropical Medicine (KCCR) for processing. The date and time of sample collection, as well as the date of transport to the KCCR laboratory, were recorded.

2.8.1. IS2404 qPCR assay for BU

Confirmation of BU by Polymerase Chain reaction (PCR) targeting the *IS2404* insertion sequence was performed using standard procedures as published previously [18,19]. Briefly, samples were transported in cell lysis solution (CLS) and DNA was extracted using the Qiagen AllPrep DNA/RNA kit (Qiagen, Hilden Germany). The extracts were then subjected to *IS2404* qPCR.

2.8.2. DNA extraction and T. pallidum and H. ducreyi multiplex qPCR assay

PCR testing for *T. pallidum* and *H. ducreyi* was performed using procedures as previously published [20]. In brief, DNA extraction was performed using the Gentra Puregene Tissue Kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. Briefly, samples stored in lysis buffer were vortexed for 2 min, swab sticks removed from the solution and then centrifuged for 1 min at 13,000 rpm to pellet the cells. Supernatant was carefully discarded. A total of 300 μL of CLS was added to the pellet after which 15 μL of lysozyme was added and mixed by inverting 25 times. It was then incubated at 37 °C for 30 min and at 80 °C for 5 min to lyse the cells. Proteins were precipitated by adding 100 μL of protein precipitation solution (Qiagen, Hilden, Germany) to the sample, vortexed 20 s and centrifuge at 13,000 rpm to pellet. Supernatant was transferred into a pre-labelled 1.5 mL tube (Eppendorf AG, Hamburg, Germany), containing 300 μL isopropanol and 2 μL glycogen. This mixture was mixed by inverting gently 50 times and then centrifuged for 1 min at 13,000 rpm to pellet DNA. The supernatant was carefully discarded and 300 μL of 70 % ethanol added to wash the pelleted DNA and then centrifuged for 1 min. Alcohol supernatant was carefully discarded and DNA air dried for 5 − 30 min. The extracted DNA was resuspended in 100 μL DNA Hydration Solution (Qiagen, Hilden, Germany), vortex for 5 s and incubated at 65 °C for 1 hr. Extracted DNA was stored at –20 °C till it was used for the multiplex real-time PCR (qPCR).

The multiplex qPCR assay was performed with RealCycler® - universal kit (Progenie molecular, Valencia, Spain) for the detection of the *PolA* and *HgbA* specific genes for *T. pallidum* and *H. ducreyi,* respectively, on a Bio-Rad CFX96 Real-time PCR detection system (Bio-Rad Laboratories, Paris, France) according to manufacturer instructions. Briefly, 14 μL of RealCycler® Universal AmpliMix (qPCR mastermix reagent) was pipetted into reaction tubes and 6 μL of DNA template was used to yield a reaction volume of 20 μL. Positive and negative controls were also included in each run. Cycling conditions of PCR are as follows one (1) cycle of initial denaturing at 95 °C for 15 min followed by 45 cycles of 95 °C (denaturing) for 5 s, 60 °C (annealing) for 30 s and 72 °C (extension) for 30 s.

2.8.3. Quality assurance processes

The KCCR laboratory is a member of the WHO network of laboratories for confirmation of BU (BULABNET) and participates in multiple rounds of external quality assessments (EQA) each year. The laboratory has consistently attained excellent results during the EQA by the BULABNET [14]. Additionally, there are in-house standard operating procedures and internal quality assurance processes for all testing. The laboratory equipment undergoes periodic calibration by the Ghana Standards Authority. For every PCR run, there are positive and negative controls. An exogenous internal positive control (Invitrogen, Karlsruhe, Germany) is included in all *IS2404* assays to exclude false negatives. The PCR's cycling conditions are observed as required.

2.9. Reporting of results

After processing at the KCCR, all results of laboratory confirmed cases were electronically sent via WhatsApp and E-mail to the designated focal persons (disease control officers) and the Director of Health Services in the district noting the date of dispatch of the results. Results were sent to the National Control Programmes monthly. The date of result reporting to districts was recorded.

2.10. Patient care

The diagnosis of leprosy was confirmed clinically. Demographic and clinical information were collected on the WHO recommended skin NTD −BU 01 forms [21]. All confirmed cases of BU, yaws and leprosy were treated at the various districts as per recommendations of the WHO [22–24]. Other wounds were referred to the nearest health facility for management.

2.11. Mapping

GPS coordinates were taken by trained field staff before sample collection using the My GPS Essentials App (GPS Essentials, Seattle, USA). Maps were made using the software QGIS version 3.30.3 (Open-Source Geospatial Foundation, Oregon, USA).

2.12. Monitoring and supervision

To maintain the skills and knowledge of the trained health personnel, a quarterly monitoring and supervisory visit was instituted to all seven districts under the study. During the field visits, personnel skills and adherence to protocols and quality assurance procedures in sample collection and labelling after clinical examination, documentation, storage, handling, and transport to the WHO reference laboratory were assessed. A monitoring tool was used and feedback was provided to the district staff. Periodic reports on all activities were sent to the national control programmes for Buruli ulcer and yaws and ANESVAD for onward submission to the WHO.

2.13. Statistical analysis

The data were recorded in Excel 2016 and analyzed using GraphPad Prism version 6.0 (GraphPad Software, San Diego California USA) and Stata 12 (Statacorp 2011 statistical software Release 12. College Station, TX: StataCorp LP). We performed a descriptive analysis of clinical and laboratory variables.

3. Results

While the implementation programme was intended for 3 skin NTDs (BU, leprosy and yaws), the present analysis includes data on participants suspected to have BU or yaws, the two conditions that required laboratory confirmation. From July 2018 to October 2022, a total of 18,683 individuals including suspected yaws (10,995) and suspected BU (976) were seen. Eight hundred and fourteen of the individuals with suspected yaws had RDT positive tests (probable yaws) and 480 (4.4 %) were confirmed to have active yaws on DPP testing (Fig. 1).

Samples from 976 individuals [median age 35 (IQR 17–54) years] were received at the KCCR for confirmation of Buruli ulcer. Of the suspected BU cases, 164 were confirmed by *IS2404* PCR giving an overall positivity rate of 16.8 %. Asante Akim North (38.3 %) and Upper Denkyira East (44.4 %) districts had relatively higher BU PCR confirmation rates compared to Nkoranza South (3.4 %) and Wa Municipal (1.2 %). BU lesions were ulcers (78.7 %), nodules (7.9 %), plaques (11.6 %) and oedema (1.8 %); category I (37.2 %), category II (36 %) and category III (26.8 %). Most of the individuals with ulcerative BU had category I (35.7 %) or II (34.1 %) disease. BU lesions were commonly located on the lower limbs (59.2 %) and upper limbs (36.8 %); one participant had the lesion on the buttocks. Eleven individuals with BU (7 with category III ulcers) had a limitation in movement of the joint. Cases of BU were identified in all the study districts. Most cases of BU were reported from the Asante Akim North (29 %), Upper Denkyira West (19 %) and Upper Denkyira East districts (15 %). The characteristics of individuals with BU are detailed in Table 1.

The median (IQR) interval from sample collection to receipt at the laboratory was 6 (2 – 18) days. When the farthest sample collection location (Wa Municipal) was excluded, the median sample transportation time decreased to 5 (1 – 11) days. BU PCR results were sent back to the districts within a median (IQR) of 5 (3 – 9) days after receipt of samples at the KCCR laboratories.

At the initiation of the project in 2018, more category III and II BU lesions than category I lesions were reported. Over time however, category I lesions predominated and there was a decreasing trend in the proportion of category III lesions (Fig. 2).

Yaws and BU were found in all 7 study districts (Fig. 3).

Samples from 75 (15.6 %) of 480 individuals [median age 11 (IQR

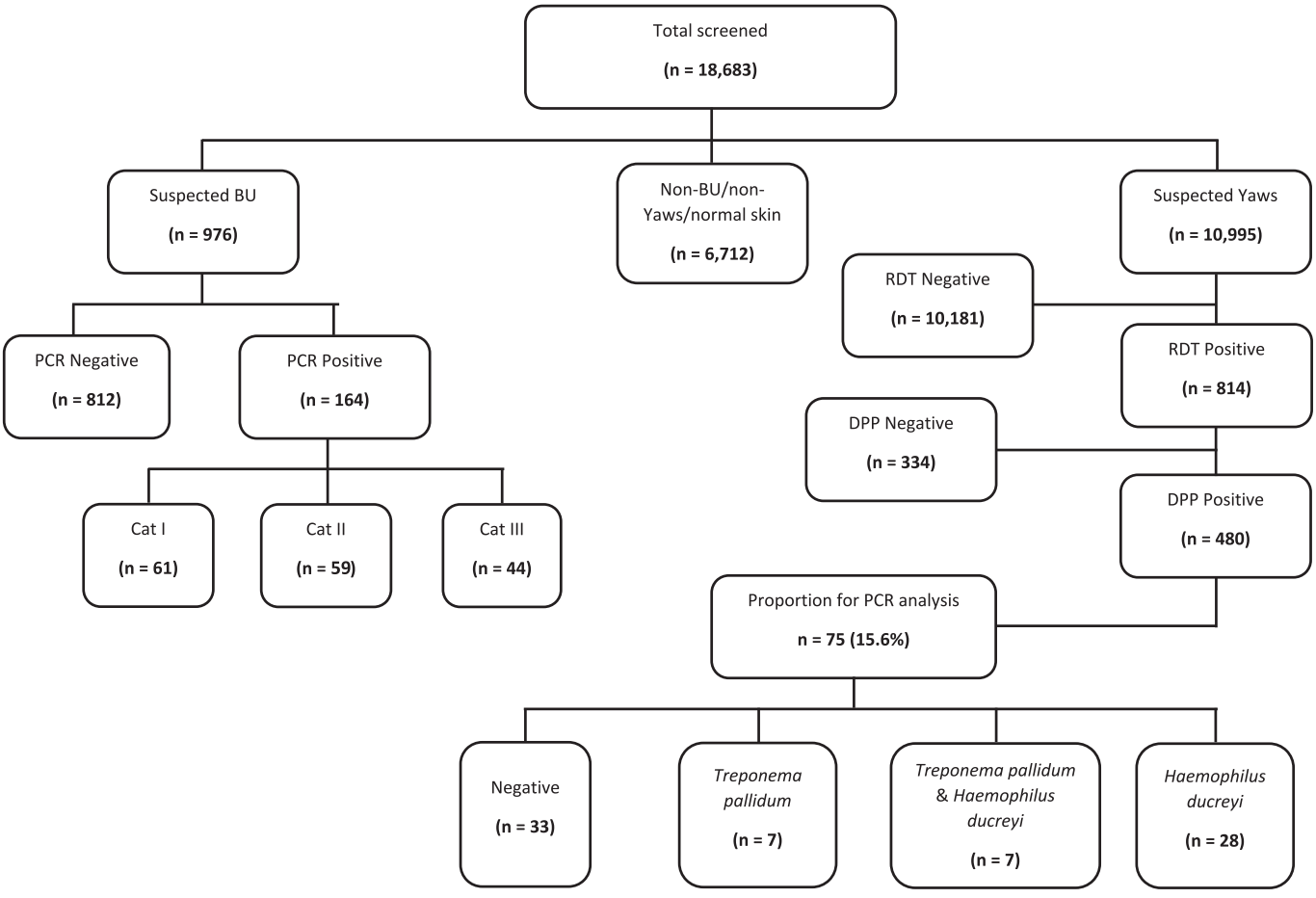

Fig. 1. Flow chart of study participants.

Table 1
Clinical and demographic characteristics of individuals with suspected and confirmed Buruli ulcer.

Variable	Study district							
	All	ASN	SAP	UDE	UDW	WAE	NKS	WM
Suspected cases, n (%)	976 (100)	136 (13.9)	76 (7.8)	54 (5.5)	242 (24.8)	208 (21.3)	175 (17.9)	85 (8.7)
Confirmed cases, n (%)	164 (100)	52 (31.7)	13 (7.9)	24 (14.6)	31 (18.9)	37 (22.6)	6 (3.7)	1 (0.6)
% PCR positivity	16.8	38.2	17.1	44.4	12.8	17.8	3.4	1.2
Median age of confirmed BU in years (IQR)	24.0 (12.0—37.8)	22.0 (9.0—35.0)	16.0 (16.0—32.0)	23.5 (10.0—40.0)	26.0 (19.0—37.0)	27.0 (14.0—45.5)	26.5 (12.5—47.3)	56
Sex, n (%)								
Male	77 (47)	23 (44.2)	10 (41.7)	17 (54.8)	17 (45.9)	2 (33.3)	7 (53.8)	1(100)
Female	87 (53 %)	29 (55.8)	14 (58.3)	14 (45.2)	20 (54.1)	4 (66.7)	6 (46.2)	0 (0)
WHO category, n (%)								
I	61 (37.2)	23(44.2)	3 (12.5)	14(45)	11(29.7)	2 (33.3)	8(61.)	0
II	59 (36)	20(38.5)	14(58.3)	10(32)	11(29.7)	1 (16.7)	3(23.1)	0
III	44 (26.8)	9 (17.3)	7 (29.2)	7 (22.5)	15(40.6)	3 (50)	2(15.4)	1 (100)
Clinical form, n (%)								
Ulcer	129 (78.7)	24 (46.2)	22 (91.6)	29 (93.5)	36 (97.3)	5 (83.3)	12 (92.3)	1 (100)
Nodule	13 (7.9)	11 (21.2)	0 (0)	2 (6.5)	0 (0)	0 (0)	0 (0)	0 (0)
Plaque	19 (11.6)	16 (30.7)	1 (4.2)	0 (0)	1 (2.7)	0 (0)	1 (7.7)	0 (0)
Oedema	3 (1.8)	1(1.9)	1 (4.2)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)

ASN- Asante Akim North, SAP- Sekyere Afram Plains, UDE- Upper Denkyira East, UDW- Upper Denkyira West, WAE- Wassa Amenfi East, NKS- Nkoranza South and WM- Wa Municipal.

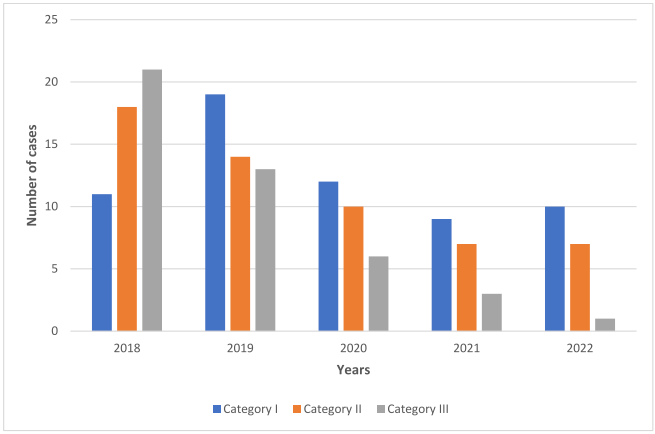

Fig. 2. Trends in number of confirmed BU cases by lesion category.

9–14) years] confirmed to have active yaws (based on DPP positive test) were further subjected to a multiplex PCR analysis. The multiplex PCR assay identified 7 DPP-positive yaws cases as *Treponema pallidum*, 28 as *Haemophilus ducreyi* (71.4 % of these were from Sekyere Afram Plains district) and 7 as *Treponema pallidum/Haemophilus ducreyi* coinfection (Table 2).

A total of 114 personnel including health staff, laboratory technicians and CBSVs received training. Further, standard operating procedures were established at all stages of the project. We observed that the training had an impact on sample collection, storage and transport. Following the trainings, samples received at the laboratory were in a better condition, adequate, and there was an improvement in the completion of accompanying forms. We analysed the percentage completion of specific details on the skin NTDs-BU01 forms at baseline (2018) and at the end of the implementation (2022). There was an increase in the completion of information on participant'demographics and contact' (50 % in 2018 vs 95 % in 2022) and 'clinical form of BU' (75 % in 2018 vs 95 % in 2022). The most improvement was seen in completion of BU 'lesion category' (10 % in 2018 vs 95 % in 2022). Compared to previous periods, the financial support given to the districts facilitated more timely transport of samples to the laboratory.

4. Discussion

We report on laboratory support for the implementation of an integrated skin NTD programme in selected districts in Ghana. One hundred and sixty-four individuals were confirmed to have Buruli ulcer and 480 were with active yaws. Among 75 individuals in the DPP positive yaws population, multiplex PCR analysis revealed 28 individuals with *Haemophilus ducreyi* (*H ducreyi*), and 7 with *Treponema pallidum* and *H ducreyi* infection. Our data provide evidence for the essential role of laboratories in the implementation of integrated approaches for the control of skin NTDs in endemic countries.

Ghana is one of the countries recognised by World Health Organization to be endemic for BU. In the current study, at least one case of BU was recorded in each of the study districts. Further, there was a decreasing trend in the number of confirmed cases of BU over the period. The high proportion of individuals with category III BU at the beginning of the study in 2018 is concerning and may be a reflection of limited case detection activities prior to this study. Early BU lesions are associated with less disability and lower socio-economic costs for affected individuals and families [25]. Furthermore, early disease may result in less stigmatization of affected individuals. With the implementation of the intervention which provided support to the districts for case searches, the proportion of individuals with category I disease increased. Most lesions (73 %) referred for BU confirmation were either category I or category II lesions. The findings from the study show that active case search activities can lead to an increase in the detection of early lesions; these may be associated with better treatment outcomes, less disability and disease related stigma.

The median time taken for samples to arrive in the laboratory after collection was 6 days. Control programmes should address the issue of sample transportation to reference laboratories using modalities including courier, dedicated cars, or drones. Timely delivery of samples to the laboratory for processing can facilitate quicker confirmation of cases and result in early initiation of treatment for cases. We have further shown that it is possible to provide PCR results to districts in a short time (median of 5 days) using electronic communication tools such as WhatsApp and email to facilitate timely decision making on patient management.

The PCR positivity rate was relatively high (about 40 %) in the Asante Akim North and Upper Denkyira East districts. This finding is most probably due to the continuous training of health staff, long experience in management of BU including previous involvement in clinical trials and low staff attrition in these two districts.

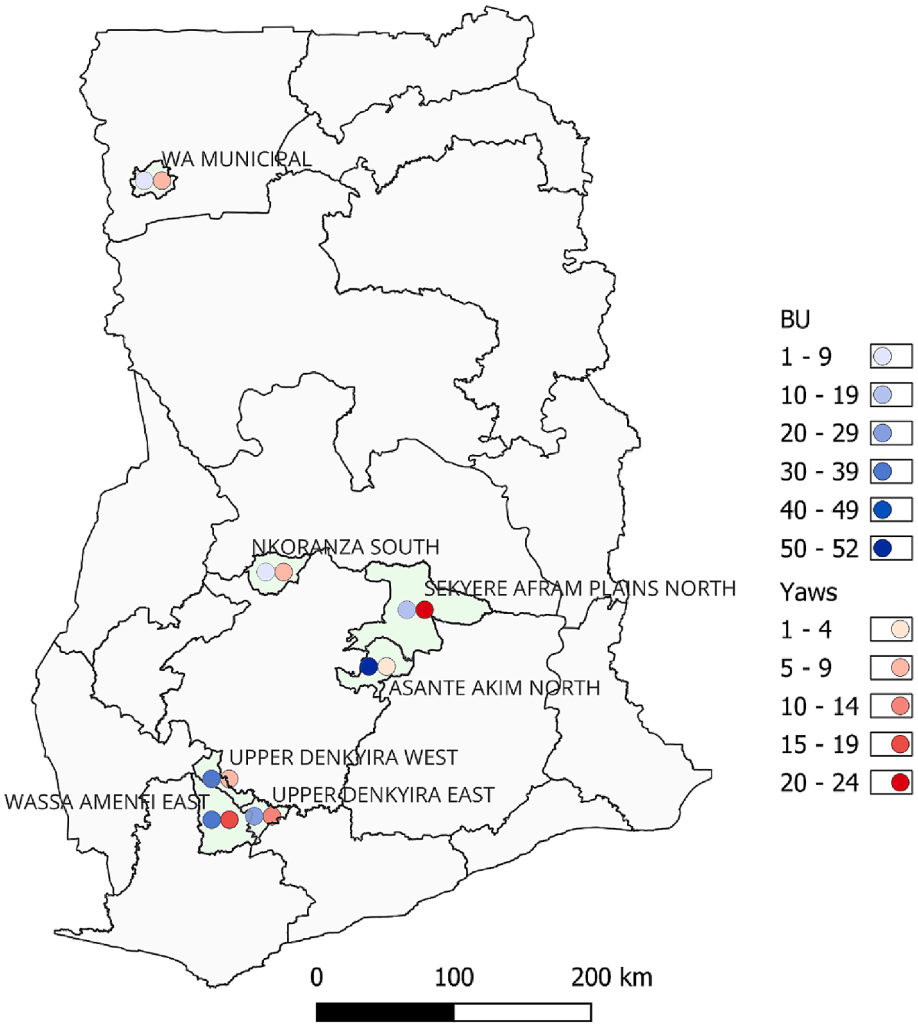

Fig. 3. The distribution of BU and Yaws in study districts. Numbers represent the absolute number of cases of either BU (blue colour) or Yaws (red colour) confirmed during the study period. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Characteristics of DPP-positive yaws cases subjected to multiplex PCR testing.

Variable	Study district							
	All n = 75	**ASN** n = 2	**SAP** n = 24	**UDE** n = 14	**UDW** n = 6	**WAE** n = 16	**NKS** n = 7	**WM** n = 6
Median age in years (IQR)	11(9–14)	4.5 (3–4.5)	11 (7.2–7.8)	11(9.5–14)	17.5 (9.5–25)	11.5 (9.3–13)	12(9.8–15.3)	12.5 (8.8–25)
Sex, n (%)								
Male	43 (57.3)	1 (50)	10 (41.7)	8 (57.1)	5 (83.3)	13 (81.3)	3 (42.9)	3 (50)
Female	32 (42.7)	1 (50)	14 (58.3)	6 (42.8)	1 (1.7)	3 (18.7)	4 (57.1)	3 (50)
Clinical form, n (%)								
Ulcer	59 (78.7)	2 (100)	24 (100)	12 (85.7)	4 (66.6)	7 (30.8)	7 (100)	3 (50)
Papilloma	15 (20)	0 (0)	0 (0)	2 (14.3)	1 (16.7)	9 (69.2)	0 (0)	3 (50)
Ulcer/Papilloma	1 (1.3)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Multiplex PCR results, n (%)								
T. pallidum	7 (9.3)	0 (0)	0 (0)	1 (7.1)	1 (16.7)	2 (12.5)	0 (0)	3 (50)
H. ducreyi	28 (37.3)	0 (0)	20 (83.3)	5 (35.7)	0 (0)	3 (18.8)	0 (0)	0 (0)
T. pallidum/ H. ducreyi	7 (9.3)	0 (0)	0 (0)	2 (14.3)	1 (16.7)	4 (25.0)	0 (0)	0 (0)
Negative	33 (44.1)	2 (100)	4 (16.7)	6 (42.9)	4 (66.6)	7 (43.7)	7 (100)	3 (50)

ASN- Asante Akim North, SAP- Sekyere Afram Plains, UDE- Upper Denkyira East, UDW- Upper Denkyira West, WAE- Wassa Amenfi East, NKS- Nkoranza South and WM- Wa Municipal.

All the study districts were found to be endemic for yaws. About 4 % of individuals with suspected yaws were confirmed to have active yaws. Indeed, Ghana is known to be highly endemic for yaws and previously reported the highest number of yaws cases globally [26]. This calls for strengthening of the surveillance system to fight the scourge of yaws in the population. There is the need to enhance case search activities, screen at-risk populations and treat all identified yaws cases and their contacts. Mapping of yaws endemic communities will be essential to guide policy planning and resource deployment in this regard.

Furthermore, some individuals with DPP positive yaws were found to

have infection with *Treponema pallidum*, *Haemophilus ducreyi* or co-infection with *Treponema pallidum/ Haemophilus ducreyi* following PCR. This observation is consistent with previous studies [27–29] and is most probably due to individuals with latent stage of yaws having a positive syphilis serological test but with other organisms implicated in the same lesion [27]. *Haemophilus ducreyi* ulcers were particularly common in the Sekyere Afram Plains district. Azithromycin is effective for treating genital strains of *H. ducreyi* and some studies have shown some good outcomes for cutaneous disease [30]. Further studies using molecular detection techniques for surveillance strategies are needed to assess treatment outcomes and possible resistance to facilitate global yaws eradication.

While BU, yaws and *Haemophilus ducreyi* were confirmed to cause skin lesions in this study population, the aetiology of majority of the lesions could not be ascertained. Indeed, cutaneous leishmaniasis has been reported among rural communities in the Oti region of Ghana [31,32]. Further studies of skin ulcers should employ multiplex assay to help decipher the aetiologies of atraumatic skin lesions in the Ghanaian population.

4.1. Challenges

There was an observed improvement in the quality of samples transported to the laboratory. Nevertheless, some samples were sent to the laboratory without accompanying forms and in some cases the skin NTD 01 forms were inadequately completed. This calls for continuous training of staff in the necessary protocols to maintain optimal standards in these areas. High turnover of staff in some districts also presents a challenge for control efforts. There is a need for continued capacity building of staff on skin NTDs to promote the attainment of the objectives of the WHO 2030 roadmap and the Sustainable Development Goal 3.3 on ending the epidemic of neglected tropical diseases [9].

5. Conclusion

The implementation of integrated diagnostic confirmation for skin NTDs is feasible with the provision of timely results to stakeholders within a week. Multiplex diagnostic tools differentiated *Treponema pallidum* and *Haemophilus ducreyi*. Sustained outreach activities are important for detection of early lesions of BU and yaws. There is a need to sustain active case search activities, enhance health worker training, and improve laboratory confirmation of cases as part of the overall strategy for the integrated control of skin neglected tropical diseases.

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Ethical statement

This study presents the outcome of an implementation activity under the auspices of the Ghana Health Service. Approval was obtained from the Committee on Human Research and Publication Ethics of the School of Medicine and Dentistry of the Kwame Nkrumah University of Science and Technology (approval number: CHRPE/AP/335/19). Further, permission was sought from local authorities and school heads. All participants provided informed consent.

CRediT authorship contribution statement

Abigail Agbanyo: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bernadette Agbavor:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Solomon Gyabaah:** Writing – review & editing, Validation, Project administration, Methodology, Investigation. **Michael Ntiamoah Oppong:** Writing – review & editing, Project administration, Investigation, Data curation. **Olivia Dornu:** Writing – original draft, Project administration, Data curation. **Philemon Boasiako Antwi:** Writing – review & editing, Visualization, Project administration. **Aloysius Dzigbordi Loglo:** Writing – review & editing, Supervision, Project administration. **Kabiru Mohammed Abass:** Writing – review & editing, Visualization, Investigation. **George Amofa:** Writing – review & editing, Visualization, Investigation. **Nana Konama Kotey:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Benedict Quao:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Michael Frimpong:** Writing – review & editing, Supervision, Project administration, Methodology. **Kingsley Asiedu:** Writing – review & editing, Visualization, Resources. **Yaw Ampem Amoako:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Richard Odame Phillips:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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