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COST-EFFECTIVENESS AND EASE OF TEST PERFORMANCE OF DIRECT AGGLUTINATION TEST AND THE RK39 RAPID DIAGNOSTIC TEST FOR VISCERAL LEISHMANIASIS IN WAJIR COUNTY, KENYA

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ABSTRACT

Background: **Testing for Visceral Leishmaniasis (VL)or Kala-Azar (KA) in low resource areas like Wajir was predominantly by serological tests like the Direct Agglutination Test (DAT) and the rapid diagnostic test (rK39). DAT was difficult to use in Wajir because it required unavailable specialized laboratories. Also, the Kenyan Ministry of Health (MOH) during part of this period did not recognize rK39 as a baseline test whereas it is available in Wajir. This resulted in systemic confusion in testing.**
Objective: **To determine the cost-effectiveness and ease of performance of DAT and rK39 which were randomly used to test for KA in Wajir.**
Methods: **Analytical study of laboratory records of newly tested patients by way of desk review was done. Quota sampling yielded 65 for study. The clinical decision analysis and diagnostic odds ratio were used for analysis.**
Setting and Study Subjects: **The study was done on the Wajir County Hospital records of the year 2008-18 of patients newly tested by DAT, rK39 and splenic aspiration.**
Outcome measures: **Cost-Effectiveness Ratio (CER) and ease of test performance of the DAT and rK39 tests.**
Results: **The study found a lower average CER of rK39 (57) compared to DAT (812) equivalent to ratio of 0.07:1. It also found performing rK39 required fewer and simpler resources than DAT.**
Conclusions and Recommendations: **The findings correlated well with similar studies done in other KA endemic areas. It was recommended that the rK39 test be adopted as the first-line diagnostic test for KA in Wajir and similar settings.**

INTRODUCTION

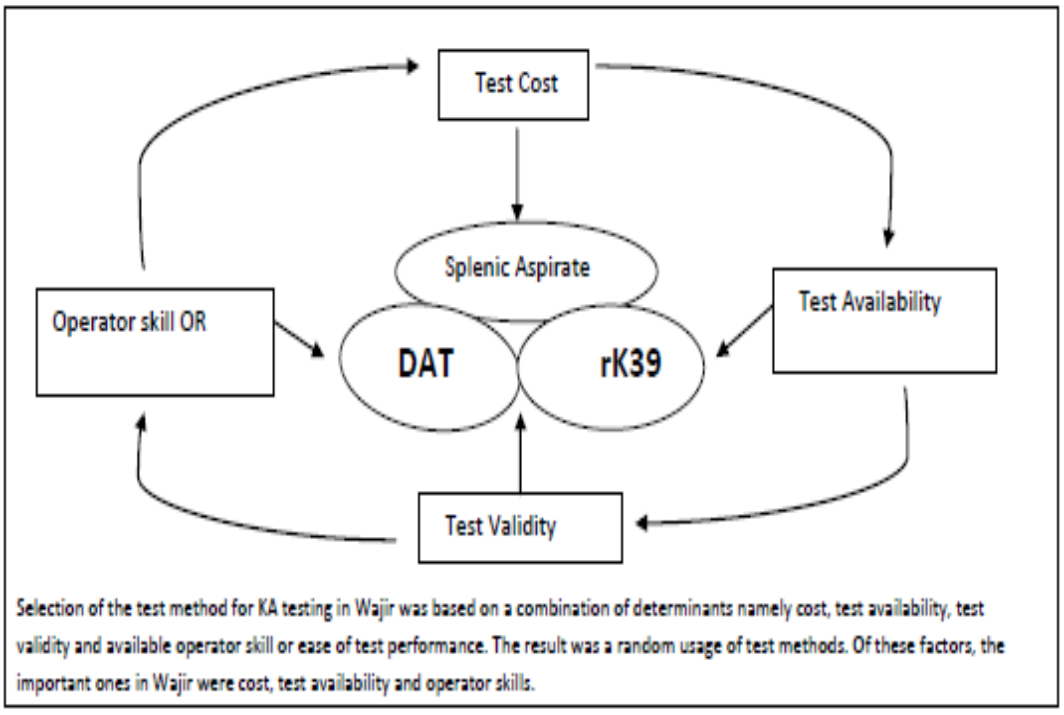
Visceral Leishmaniasis (VL) or Kala-Azar (KA) is a chronic human and canine disease. The common tests in low resource countries are Direct Agglutination Test (DAT) and the newer rapid diagnostic tests based on the rK39 protein like rK39 and its variants like rK9, rK26 and rKE16 [1]. DAT was difficult to use in remote field conditions [2] and particularly in Wajir County because it required that patient specimens be shipped to Nairobi where the required laboratory was available [3]. Also, the Kenyan MOH did not recognize the rK39 test as diagnostic [4]. This led to random use of the DAT and rK39 tests in Wajir.

KA is endemic in East Africa [5]. Outbreaks occurred in Sudan (1984–1994 and 1996-1997), Kenya and Ethiopia (2000–1) and Ethiopia and Eritrea (1997–1998) [6]. It is estimated that VL in East Africa causes at least 4 000 deaths annually [7]. The most recent outbreak in Kenya was in Wajir and Isiolo counties in 2008 [3]

The world needs cost-effective health care. The relationship between the cost of health care and benefits has come under scrutiny because new expensive technologies may contribute to a rapid increase in health insurance premiums while providing little or no benefit to the patient [12].

The study was important because in Wajir DAT and rK39 were used randomly for diagnosis due to inadequate policy and local knowledge to aid in test selection. This may have caused confusion among health workers and increased healthcare expenses. The Wajir scenario was best represented in the study conceptual framework below (Figure1).

Figure 1: Conceptual Framework



Source: Original work by the author

The objectives of the study were to determine the cost of testing with DAT and rK39 in Wajir, evaluate the ease of

performing the tests and to calculate and compare their cost-effectiveness ratios.

MATERIALS AND METHODS

Study Design: This was a hospital-based analytical study. Clinical decision analysis (CDA) approach and diagnostic odds ratio (DOR) were used for analysis. A decision tree describing the possible alternative testing strategies together with their probabilities was used to make a judgment of clinical and economic consequences of the testing options (Figure 11).

Variables: Test validity, cost and ease of performance for DAT and rK39, with splenic aspirate as the gold standard test hence the control.

Study Setting: The study was carried out in 2018 in Wajir County Hospital which is the county referral hospital. The cost of the study was borne by the researcher.

Study Population: Laboratory records of years 2008-18 of all the patients suspected of and tested for KA with a splenic aspirate, DAT and rK39 tests.

Inclusion-Exclusion Criteria

Records of patients who presented for the first time at the Wajir County Hospital from 2008-18 suspected of KA and were tested with the rK39, DAT and splenic aspirate tests were included in the study. The indeterminate result, re-lapsed and re-treated patient records were excluded.

Sampling Procedure and Size: Quota sampling was done on laboratory records of all KA suspected patients who met the inclusion-exclusion criteria on the 3 tests (quotas). 65 records met the criteria and were all studied.

Data Type and Collection: Data was collected using researcher designed data sheets (Table 1-2). Dichotomous, nominal, quantitative data was collected thus: -

1. Test results for splenic aspirate, DAT and rK39 from which tests validity was determined.
2. Costs of the tests from which average costs of testing for DAT and rK39 were calculated.
3. Procedural steps and equipment used from which ease of test performance was determined.

Table 1

Test validity

Positive test (+ve)	**True Positive (A)** **Splenic Aspirate (SA) +ve but rK39 or DAT +ve**	**False Positive (B)** **SA –ve, rK39 or DAT +ve**	**All Positives (A+B)**
Negative test (-ve)	False Negative (C) Splenic Aspirate (SA) +ve but rK39 or DAT –ve	True Negative (D) SA –ve, rK39 or DAT –ve	All Negatives (C+D)
Population	All infected (A+C)	All uninfected (B+D)	All population (A+B+C+D)

Table 2

Cost and Ease of test performance

	DAT test				**rK39 test**			
No.	Cost KES)	Test steps	Process Time	Special Skills & Equipment (Yes/No)	Cost (KES)	Test Steps	Process time	Special skills & equipment
1								
2								
Average								

Data Processing and Analysis: CDA and DOR were used to analyze data. CER was

calculated as the cost per morbidity averted. Ease of test performance (ETP) was an additional measure.

Minimization of Error and Study Limitations: Minimization of error was done at multiple levels namely: -

- Selection – Entire sampling frame was studied.
- Recall - Data was collected from existing records only.
- Matching – The study subjects selected had undergone all three tests.
- Data analysis - Operator error reduced by using statistical software SPSS.
- Confounders – Relapse and re-treatment cases were excluded from the study because of the risk of false positivity due to lingering antibodies.

Limitations of the study: Prevalence of KA in Wajir was unknown preventing the precise calculation of predictive values which would determine the public health significance of the tests in the local context.
Qualified medical staffs in Wajir were inadequate and so only a few splenic aspirates were done thus limiting the sampling frame and sample size.
Some aspects of costing were like labour, depreciation of equipment, utilities and time were difficult to determine because of unavailable records.
Ethical Issues: Authority for the study was obtained from the Ethics Committee of the University of Nairobi and the Kenyatta National Hospital. At the Wajir County Hospital, the authority of the hospital management committee was obtained. The study was done on patient's records only where standard research guidelines of confidentiality, accountability and feedback were observed.

RESULTS

Result I – Test Validity: 65 study cases whose KA test results were studied are summarized in Table 3.

Table 3
KA testing results

	Result Description	**DAT (titre >1:3200)**	**rK39**
1	True +ve	50	48
2	False +ve	2	5
3	All +ve	52	53
4	True –ve	12	9
5	False –ve	1	3
6	All –ve	13	12
7	Total	65	65

Test validity was the calculated sensitivity, specificity and predictive values.
Sensitivity = (True +ve / True +ve + False –ve) x 100%
Sensitivity of DAT = (50/ (50+1) x 100% = 98%
Sensitivity of rK39 = (48/ (48+3) x 100% = 94.7%
Specificity = (True –ve / True –ve + False +ve) x 100%
Specificity of DAT = (12/ (12+2) x 100% = 86%
Specificity of rK39 = (9/ (9+5) x 100% = 64%
Positive Predictive Value (PPV) = TP/(TP+FP)
The positive predictive value of DAT = 50/ (50+2) =96%

The positive predictive value of rK39 = 48/(48+5) = 91%

Negative Predictive Value (NPV) = TN/(TN+FN)

The negative predictive value of DAT = 12/(12+1) = 92%

The negative predictive value of rK39 = 9/(9+3) = 75%

Result II – Cost and Ease of Test Performance

The cost of the KA tests and the ease of performance of the tests are summarized in Table 4.

Table 4

KA Cost and Ease of Performance

	Item Description (per patient/test)	**Cost/Value** Note: - All cost in Kenya shillings (KES)		
		DAT	**rK39**	**Splenic Aspirate (SA)**
1	Average cost of health service (laboratory) costs.	KES 747	KES 45	KES 900
3	Ease of test performance (steps involved)	4	2	5
4	Ease of test performance (time spent) in minutes (min)/days.	1445 min	10 min	3 days
5	Ease of test performance (number of specialised skills)	2	1	6
6	Ease of test performance (number of specialised equipment)	7	0	7

Operational definitions: -

1. *Health service costs or laboratory costs – the cost of material used by the laboratory to perform a test.*
2. *Specialised skills – skills that the staffs at the laboratory required to undertake the test.*
3. *Specialised equipment – laboratory equipment exclusively used for testing KA.*

Result III - Cost-Effectiveness Analysis (CEA): CEA took into account test validity, test effectiveness and test cost.

Test Validity: The validity probabilities for DAT and rk39 are summarized in Table 5.

Table 5

Validity and Probabilities

	Test	Wajir (Baseline) DAT Titre >1:3200	Plausible/Reference Range from literature review. DAT Titre 1:400–6400	References for plausible range from literature review
1	Specificity DAT	0.86	0.6 - 1	[9]
2	Sensitivity DAT	0.98	0.8 - 1	[10]
3	Specificity rK39	0.64	0.6 – 0.85	[6]
4	Sensitivity rK39	0.94	0.7 – 0.95	[9]

Test Effectiveness: Effectiveness of KA test in this study was considered as morbidity averted relative to the obligatory terminal morbidity associated with the absence cure of KA. The test which averted morbidity the most was the most effective.

Since morbidity could not be quantitatively measured, an outcome's effectiveness was determined by stating either: -

Yes = that KA morbidity was averted as a result of a correct diagnosis, or

No = that KA morbidity was not been averted as a result of incorrect diagnosis.

This allowed for a decision tree for the results to be constructed with their respective probabilities as is presented in Figure 11. The average effectiveness of each outcome was calculated from its pay-off

value weighted by its probability. Because the effectiveness, in this case, was a qualitative result (yes or no) it was equated to a score of one (1) to make weighting by probabilities mathematically possible (Table 6).

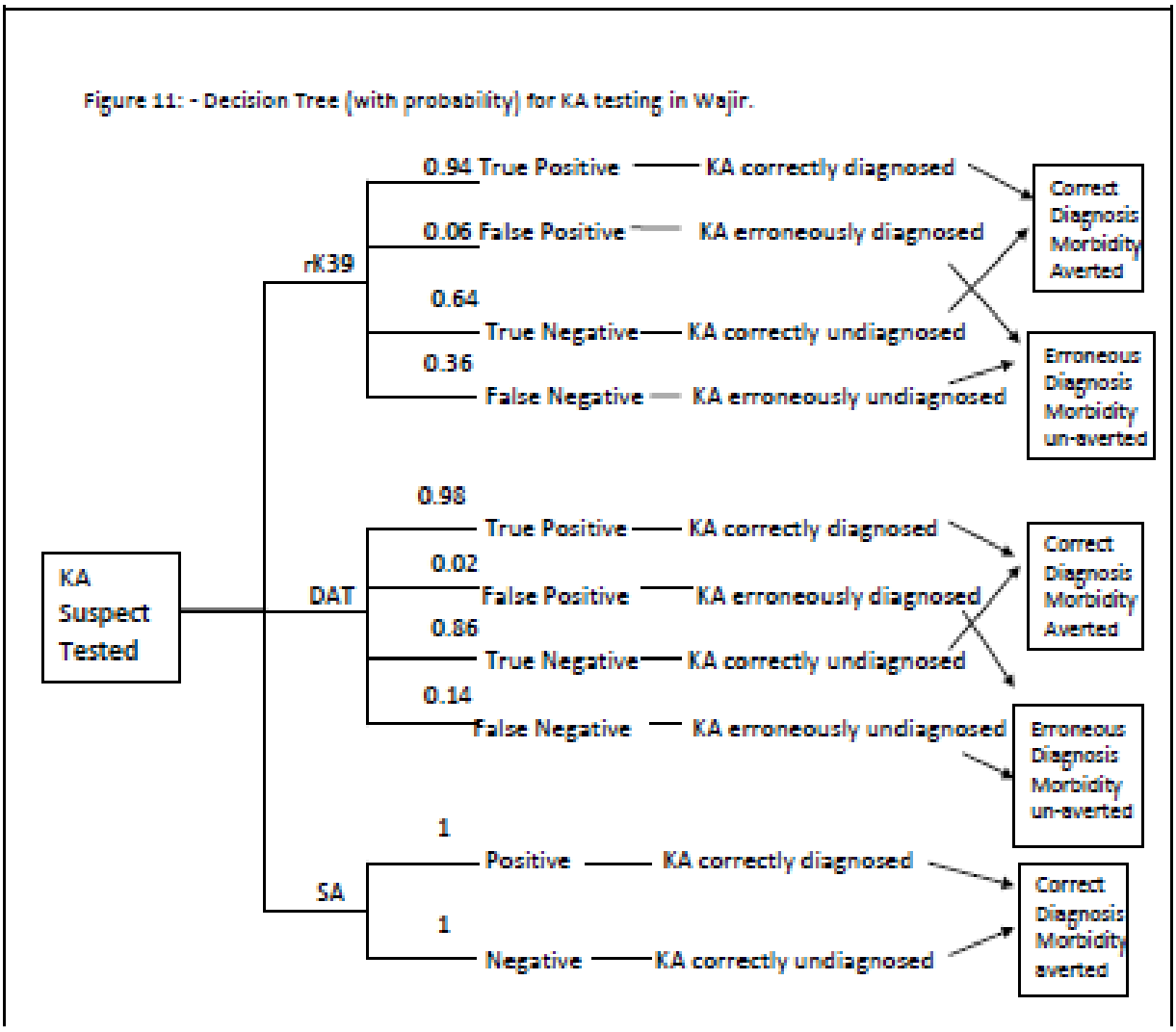


Table 6

Effectiveness of KA test outcomes

	Test Result	Test Outcome	Effectiveness (pay-off)	Probability	Weighted Effectiveness (expected value)
DAT					
1	True Positive	KA correctly diagnosed	Yes (1)	0.98	0.98
2	True Negative	KA correctly undiagnosed	Yes (1)	0.86	0.86
3	1+2	Correct diagnosis. Morbidity Averted	Yes (1)	0.92	0.92
4	False Positive	KA erroneously diagnosed	No (-1)	0.02	-0.02
5	False	KA erroneously	No (-1)	0.14	-0.14

	Negative	undiagnosed			
6	4+5	Erroneous Diagnosis. Morbidity Un-averted	No (-1)	0.08	-0.08
rK39					
1	True Positive	KA correctly diagnosed	Yes (1)	0.94	0.94
2	True Negative	KA correctly undiagnosed	Yes (1)	0.64	0.64
3	1+2	Correct diagnosis. Morbidity Averted	Yes (1)	0.79	0.79
4	False Positive	KA erroneously diagnosed	No (-1)	0.06	-0.06
5	False Negative	KA erroneously undiagnosed	No (-1)	0.36	-0.36
6	4+5	Erroneous Diagnosis. Morbidity Un-averted	No (1)	0.21	-0.21
SA					
1	Positive	KA correctly diagnosed	Yes (1)	1	1
2	Negative	KA correctly undiagnosed	Yes (1)	1	1

Average Cost Effectiveness: The average cost-effectiveness ratio (ACER) for each test was calculated as shown in Table 7.

Table 7

Average Cost-Effectiveness Ratios of Screening KA

	Description	**Test type DAT**	**Test type rK39**
1	Average cost per test	747	45
2	Average Effectiveness per test	0.92	0.79
3	Average Cost-Effectiveness Ratio (ACER) (Cost per KA Morbidity Averted) = (1/2)	812	57

Sensitivity Analysis of Critical Components: Univariate sensitivity analysis was performed on test parameters susceptible to uncertainty. Four critical components were considered – test validity, effectiveness, cost and patient heterogeneity.

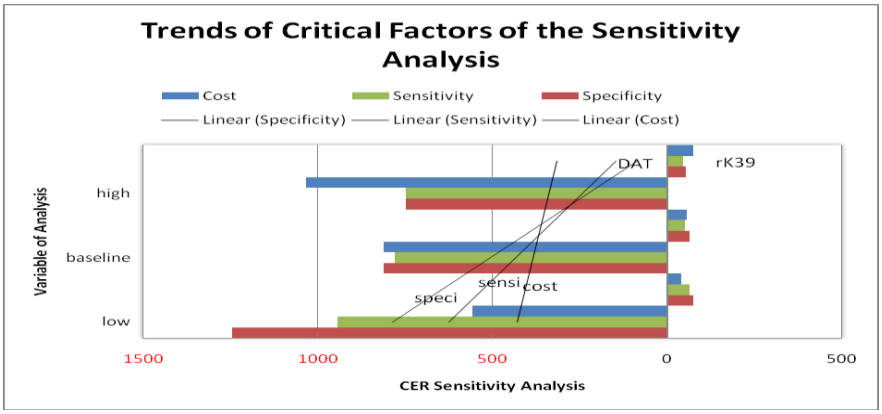
The plausible ranges for sensitivity and specificity were used for test validity sensitivity analysis. Sensitivity analysis of the cost of the test was done by assuming price changes in either direction at fixed measures of 15% and 30%. The sensitivity analysis considering all the critical components were summarized in Table 8 and tornado diagram in Figure 111. The finding was that the ACER of DAT was significantly higher than that of rK39 in all components.

Table 8

Aggregated Sensitivity Analysis by Test Validity, Cost and Effectiveness

Description		DAT	rK39
	Baseline		
1	Average cost per test	747	45
2	Average Effectiveness per test	0.92	0.79
3	Average Cost-Effectiveness Ratio (ACER)	812	57

	Analysis 1 (price 30% less)		
1	Average cost per test	523	32
2	Average Effectiveness per test	0.92	0.79
3	Average Cost-Effectiveness Ratio (ACER)	568	41
	Analysis 2 (price 15% less)		
1	Average cost per test	635	38
2	Average Effectiveness per test	0.92	0.79
3	Average Cost-Effectiveness Ratio (ACER)	690	48
	Analysis 3 (price 15% more)		
1	Average cost per test	859	52
2	Average Effectiveness per test	0.92	0.79
3	Average Cost-Effectiveness Ratio (ACER)	934	66
	Analysis 4 (price 30% more)		
1	Average cost per test	971	59
2	Average Effectiveness per test	0.92	0.79
3	Average Cost-Effectiveness Ratio (ACER)	1055	75
	Analysis 5 (sensitivity low extreme plausible range)		
1	Average cost per test	747	45
2	Effectiveness per test	0.8	0.7
3	Average Cost-Effectiveness Ratio (ACER)	934	64
	Analysis 6 (sensitivity high extreme plausible range)		
1	Average cost per test	747	45
2	Effectiveness per test	1	0.95
3	Average Cost-Effectiveness Ratio (ACER)	747	47
	Analysis 7 (specificity low extreme plausible range)		
1	Average cost per test	747	45
2	Effectiveness per test	0.6	0.6
3	Average Cost-Effectiveness Ratio (ACER)	1245	75
	Analysis 8 (specificity high extreme plausible range)		
1	Average cost per test	747	45
2	Effectiveness per test	1	0.85
3	Average Cost-Effectiveness Ratio (ACER)	747	53
	Analysis 9 (effectiveness low extreme plausible range)		
1	Average cost per test	747	45
2	Average Effectiveness per test	0.7	0.65
3	Average Cost-Effectiveness Ratio (ACER)	1067	69
	Analysis 10 (effectiveness high extreme plausible range)		
1	Average cost per test	747	45
2	Average Effectiveness per test	1	0.9
3	Average Cost-Effectiveness Ratio (ACER)	747	50



Key: - Speci = specificity. Sensi = sensitivity

Figure 111. Trends of Sensitivity Analysis

The trend line for specificity had the steepest gradient meaning specificity caused the most change of cost-effectiveness.

Patient heterogeneity: Heterogeneity was controlled by the study inclusion-exclusion criteria where only new suspected cases of KA were studied.

Diagnostic Odds Ratio (DOR)

DOR defined as the ratio of the odds of disease in test positives relative to the odds in test negatives [14] was calculated as DOR = (TP/FP) / (FN/TN). The confidence intervals (SE) for range estimates and significance testing was calculated as SE (log DOR) = √(1/TP +1/TN + 1/FP + 1/FN). A 95% confidence level of the log of DOR was chosen and calculated as SE=logDOR +/- 1.96SE (logDOR). Results are summarized as below.

DOR of DAT = (50/2) / (1/12) = 300

DOR of rK39 = (48/5) / (3/5) = 16

SE (logDOR) of DAT = √(1/50)+(1/12)+(1/1)+(1/2) = 1.725

SE (logDOR) of rK39 = √(1/48)+(1/9)+(1/3)+(1/5) = 0.789

A 95% confidence level for DAT = range from 1.725 - (1.96x1.725) to 1.725+(1.96x1.725) = -1.656 to 5.106

rK39 = range from 0.789 - (1.96x0.789) to 0.789+(1.96x0.789) = -0.757 to 2.335

DOR of DAT at 95% confidence level = 298.344 to 305.106

DOR of rK39 at 95% confidence level = 15.243 to 18.335

DISCUSSION

Sensitivity, Specificity and Predictive Value

In comparing the effectiveness of rK39 strip test to DAT it is noteworthy that both being serological tests may give false-positive results due to past KA infections and cross-reactions with co-infections from infectious diseases like tuberculosis, HIV, and malaria [11]. Sensitivity and specificity by region also vary due to ethnicity, environment and severity of infection or differences in antigen genotype [12]. The World Health Organization's Special Program for Research and Training in Tropical Disease (TDR) evaluated five different immuno-chromatographic tests utilizing either rK39 or rKE16. Testing was performed in East Africa, Brazil and on the Indian subcontinent, and sensitivities ranged from 36.8–100% and specificities from 90.8–100%. No test was the clear winner across all regions and conditions [13]. The Wajir study compared well having a specificity of rK39 of 64% and sensitivity of 94.7%.

In a meta-analytical Indian study, DAT was more sensitive than rK39 with results varying between 92.6 and 100% for DAT and 87 and 100% for rK39. Specificity varied widely between studies but was always lower than sensitivity results [8]. In East Africa, by analyzing results from Sudan, Kenya, and Ethiopia, the average sensitivity for DAT was found to be 92.8% and for rK39 was 79.1%, but with some results as high as 90.0%. As with Indian results, specificity varied widely for DAT and rK39. DAT had an average of 91.2% specificity while rK39 was 84.8% [8]. These studies in the Indian and East African regions correlated well with those of the Wajir study.

Diagnostic Odds Ratio

The prevalence of KA in Wajir was not known and therefore DOR was used to validate the specificity and sensitivity. The higher the value of a DOR the better the discriminatory ability of the test [14].

The estimated DOR of DAT to detect KA in Wajir at 95% confidence level was 298.344 to 305.106 while that of rK39 was 15.243 to 18.335. This meant that for DAT the odds for positivity among patients with KA is 300 times higher than the odds for positivity among subjects without KA compared to odds of 15 for rK39. This finding of the DOR agrees with that of specificity, sensitivity and predictive values that DAT was testing better than rK39.

Cost, Cost-Effectiveness and Ease of Performance.

DAT and rK39 are available at low costs hence are affordable in low-income areas [15]. Studies between 1999-2005 found DAT to cost an average of $2.50/test, including peripheral costs for materials and labour while rK39 tests were found to cost between $1 and $1.30[6][8]. rK39 therefore given its lower cost and the fewer additional requirements was more feasible, cost-effective and easy to use in the field [15]. The Wajir study found similar results that rK39 was more cost-effective than DAT. It also found performing rK39 was also easier compared to DAT.

CONCLUSIONS AND RECOMMENDATIONS

The Wajir study like other studies done elsewhere found that DAT was more sensitive and specific than rK39 but less cost-effective and harder to use in the field compared to rK39. It was therefore recommended that rK39 be used as the first-line test for diagnosis of KA in Wajir. There however remained questions on how much both DAT and rK39 results can be accepted as confirmatory in Wajir because of inadequate data on local KA and other infectious diseases prevalence hence the possibility of significant false test results and more research was recommended on prevalence.

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