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# Diagnostic accuracy of swine echinococcosis cytopathological tests and challenges for a differential diagnosis: slaughterhouse data

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**Summary**

Echinococcosis disease shows clinical signs similar to many diseases. Hence we report cases that need to be confirmed using appropriate tests. A confirmatory study has been conducted to assess the accuracy of two cytopathological tests, with the histopathology test as the reference standard. The first cytopathological test evaluates the Ziehl Neelsen staining with an epifluorescence microscope (cytopath 1). The second cytopathological test uses the same staining followed by a transmitted light microscope examination (cytopath 2). Of a total of 2524 inspected pigs, 101 suspected cases of echinococcosis were detected, of which 67 were found positive with the two cytopathological tests and the histopathological one. The specificity of cytopath 1 (100 % [95 % CI 100 – 100]) and cytopath 2 (100 % [95 % CI 100;100]) were similar, as well as their respective positive predictive values: 100 % [95 % CI 100 – 100] vs. 100 % [95 % CI 100 – 100]. The sensitivity of cytopath 1 is 79.66 % [95 % CI 69.39 – 89.93], while cytopath 2 equals 66.10 % [95 % CI 54.02 – 78.18]. The difference in sensitivity of both tests was not significant. Negative predictive values found for cytopath 1, and cytopath 2 were 40 [95 % CI 18.53 – 61.47] and 28.57 [95 % CI 11.84 – 45.3], leading to the Generalized Estimating Equations (GEE) Model estimate for an odds ratio of 1.4 [95 % CI 0.41 – 5.2], p = 0.06. Cytopath 1 and cytopath 2 are equivalent in terms of specificity (100 % [95 % CI 100 – 100] vs. 100 % [95 % CI 100;100]) and positive predictive value (100 % [95 % CI 100 – 100]. Cytopath 1 is more sensitive than cytopath 2 but not significant (79.66 % [ 95 % CI 69.39 – 89.93] vs. 66.10 % [95 % CI 54.02 – 78.18]). However, the negative predictive value of cytopath 1 is better than that of cytopath 2: 40 % [95 % CI 18.53 – 61.47] vs. 28.57 % [95 % CI 11.84 – 45.3].
**Keywords:** *Echinococcus* spp; Epifluorescence; Histopathology; Pig

## Introduction

Hydatidosis is of interest in many developing countries. It remains a public health concern and is on the list of neglected tropical zoonotic diseases (Moro & Shantz, 2009). Hydatidosis is caused by parasites of the genus Echinococcus (Craig *et al*., 2015), of which 4 are zoonotic species: *Echinococcus multilocularis*, *Echinococcus granulosus*, *Echinococcus oligarthrus* and *Echinococcus vogeli* (causing Polycystic Echinococcosis); and of two recently discovered species *Echinococcus shiquicus* in small mammals from the Tibetan plateau and *Echinococcus felidis* found in African lions (Eckert *et al*., 2011). The confirmation of suspected cases using adequate methods giving accurate, reliable results available on the same day of testing, is needed and essential. The tests, especially cytopathological ones, require the presence of suspected hydatid fluid. In clinical samples, many studies demonstrated the acid

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fast characteristics of *Echinococcus* spp. hook. Unfortunately, in many cases, the fluids are insufficient, no longer available, or poor around the hook. Therefore, the histopathological test is not the only remaining reference standard test (WHO/OIE, 2001; Zhang & McManus, 2006) for Cystic Echinococcosis (CE) and Alveolar Echinococcosis (AE) that demand tissue with suspected parasitic parts (Craig *et al*., 2015; Brunetti *et al*., 2010) but it is the best diagnostic method for differential diagnosis between relevant diseases such as tuberculosis, benign or malignant tumors, abscesses, or *Ascaris suum* infection. Moreover, histopathological tests and Polymerase Chain Reaction (PCR) accurately differentiate *Echnicoccus granulosus* and *Echinococcus multilocularis*, whereas serologic tests such as ELISA cannot (Georges *et al*., 2004, Thiaoying *et al*., 2008). Expensive PCR techniques and Western blot tests can differentiate *Echnicoccus granulosus* from *Echinococcus multilocularis* in 76 % of cases (Liance *et al*., 2000).

Macroscopic observation can detect *Echinococcus multilocularis* with multilocular cysts and smaller in size. The histopathology examination reveals the parasitic cyst on an outer laminated layer that generally calcifies and in an inner germinal layer with most often absent protoscolices. There is also a weak or no striated laminated layer of parasite membrane. Generally, vital protoscolices are absent and don't contain calcification (Reinehr *et al*., 2019). The acellular laminated layer is either non-disrupted or lines the cystic parasite vesicle, which is fragmented and displays a convoluted architecture (Keutgens *et al*., 2013). In detail, there is noteworthy cells budding in the germinal layer; protoscolices contain calcareous corpuscles and hooks. An essential feature of vesicles is the presence of fine reticular tissue in which broad capsules contain fully developed and embedded protoscolices. Another hallmark is the presence of a germinal layer infiltrating cellular protrusion in the distant metastatic foci (Miyauchi *et al*.,1984, Thiaoying *et al*., 2008).

Macroscopic examination of *Echinococcus granulosus* shows all simple or unilocular cysts, much larger and fertile. Histopathologically, there is a typical trilayered cyst wall: inner germinal, intermediate laminated, and outer adventitial layer. The additional feature is the presence of vital protoscolices having calcification some-

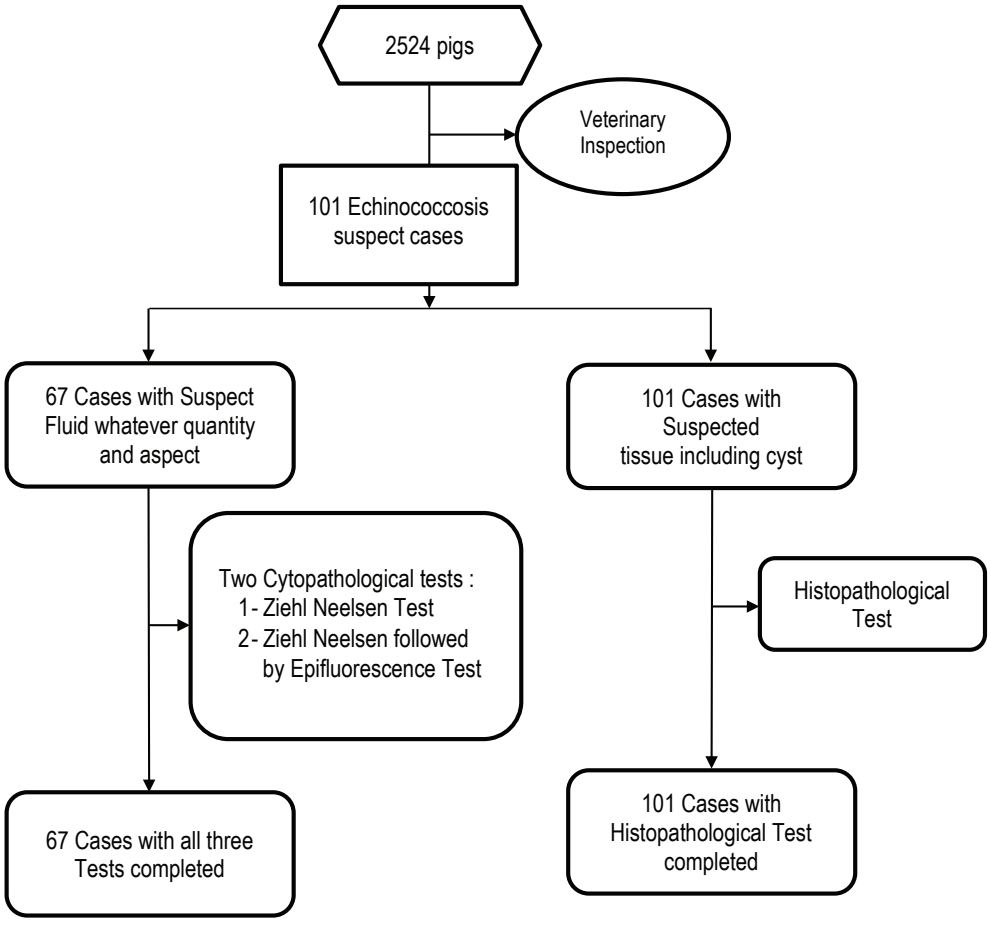


Fig. 1. Flow chart of the diagnostic accuracy study.

Table 1. Nature of infected organs in swine echinococcosis diagnosed in Côte.

| Parasite | Infected organs | | |
|---|---|---|---|
| | Liver (%) | Lung (%) | Kidney (%) |
| *Echinococcus granulosus* | 1 | 7 | 53 |
| *Echinococcus* spp. | 0 | 0 | 2 |
| Total | 1 (1.6) | 7 (11.1) | 55 (87.3) |

Values in parentheses are percentages.

times but no calcification in the striated laminated layer (Reinehr *et al*., 2019). Neither brood capsule nor calcareous corpuscles are observed.

The current study aims to assess the diagnostic accuracy of two index diagnostic methods: cytopathological test 1, in which methanol is fixed followed by Ziehl Neelsen stain followed by examination under transmitted light microscopic, and cytopathological test 2, which utilizes the same stain but examination is performed with epifluorescence microscopic, and compare both with the standard histopathological method. The second goal of this study is to detect species of *Echinococcus* spp involved in positive swine echinococcosis, and the third objective is to demonstrate how vital differential diagnosis is.

## Materials and Methods

*Study design*

This is a prospective, mono-center, paired-cohort confirmatory study. The methodology fulfills the level 1b evidence for diagnostic test performance as published in the international reference guideline: Standards for Reporting Diagnostic Accuracy (STARD) (Bossuy *et al*., 2015). Moreover, STARD (Standards for Reporting of Diagnostic Accuracy Studies) is endorsed by the major outstanding medical and scientific journals such as The New England Journal of Medicine, The Lancet, Nature, and Science.

*Study area and data collection*

Current study involved pigs from the southeast region of Côte d'Ivoire, representing around 90 % of the national pig production (FIRCA, 2019). Pigs intended to be slaughtered irrespective of sex, and age, except pregnant sows and sick animals, were recruited between October and December 2019. At the SIVAC slaughterhouse, during post-mortem inspection, suspected cysts were incised into two parts and diagnostics were followed by sample collection. Samples were of fluid inside the cyst, where half of the suspect cyst was preserved in a 10 % formalin-fixed solution, and the second half was collected in a sterile plastic vial. Unfixed samples and the portion of samples placed in formalin solution were kept at +4°C in the abattoir before the same-day transportation to the Central Vet Lab.

*Definition of swine clinical echinococcosis*

The cyst-like mass from 4 mm to 1 cm for young cysts and up to 10 cm for old ones should be present in organs such as the liver, lung, and kidney. After palpation and incision, the Veterinary-Inspector should detect fluid in the partially or filled cysts. Fluid should be clear, lemon juice-like, or brown, where all cyst types represent a suspect case of echinococcosis (CDC, 2020; Jeffrey *et al*., 2012, Bacciarini *et al*., 2004).

In the current study, a confirmed case with the index tests is present when one of the following structures is revealed by Ziehl Neelsen staining in hydatid fluid: *Echinococcus* spp pathognomic hooklets, protoscolices, or protoscolices components. Then, these structures are observed with an Epifluorescence microscope (Cytopathopathological test 1) or transmitted light microscopy (Cytopathological test 2).

Following WHO-expert consensus, *Echinococcus granulosus* or *Echinococcus multilocularis* infection is confirmed using histopathology test and/or detection of nucleic acid sequence in a clinical specimen (Brunetti *et al*., 2010).

*Sample size for sensitivity and specificity estimations*

The sampling strategy followed the simple two phases design (Obuchowski & Zhou, 2002). In this design, the Veterinary-Inspector first examines all the subjects (N) recruited. After this first phase, only positive subjects undergo the two index diagnostic tests and the reference standard test in the second phase. In a performed prospective study that estimates diagnostic test accuracy, this strategy minimizes the sample size to the strict necessary subjects. Indeed, In the first phase, pigs not having suspect cysts, whatever their size, are classified as negative. Under this design, conditions are met because the expected prevalence of echinococcosis is low (equals or less to 10 %), the expected specificity of index tests is high (>80 %), the expected sensitivity is moderate to better (~70 %) and cost of a tested subject is greater than the cost of the test (Obuchowski & Zhou, 2002). Based on slaughterhouse registry data, because there is no nationwide data, the expected prevalence p is of around 6 %, with 5 % precision, α risk at 5 % (two-sided), one-half width L of confident interval (CI) of 95 % being 0.10, the expected sensitivity $S_e$ is 70 %, to assess the accuracy of each cytopathological test relative to histopathological reference standard test, The minimum sample size calculated is N = 1345 subjects.

$$N = \frac{(1-S_e)S_e}{PL^2} \times Z^2_{1-\alpha/2}$$ (Obuchowski & Zhou, 2002)

N: sample size
P: expected prevalence
L: width of the confident interval; Se: expected sensitivity;
$Z^2_{1-\alpha/2}$ : Statistical critical value to be read in the table;

*Laboratory assays*

Cyst fluid examination

All collected fluid samples were screened with a stereomicroscope (ZEISS, Germany) at 600 X magnification to detect infection caused by *Cysticercus tenuicollis* and *Taenia hydatigena* parasitic larvae stage. Then, for the cytopathological examination, those samples were centrifuged at 500 g for 10 minutes (Z 306, Germany), and the pellets were resuspended in a plastic vial. Then 1 – 2 ml were placed on a glass slide for a microscopic examination. The suspended pellet was dried on air on the second slide for about 5 minutes. Then, after using methanol and hot carbol-fuchsin for 10 min, 3 % HCl in 95 % ethanol for 30 s fixation staining with 1 % methylene blue for 30 s, and Ziehl Neelsen stain followed. The stained slides were observed with an epifluorescence microscope (OLYMPUS CX 23, Japan), at 250 magnification for the first index test (Cytopathological test 1) and with a transmitted light microscope (ZEISS, Germany) equipped with phase contrast for the second index test (Cytopathological test 2). Concerning epifluorescence, the sole set available is characterized by an excitation filter wavelength of 436 nm with a long-pass filter of 520 nm. Microscopic observation and results reading were carried out blindly by two different lab technicians for cytopathological test 1 and cytopathological 2 index tests.

Fixed formalin cyst tissue examination

Histopathological and macroscopic examinations of affected organs containing suspect cysts have been performed at the central

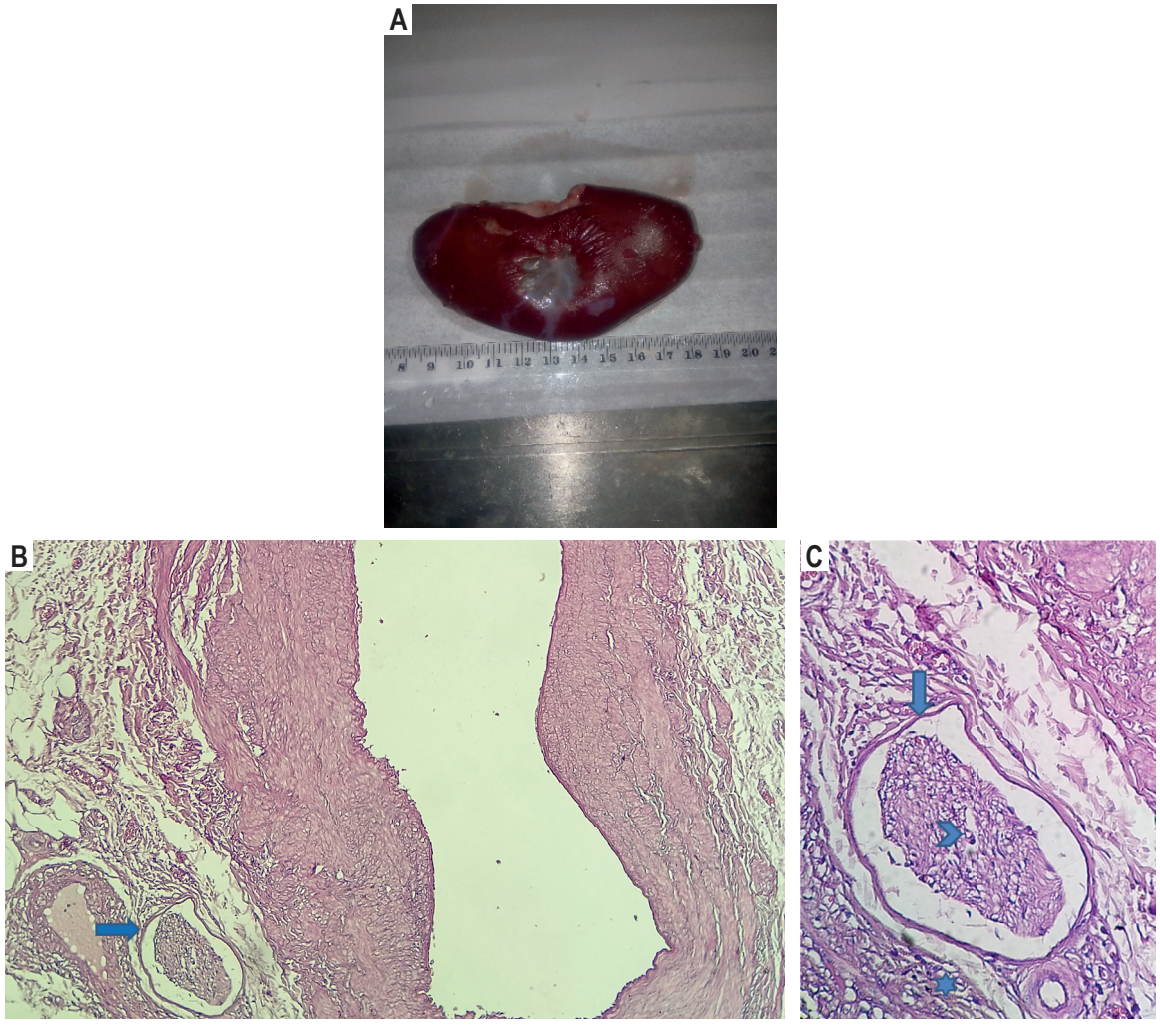


Fig. 2. Kidney echinococcosis caused by *Echinococcus granulosus*

**A:** Hydatid cyst in kidney, swine. **B:** Broad capsule of hydatid cyst with typical striated and trilaminated layer and that do not contain calcification. Note a protoscolisce (arrow). HES stain.**C:** Protoscolice (arrow), with inside little calcareous corpuscles (arrow head). Protoscolisce is surrounded by almost no inflammatory reaction composed of few lymphocytes and macrophages (stars). There are no neutrophils.

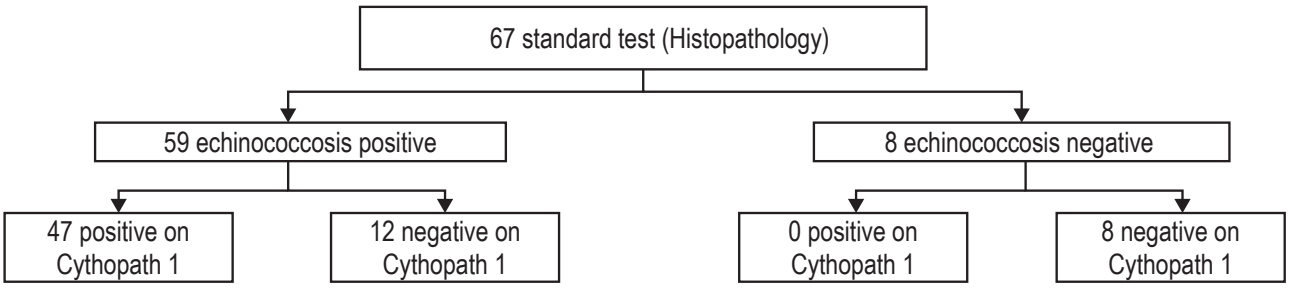


Fig. 3. Diagnostic accuracy for detection of swine echinococcosis between histopathological test and cytopathological test 1 (Ziehl stain with epifluorescence microscope).

veterinary laboratory of Bingerville (Côte d'Ivoire). This method permitted to accomplish the first differential diagnosis between *Echinococcus granulosus* and *Echinococcus multilocularis* lesions or cysts presentation as described by Bacciarini *et al*., 2004, Miyauchi *et al*., 1984; Chiou *et al*., 2001, and detect the pathologies such as neoplasms, abscess, Mycoses, *Ascaris suum* parasitic infections, cirrhosis that could happen. Tissue samples, including cysts, were embedded in paraffin, cut to 3 – 5 µm in diameter, and stained with classical Hematoxylin Eosin stain. Special stains such as PAS, Gram, and Ziehl Neelsen have been carried out to fulfill differential diagnosis requirements.

Additional assay

If the histopathological diagnosis were a bacterial or viral disease origin, samples have been submitted to a culture test or conventional PCR test. All the histopathologically positive or negative samples of *Echinococcus* spp, including *Echinococcus granulosus* or *Echinococcus multilocularis,* have been submitted to the immunohistochemical test to get second confirmatory results according to the procedure described by Reinehr *et al*. (2020).

*Statistical analysis*

The determined diagnostic performances were as follows: diagnostic sensitivity, diagnostic specificity, Negative Predictive Value (NPV), and Positive Predictive Value (PPV) of the two index cytopathological tests analyzed with R software (http://www.r-project.org) package DTComPair. The sensitivity and specificity of the two index tests were compared by the Mac Nemar test (Mac Nemar, 1947). For the epidemiological study, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were compared using the mean of General Estimating Equation (GEE) and logistic regression model (Leisenring *et al*., 2000; Kosinski, 2013; Moskowitz & Pepe, 2006). The odds ratio is defined by the odds of each test correctly detecting the presence or absence of echinococcosis disease. Therefore, the ratio of cytopathological test 1 and cytopathological test 2 was determined. The Kappa parameter of each index test regarding the reference test and their respective statistical Z test have been evaluated.

**Ethical Approval and/or Informed Consent**

Considering ethical issues, adverse events to animals from performing the index and reference standard tests are not applicable because they were performed as post-mortem examinations. Moreover, as in any abattoir, pigs are not slaughtered for study purposes. Therefore, formal consent is not required.

**Results**

Between October and November 2019, 2524 pigs were examined by a Veterinary-Inspector for suspected echinococcosis cases, and 101 were found positive. A total of 67 suspected cases out of 101 positive samples complied with the requirements for cytopathological index tests and standard reference test examination with fluid in the cystic lesions. These 67 cases were submitted to reference histopathological test, and 63 were found positive (Fig. 1). Among these 63 were actual echinococcosis cases, there were 61 positive for *Echinococcus granulosus* (Table 1; Fig. 2), and 2 were positive cases for *Echinococcus* spp. (Table 1). Considering affected organs, most of the infestation occurred in the

Table 2. Diagnostic accuracy of cytopathological test 1 (Ziehl staining with the epifluorescence microscope) and cytopathological test 2 (Ziehl Neelsen staining with the transmitted light microscope) in the detection of clinical swine echinococcosis.

| | Cythopath 1<br>% [95% CI] | Cythopath 2<br>% [95% CI] | Test ratio<br>[95% CI] | p value |
|---|---|---|---|---|
| Specificity | 100 [100 – 100] | 100 [100 – 100] | 1 [1 – 1] | – |
| Sensitivity | 79.66[69.39 – 89.93] | 66.10 [54.02 – 78.18] | 1.2 [0.88 – 1.66] | 0.059 |
| PPV | 100 [100 – 100] | 100 [100 – 100] | 1 [1 – 1] | – |
| NPV | 40 [18.53 – 61.47] | 28.57 [11.84 – 45.3] | 1.4 [0.41 – 5.2] | 0.06 |

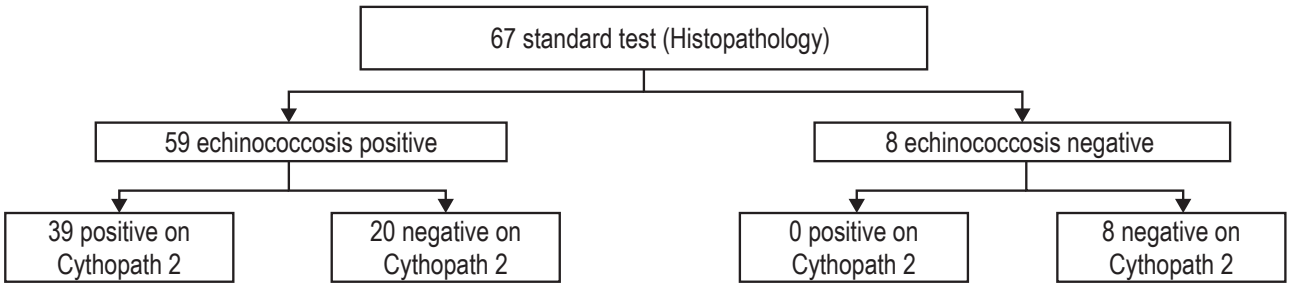


Fig. 4. Diagnostic accuracy for detection of swine echinococcosis between histopathological test and cytopathological test 2 (Ziehl Neelsen stain with transmitted light microscope).

kidneys (55 cases; 87.3 % [95 % CI 76.5 – 94.35]), followed by the lungs (7 cases; 11.1 % [95 % CI 4.59 – 21.56]) and liver (1 case; 1.6 % [95 % CI 0.04 – 8.53]) ($p < 0.001$) (Table 1). All the positive histopathological cases were immuno-histochemically positive for *Echinococcus granulosus*. Also, the negative histopathological cases were negative for the immuno-histochemical test.

*Cytopathological diagnostic tests accuracy*

Methanol fixed, followed by Ziehl Neelsen staining, was examined with an epifluorescence microscope on the 67 fluids, and 47 were positive (70.1 %) (Fig. 3).

With the methanol-fixed fluids followed by the Ziehl Neelsen test, 39 out of 67 samples were positive (58.2 %). Neither index tests had intermediate or doubtful cytopathological results (Fig. 4).

Both cytopathological tests are equivalent in accuracy regarding specificity (100 % [95 % CI 100 – 100] vs. 100 % [95 % CI 100 – 100]; Mac Nemar test ratio =1[95 % CI 1 – 1]). For the PPV, cytopathological test 1 had the same accuracy as the second test (100 % [95 % CI 100 – 100] vs. 100 % [95 % CI 100 – 100], GEE Model estimate for odds ratio 1[95 % CI 1 – 1]). (Table 2).

Examination regarding sensitivities showed that cytopathological test 1 is more sensitive than cytopathological test 2 (79.66 % [95 % CI 69.39 – 89.93] vs. 66.10 % [95 % CI 54.02 – 78.18]; Mac Nemar test ratio 1.2 [95 % CI 0.88 – 1.66]). Nevertheless, the difference in sensitivity was not significant. The NPV parameter conferred a better accuracy to the cytopathological test 1 on cytopathological test 2 (40 [95 % CI 18.53 – 61.47] vs. 28.57 [95 % CI 11.84 – 45.3]; GEE Model estimated the odds ratio 1.4 [95 % CI 0.41 – 5.2], $p = 0.06$). Concerning conformity of both index tests with the reference one, the cytopathological test 1 showed 82 % vs. 70.1 % for the cytopathological test 2. The Kappa parameters were 48 % [95 % CI, 22 – 75] and 34 % [95 % CI, 9 – 58]. In consequence, the corresponding Z test was $Z= 3.624$ ($p < 0.001$) for test 1 and $Z = 2.69$ ($p < 0.01$) for test 2.

*Diseases of differential diagnosis detected*

Many diseases have been diagnosed when swine echinococcosis is not confirmed. These diseases can be divided into parasitic,

Table 3. Occurrence of diseases that could be misdiagnosed as swine echinococcosis in Côte d'Ivoire

| Diseases | Involved organs | | |
|---|---|---|---|
| | Liver | Lung | Kidney |
| *Ascaris suum* infection or migration | 4 | 2 | |
| *Cysticercus tenuicollis* hepatitis | 1 | | |
| Fasciolosis | 1 | | |
| *Cryptococcus neoformans* infection | 1 | | |
| Cirrhosis | 3 | | |
| Neoplasms | 1 (Hepatocellular carcinoma) | 0 | 1 (Small clear cell carcinoma) |
| Hydronephrosis | | | 4 |
| *Actinobacillus* spp *or Mycoplasma* spp. Pneumonia or Pleuropneumonia | | 11 | |
| Inflammatory conditions of unknown aetiology | | | 2 |
| *Abscess* | | 3 | |
| Total | 11 | 16 | 7 |

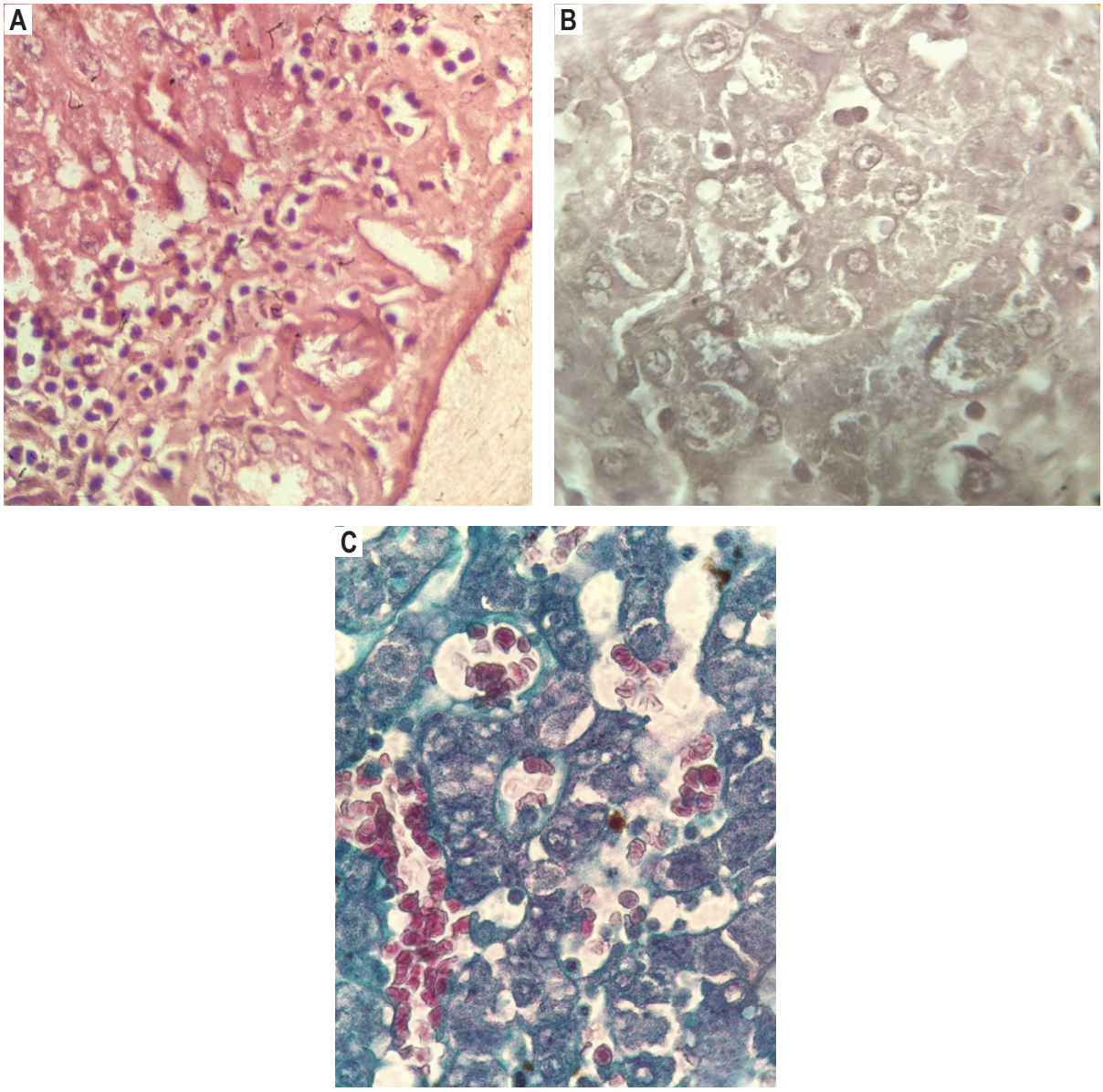


Fig. 5. *Cryptococcus neoformans* hepatitis (5.A: HES Stain; 5.B: PAS stain: There are many budding yeasts (arrow); 5.C: Grocott Methenamine Silver stain).

non-parasitic infections, and miscellaneous conditions such as neoplasm and cirrhosis. Indeed, diseases such as *Cysticercus tenuicollis* hepatitis, *Ascaris suum* multifocal and granulomatous hepatitis, hepatocellular carcinoma, liver cirrhosis, and other pathological conditions have been diagnosed (Table 3). Nevertheless, in one confirmed cystic echinococcosis case, it's noteworthy to mention that associated peri-portal amyloidosis affected the liver. In this challenging case, there was a yeast infection present due to *Cryptococcus neoformans* (Fig. 5)

## Discussion

One of the biggest challenges in meat inspection is confirming the diagnosis within hours. An accurate, less expensive and rapid test is essential for such purpose. Suppose cytopathological test results for echinococcosis diagnostic are available after several hours. In that case, it is essential to estimate their accuracy with the histopathological test as a reference standard because of the scarcity of standard gold tests. Whatever the infected organs, and whenever a cytopathological test cannot be conducted due to lack of fluid or insufficiency (< 3ml), the histopathological reference test from tissue samples has always performed well.

To the best of our knowledge, this is the first study to report the cytopathology test's diagnostic performance for the detection of swine echinococcosis. Consideration of another diagnostic test utilizing crude hydatid cyst fluid (HCF) from a specimen, such as

IHA, Craig *et al.* (2015) reported a lower sensitivity and specificity between [25 % and 50 %] and [40 % and 60 %], respectively. For the immunoelectrophoresis test performed on the same specimen, the sensitivity estimate was 23.8 % and specificity was from 61 % to 89 % if the target population is 1 year or older. Utilizing ELISA in which recombinant antigen is based on HCF, sensitivity was 89.2 %, and specificity was 89.5 %. For many reasons, cytopathological tests' performances are superior to these tests. First of all, with cytopathological tests, false positives are avoided. It has to keep in mind that false positive implies organ condemnation with the consequence of unjustified economic losses. This is the case with IHA, ELISA, and immunoelectrophoretic tests showing 11.5 % to 60 % false positivity. Secondly, sensitivities are similar to ELISA sensitivity or superior to immunoelectrophoresis or IHA sensitivities. Here the advantages of cytopathological tests, sensitivities, and specificities reside in the detection of true positive subjects, whereas in serological tests sensitivities and specificities are false positives due to cross-reactions. These cross-reactivities have been observed in humans infected with parasitic diseases such as cysticercosis (Hermelin *et al*., 2019). Checking the false negative's importance, cytopathological tests are equivalent to ELISA but superior to IHA and immunoelectrophoresis.

Concerning the applicability of index tests, they are easy to perform, results are available after several hours. In that way, the Veterinary-Inspector can rapidly provide balanced and rapid decisions. The finer point of the Veterinary-Inspector decision lies within three constraints: The necessity to preserve public health, the avoidance of unjustified organ destruction, and the need for an urgent decision to avoid keeping (in many developing countries, there is no facility to do that and if available it is very expensive) animal carcass or organs for several days pending laboratory results. Each of these aspects has double components: economic and ethical.

The major disadvantages of cytopathological tests are attributable to the disease's physiopathological state, host immune response and possible previous and intermittent treatments received by a pig. In particular, these drawbacks preferably impact the sensitivity. Firstly, there is a paucity of hydatid fluid in characteristic hooklets and protoscolices when cysts are very young. Secondly, there is an insufficient volume of hydatid fluid (< 0.3 ml) to perform the cytopathological test. The reason for that is the physiopathological standpoint; when after the host infection, the cyst grows 1mm per month (Mihmanli *et al*., 2016). This situation mainly occurs in earlier or more aged cysts in which water content becomes gelatinous because of its resorption and mineralization of dead protoscolices, including hooklets (Chiou *et al*., 2001). Third in more advanced chronic states, hydatid fluid can be very dirty due to the strong inflammatory response of the host. Consequently, this immune response causes parasite starvation (Thompson & Lymbery, 1990). In addition, many hooklets could be hidden or wrapped. Yet, it is noteworthy that the centrifugation step in our study highly overcomes this hindrance. The Kappa parameter also shows the usefulness of the two index tests. However, this is not the case for concordance coefficient, which could not be sufficient in situations when Kappa value is less than 80 %: This means there is more than 20 % of disagreement or uncertainty. So, the health and economic impacts of these incorrect results could be inacceptable for medical or veterinary practices. In all cases of hydatid fluid shortage, we recommend adding a cyst specimen to perform a simultaneous histopathological test as a guideline. This advice could be relevant in human or canine surgical treatment for echinococcosis infection.

Chemotherapy's impact on the sensitivity of index tests is primarily explained in most African regions by frequent farmers' auto medication with drugs such as albendazole, mebendazole, and praziquantel. Indeed, these are the three most used drugs in pigs from the south region. These prescriptions could significantly lower parasitemia by rendering cysts sterile, so their mild hooklet and protoscolices contents (WHO, 2001; Arif *et al*., 2008). Apart from the auto medication practiced by farmers, a veterinary prescription is declined for piglets in one administration at weaning, two administrations during growth, then for sows in one administration two weeks before parturition, and all pigs in one administration whenever echinococcosis condition is suspected. This effect results in numerous false negatives with cytopathological tests. The same problem could occur in human cases, especially in developing countries where most people take drugs without medical prescription or surveillance. This would constitute a potential bias when designing a study.

Veterinary inspection as a diagnostic activity remains the basis on which the Veterinary Inspector decides on the fate of a carcass or an organ. In general, the Veterinary-Inspector can decide to accept or condemn the entire carcass or a given organ as safe for human consumption, or to accept a part of the carcass or a given organ, or to require a particular treatment of meat before approving it for human consumption. This decision-making process relies on the accurate diagnostic of the disease, its severity, and its extent in the inspected animal. Despite their sensitivities, both index tests with a PPV of 100 % mean that the probability of infection is 100 % for a given cytopathological positive test result. Therefore, Veterinary-Inspector decision-making on positive test results is reliable. Similarly, an NPV of 99 % for the two index tests implies that a negative pig tested has a 99 % chance of being uninfected. Preservation of public health due to zoonosis linked to CE, the Veterinary-Inspector decision-making has to consider additional testing such as histopathological test on suspect cyst membrane.

In the west of Africa, this is the first published confirmatory diagnostic of domestic livestock echinococcosis caused by *Echinococcus granulosus*. The first confirmatory canine CE in west Africa was recently published (Mauti *et al*., 2016). This case involves genotype 6. Nevertheless, the first human cases published concern Côte d'Ivoire (Schmidt *et al*., 1978), Senegal (Hane *et al*., 1989), Ghana (Schneider *et al*., 2010) and Mauritania (Maillard *et al*., 2007; 2009). These human CE cases also involved genotype

6. It is well known that Côte d'Ivoire imported many pig breeds decades ago. Besides this, considering the use of dogs for hunting in the neighboring wild environment, the epidemiologic role mainly especially as an intermediate host of farmers' dogs in *Echinococcus multilocularis* and neotropical *Echinococcus* species*: Echinococcus oligarthrus* (Lühe, 1910) and *Echinococcus vogeli* Rausch and Bernstein, 1972 has to be addressed in the African continent. Yet, previous studies in other countries have demonstrated the role of domestic dogs in the *Echinococcus multilocularis* cycle (Eckert, 1997; Gunn & Pitt, 2012), even if foxes play a major role (Kamiya *et al*., 1987). Moreover, the suburban location of this study, originating farms, reinforces this role exerted by farmers' dogs. Concerning *Echinococcus vogeli*, the causative agent of polycystic echinococcosis, naturally infected domestic dogs have been diagnosed (D'Alessandro *et al*., 1981), and the bush dog is established to be a definitive natural host (Eckert *et al*., 2011).

Our study and many other studies show that the main locations of cystic echinococcosis involve the kidney and lungs (Chiou *et al*., 2001). A retrospective study between 2008 and 2015 in Côte d'Ivoire on most cattle suspect cases reported the kidneys are affected most (Acapovi *et al*., 2019). In Nigeria, a sole old study has shown the prevalence of suspect swine cases to be 56 % (Arene, 1985). This high prevalence could be due to many false positive cases. Nevertheless, the liver remains a potential site of infection (Moro & Shantz, 2009) even if only one case has been diagnosed in the current study. The reason for which swine kidney is preferably infected in the current study remains unknown. Further studies are needed to substantiate this renal affinity in swine.

The authors acknowledge that amyloidosis is observed for the first time in pig cystic echinococcosis, where the liver is the sole organ affected. The subtype AA, AL, or LL has not been characterized. This type of lesion has explicitly been diagnosed for *Echinococcus multilocularis* species infection in Cynomolgus Monkeys (*Macaca fascicularis*) (Bacciarini *et al*., 2004) and human patients (Ali-Khan & Raush, 1987). The amyloid subtype was AA, and the Disse space of the liver was affected. Additional infection, in this case owing to *Cryptococcus neoformans,* can be explained by pig exposure via inhalation to contaminated bird feces. Therefore, liver infection by this yeast could reveal the disease' disseminated stage. Nevertheless, amyloidosis might be the consequence of severe and chronic CE infection.

Amongst the limitations of the current study, the histopathological reference test is to be refined with the gold standard test that rarely exists when failed to detect infected animals that have not yet developed characteristic cystic lesions. So, these animals will be tested false negative. Moreover, diagnostic accuracy between subgroups such as sex, age and breed has not been assessed yet. This aspect will be evaluated in further studies. The interval time report from the index tests and the standard reference test is not applicable because tests have been done after animals' death. However, this interval time factor must be carefully assessed in human cases or living diseased animals because the physician or the research team often doe not get information about the drug administration.

In terms of the study added value, it should be noted the improvement in the diagnostic activity of the Veterinary Inspector who submits more or less samples to the laboratory to confirm his suspected cases

Then, based on differential diagnosis results and the macroscopic lesions generated, the college of Veterinary-Inspector brushes up its diagnostic skills before submitting samples to the laboratory. The necessity to improve disease control implies generating sound diagnostic data. It is the cornerstone condition that prevents or plans public health interventions to eradicate diseases.

**Key Findings**

- Cytopathological test using Ziehl Neelsen stain is a reliable test to detect and confirm swine echinococcosis rapidly.
- There is an enhancement of cytopathological test performance by using the epifluorescence microscope.
- If the amount of cyst fluid is insufficient to perform a cytopathological test, it is recommended to take a histopathological test on cystic tissue.
- Many diseases could be incorrectly diagnosed with echinococcosis, so it is essential to confirm each suspect case.
- Liver amyloidosis has been observed for the first time in swine echinococcosis due to *Echinococcus granulosus* associated with *Cryptococcus neoformans* infection.

**Conflict of Interest**

None declared.

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**Author Contribution**

Alassane TOURE conceived and designed the study. He wrote the article. Lisette TOURE and Genevieve ACAPOVI conducted data gathering. Brice SENIN did and supervised the veterinary examination of pigs. Naferima KONE and Lisette TOURE did some

of the statistical calculations. Emmanuel COUAC-HYMANN supervised laboratory diagnostics and reviewed the article. Malika KACAHANI also supervised laboratory diagnostics.

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