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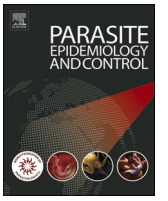

An update on epidemiology and clinical aspects of besnoitiosis in livestock and wildlife in sub-Saharan Africa: A systematic review

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ABSTRACT

Besnoitiosis is a parasitic disease of economic importance caused by cyst-forming protozoa from the genus *Besnoitia*. The disease affects the skin, subcutis, blood vessels, and mucous membranes of the animals. It is traditionally endemic in the tropical and sub-tropical regions of the world, and causes enormous economic loss associated with impaired productivity and reproduction, as well as skin lesions. Therefore, knowledge of the epidemiology of the disease, including the current *Besnoitia* species occurring in sub-Saharan Africa, the wide range of mammalian species hosts they use as intermediate hosts, and the clinical signs manifested by infected animals is crucial in developing effective prevention and control measures. This review collected information from peer-reviewed publications involving the epidemiology and clinical signs of besnoitiosis in sub-Saharan Africa using four electronic databases. Results showed that *B. besnoiti*, *B. bennetti, B. caprae, B. darlingi-like* and unidentified *Besnoitia* spp. were found naturally infecting livestock and wildlife across nine reviewed sub-Saharan African countries. *Besnoitia besnoiti* was the most common species, occurring in all nine reviewed countries, and utilised a wide range of mammalian species as intermediate hosts. Prevalence of *B. besnoiti* ranged from 2.0 to 80.3%, and *B. caprae* 5.45–46.53%. Infection rate was high with serology compared to other techniques. Some of the typical signs of besnoitiosis included sand-like cysts on the sclera conjunctiva, nodules in the skin, thickening and wrinkling of the skin and alopecia. Inflammation, thickening and wrinkling of the scrotum were observed in bulls, and lesions on the scrotum deteriorated progressively and became generalized in some cases in spite of treatment. There is still a need for surveys focusing on detecting and identifying *Besnoitia* spp. using molecular techniques in combination with serological, histology and visual observation, and scoping their natural intermediate and definitive hosts, as well as assessing the burden of the disease animals reared on different husbandry systems in sub-Saharan Africa.

1. Introduction

Besnoitiosis, also known as the elephant skin disease, is an acute or chronic parasitic disease caused by cyst forming coccidian protozoa from the genus *Besnoitia* (Apicomplexa: Sarcocystidae) (Njagi et al., 1998). According to Njagi et al. (1998), *Besnoitia*

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infections are of economic importance, associated with high morbidity and low mortality in their hosts (Glover et al., 1990; Van Heerden et al., 1993). The disease affects a wide range of domestic and wildlife host species (Schulz, 1960; Njagi et al., 1998), and infections present in three distinct stages: the acute anasarca stage, subacute stage and the chronic scleroderma stages which involves the development of cysts predominantly in the superficial tissues (Bigalke, 1968; Basson et al., 1970; Oryan et al., 2011; Langenmayer et al., 2015). Clinical cases are also characterised by physical features such as the formation of cysts in the connective tissues of the skin, muscles and other organs of their hosts, as well as the thickening of the skin around the eyes, face, nose, scrotum and the limbs (Njagi et al., 1998; Schulz, 1960).

According to Oryan et al. (2014), the *Besnoitia* genus includes species which have a heteroxenous life cycle that closely resembles that of *Toxoplasma gondii*, and has been proven to multiply sexually in the intestines of the definitive hosts, or asexually in various tissues of the intermediate hosts (IHs). *Besnoitia* species are thought to have an obligatory heteroxenous life cycle, and they exploit the prey-predator relationships to guarantee transmission (Frenkel, 1977; Zango et al., 2016). Ten *Besnoitia* species have been documented to date, viz. *B. besnoiti* (Marotel, 1912) Henry 1913, *B. caprae* (Njenga et al., 1993), *B. bennetti* (Babudieri, 1932; Dubey et al., 2005), *B. jellisoni* (Frenkel, 1953), *B. tarandi* (Hadwen, 1922; Levine, 1961; Dubey et al., 2004), *B. akodoni* (Dubey et al., 2003), *B. darlingi* (Brumpt, 1913) Mandour, 1965, *B. oryctofelisi* (Dubey et al., 2003), *B. wallacei* (Tadros and Laarman, 1976) Frenkel, 1977, and *B. neotomofelis* Dubey and Yabsley, 2010 (Dubey et al., 2013). They utilise large mammalian herbivores such as cattle, goats, equids, reindeer, caribou, opossums, rabbits, rodents (Glover et al., 1990; Oryan et al., 2014), and reptilian species such as lizards (Leighton and Gajadhar, 2001; Dubey et al., 2003, 2013) as intermediate hosts globally. The life cycle of *Besnoitia* is only complete for four species i.e. *B. darlingi*, *B. oryctofelisi*, *B. wallacei* and *B. neotomofelis* out of the ten mentioned species (Dubey et al., 2003, 2013), which according to Glover et al. (1990) cats (*Felis catus*) are presumed definitive hosts. As a result, there are still uncertainties on the identity of the definitive hosts of the remaining six species as their life cycles are still not fully described and their morphological differences are poorly defined (Dubey et al., 2003).

Although the occurrence of besnoitiosis in livestock has been recorded as outbreaks in certain tropical and subtropical regions, and sporadic in some regions including endemic areas, the disease has shown to be capable of affecting a large number of the livestock herd/flock, and subsequently causing enormous economic loss (Zango et al., 2016). Authors have shown that in *B. besnoiti* infected herds, usually a small portion of the infected animals show clinical signs, with majority of the animals manifesting the inapparent sub-clinical form of besnoitiosis and often go unnoticed (Bigalke and Naude, 1962; Bigalke, 1968; Olias et al., 2011; Liénard et al., 2011; Villa et al., 2019). Furthermore, some infected cattle might show only bilateral sclera-conjunctival cysts, while majority of the cattle remain asymptomatic (Bigalke, 1968; Goldman and Pipano, 1983; Liénard et al., 2011). According to Cortes et al. (2014), the occurrences of bovine besnoitiosis often coincides with the introduction of new animals on the farm, and these may be bulls to be used for reproduction in attempt to avoid consanguinity or promote heterosis. Previous studies indicated that when infection is introduced into a cattle herd, approximately 10% of the animals are expected to acquire the disease and lose their commercial value within the next three years post-infection (Bigalke, 1968; Pols, 1960). However, Fernández-García et al. (2009) recently showed that an outbreak of besnoitiosis with almost 90% intra herd seroprevalence, approximately 50% of the infected animals show clinical signs and lesions, which according to Gutiérrez-Expósito et al. (2017), are less severe in endemically infected herds. According to Cortes et al. (2014), when the intra-herd prevalence is high, and animals start to show clinical signs more frequently after the three years. Furthermore, when the herd has high prevalence of sub-clinically infected animals, the diseased animals are commonly the naïve animals introduced for reproduction purposes (Cortes et al., 2005, 2006b).

Although arthropods are suspected to be vectors of *Besnoitia* spp. responsible of spreading the parasite from one host to another (Cortes et al., 2014), the natural route of transmission is still not clear (Zango et al., 2016). Outbreaks of bovine besnoitiosis mostly occur during seasons when biting flies are active (Cortes et al., 2014). *Besnoitia besnoiti* may be naturally transmitted mechanically through biting/bloodsucking insects such as the tsetse flies (*Glossina brevipalpis*), several tabanid species (*Atylotus nigromaculatus, Tabanocella denticornis, Haematopota albihirta*), and stable flies (*Stomoxys calcitrans*) (Bigalke, 1968; Alvarez-García et al., 2014; Liénard et al., 2013). Non-hematophagous arthropods such as *Musca domestica* and *M. autumnalis* have also been suspected to transmit *B. besnoiti* mechanically (Liénard et al., 2013) and iatrogenically within herd during herd health procedures (Pols, 1960; Bigalke, 1968).

Bovine besnoitiosis has been largely reported in sub-Saharan Africa (Bigalke and Prozesky, 2004; Chatikobo et al., 2013), Asia (Olias et al., 2011) and Europe (Cortes et al., 2005, 2006b; Alzieu et al., 2007; Mehlhorn et al., 2009; Gollnick et al., 2010; Jacquiet et al., 2010; Liénard et al., 2011; Gentile et al., 2012; Álvarez-García et al., 2013; Ryan et al., 2016; Garrido-Castañé et al., 2019; Villa et al., 2019; Delooz et al., 2021; Napoli et al., 2021; Rhodes et al., 2022). In the sub-Saharan African region, the disease has been reported in South Africa, Swaziland, Zimbabwe, Zambia, Botswana, Namibia, Angola, Tanzania, Kenya, Uganda, Sudan, Nigeria, and Cameroon (Zango et al., 2016). There are currently no chemotherapeutic agents available to treat or prevent besnoitiosis (Shkap et al., 1987; Cortes et al., 2007; Olias et al., 2011). However, live vaccines have been developed against besnoitiosis in South Africa and bovines in Israel, based on wildebeest strain (Bigalke et al., 1967, 1974b; Olias et al., 2011). Although a single dose of this vaccine has shown to protect animals against clinical disease for up to four years in South Africa, it however did not prevent against sub-clinical infections (Bigalke, 1981). Furthermore, the efficacy of the vaccine has not been scientifically proven (Olias et al., 2011). The control of besnoitiosis should be based on (i) serological examination of animals upon introducing into a *Besnoitia*-free herd, (ii) protection of animals against biting flies by using repellents, insecticides or indoor traps may help reduce the indoor winter activities of stable flies, and (iii) rapid and systematically evaluating the infection status of animals and gradually removing/culling infected animals from the herd to avoid the further spread of infection (Jacquiet et al., 2010; Liénard et al., 2011; Baldacchino et al., 2014; Gutiérrez-Expósito et al., 2017). Therefore, in this article we review available data on the species of *Besnoitia* and their hosts documented in sub-Saharan Africa, epidemiology and clinical manifestations of the disease from natural infections in livestock and wildlife.

2. Materials and methods

2.1. Search strategy

A systematic search of literature was conducted on Google scholar (https://scholar.google.com), PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Science direct (https://www.sciencedirect.com/) and JSTOR (https://www.jstor.org/) databases using the Boolean operators (OR, AND) and the following terms: *Besnoitia* OR besnoitiosis, besnoitiosis OR *Besnoitia* AND epidemiology, besnoitiosis AND clinical signs AND sub-Saharan African countries (Angola OR Benin OR Botswana OR Burkina Faso OR Cameroon OR Cape Verde OR Central African Republic OR Chad OR Comoros OR Congo OR Côte d'lvoire OR Djibouti OR Equatorial Guinea OR Eritrea OR Ethiopia OR Gabon OR The Gambia OR Ghana OR Guinea OR Guinea-Bissau OR Kenya OR Lesotho OR Liberia OR Madagascar OR Malawi OR Mali OR Maurinatia OR Mauritius OR Mozambique OR Namibia OR Niger OR Nigeria OR Réunion OR Rwanda OR Sao Tome and Principe OR Senegal OR Seychelles OR Sierra Leone OR Somalia OR South Africa OR Sudan OR Swaziland OR Tanzania OR Togo OR Uganda OR Western Sahara OR Zambia OR Zimbabwe). Literature search was limited to articles conducted between 1960 and 2021 and written in English. Relevant articles were first identified by screening through their titles and abstracts. Furthermore, the bibliography and reference lists of the selected articles were screened as potential leads for additional relevant studies to review. Full-text articles were retrieved and managed in Endnote reference manager version x9 (Clarivate Analytics, Philadelphia, PA, USA).

2.2. Selection criteria

The systematic search included epidemiological studies and case reports conducted in sub-Saharan Africa. Studies were included in the review if they were published in peer-reviewed journals clearly reporting on the epidemiology of *Besnoitia* spp. infection or besnoitiosis, clinical signs of besnoitiosis from natural infections and the identity of *Besnoitia* up to genus and/or species level.

Studies were excluded from the scoping review if they reported experimental infections, were conducted outside the sub-Saharan African countries, they reported on the general clinical signs of Sarcocystidae species, with no specific mention of *Besnoitia* species,

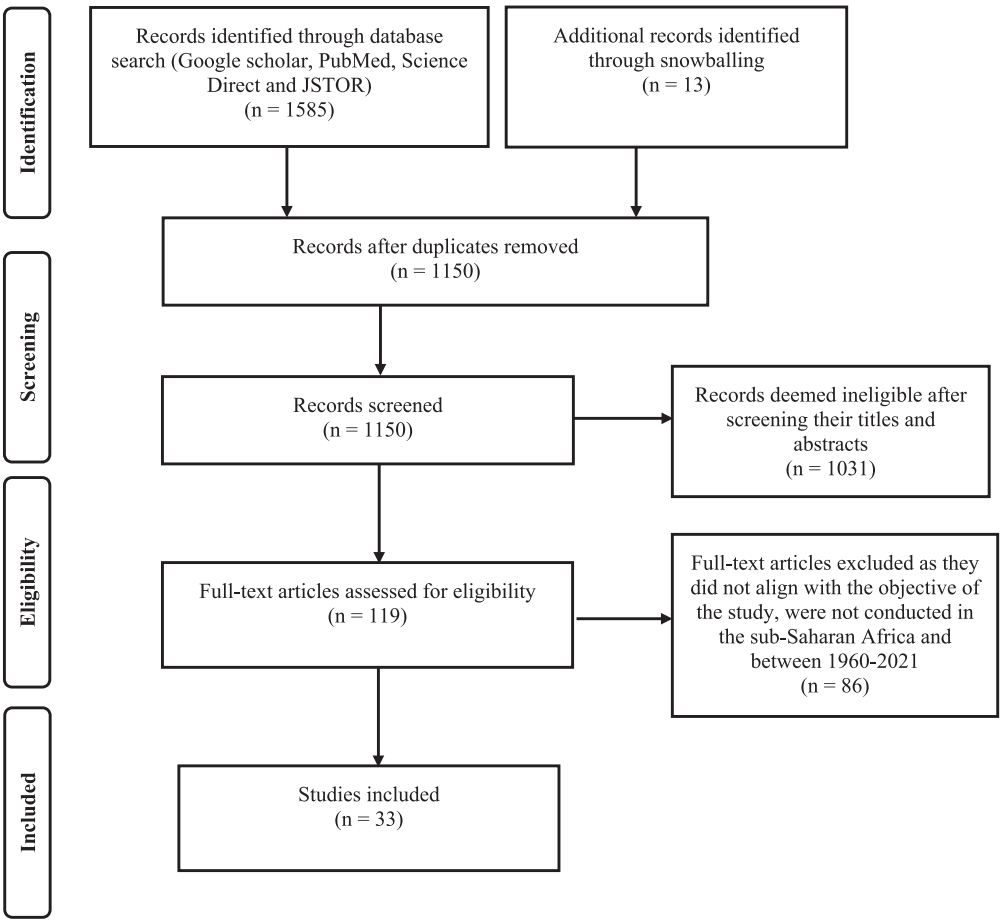

Fig. 1. PRISMA flow diagram showing selection process.

they were published reviews and books, and relevant research articles conducted and published outside the date and language limitations.

2.3. Data extraction

Data was extracted from articles that met the inclusion criteria after appraisal and recorded in a spreadsheet. Information of the authors, aim or objectives of the study, country where the study was conducted, type of study, the host species, number of animals screened, number and percentage of animals infected, the diagnostic tests used, the clinical signs and the outcomes of the study were extracted and recorded on a spreadsheet. In the case where one study reported infection in more than one host species, or using more than one diagnostic test, the prevalence of infection data was recorded separately.

3. Results

Literature search on four electronic databases yielded 1585 articles consisting of books, reviews, dissertation/thesis, case reports, field and experimental studies, abstracts, and several duplicating studies (Fig. 1), and additional thirteen articles were obtained through snowballing. Four hundred and thirty-five ($n = 435$) articles were excluded because they were duplicates, and 1031 studies were deemed ineligible after screening their titles and abstracts. Full texts of 119 studies were screened, and 86 studies were excluded as they did not meet the eligibility criteria and/or contribute to answering the review questions. A total of 33 articles met the criteria and were discussed in this review (Fig. 1).

Of the 33 reviewed, Kenya ($n = 9$) had the highest number of studies followed by South Africa ($n = 8$) and Nigeria (n = 8), Namibia ($n = 2$), Mozambique (n = 2), Sudan (n = 2), Uganda (n = 2), Zimbabwe ($n = 1$) and Rwanda (n = 1) and one study was conducted using animals from two countries (Kenya and South Africa) (Supplementary Table 1). The most reported hosts for *Besnoitia* species was cattle (Bovine) ($n = 19$), followed by goats (Caprine) (n = 8), blue wildebeest *(Connochaetes taurinus)* ($n = 4$), sheep (Ovine) (n = 2), horses (Equine) (n = 2), warthog (*Phacochoerus africanus*) (n = 1), impala (*Aepyceros melampus*) (n = 1), kudu (*Tragelaphus strepsiceros*) (n = 1), rabbits (*Oryctolagus* spp) (n = 1), African lion (*Panthera leo*) (n = 1) and cheetah (*Acinonyx jubatus*) (n = 1).

3.1. Besnoitia species and their hosts in sub-Saharan Africa

Four species of *Besnoitia* (*B. besnoiti*, *B. bennetti*, *B. caprae* and *B. darlingi*-like) and an unidentified *Besnoitia* spp. were documented in natural infections in sub-Saharan Africa (Table 1, Supplementary Table 1). Of these species, *B. besnoiti, B. bennetti* and *B. caprae* are known to infect ungulates, and *B. darlingi*-like infects small mammals. The unidentified *Besnoitia* spp. species showed host species that cut across both angulates and small mammals. Studies used histopathology/histology, examination of clinical signs, gross pathological examination/necropsy, serological tests (ELISA, IFAT, immunoblot), and PCR based on the ITS-1 primers to confirm *Besnoitia* parasite exposure and infection. Results further showed that majority of the studies utilised more than one diagnostic technique to confirm infection.

Besnoitia besnoiti was the most common species documented in nine sub-Saharan African countries (Kenya, Mozambique, Namibia, Nigeria, South Africa, Sudan, Uganda, Zimbabwe, Rwanda) (Table 1). Although natural infections of this species were predominantly found in cattle ($n = 19$), infections were also documented in blue wildebeest in South Africa (McCully et al., 1966; Ellis et al., 2000), Namibia (Seltmann et al., 2020) and Kenya (Ellis et al., 2000), African lion in Namibia (Seltmann et al., 2020), and in impala (McCully et al., 1966) and Kudu (McCully et al., 1966) from South Africa. *Besnoitia caprae* natural infections in goats (Bwangamoi et al., 1989; Njenga et al., 1999a; Njenga et al., 1999b; Ellis et al., 2000; Sambo et al., 2007) and sheep (Bwangamoi et al., 1989) were documented in Kenya. Natural infection of horses with *B. bennetti* were documented in Kenya (Bwangamoi, 1972) and South Africa (Van Heerden et al., 1993). *Besnoitia darlingi*-like species was found naturally infecting cheetah (Schares et al., 2021), and only in Namibia. *Besnoitia*

Table 1
Checklist of *Besnoitia* species and their hosts reported in sub-Saharan Africa (1960-2021).

Besnoitia species	Country where reported	Type of infections	Intermediate host	References
B. besnoiti	Kenya, Mozambique, Namibia, Nigeria, Rwanda, South Africa, Sudan, Uganda, Zimbabwe	Natural	Cattle (Bovine), blue wildebeest (*Connochaetes taurinus*), African lion (*Panthera leo*), Impala (*Aepyceros melampus*), Kudu (*Tragelaphus strepsiceros*)	Bigalke and Naude, 1962; McCully et al., 1966; Bwangamoi, 1968; Hussein and Haroun, 1975; Kumi-Diaka et al., 1981; Janitschke et al., 1984 Sekoni et al., 1992; Njagi et al., 1998; Njenga et al., 1999b; Dubey et al., 2013; Chatikobo et al., 2013; Sambo et al., 2014; Zango et al., 2016; Alsacia et al., 2017; Habarugira et al., 2019; Seltmann et al., 2020
B. caprae	Kenya	Natural	Goats (Caprine), sheep (Ovine)	Bwangamoi et al., 1989; Njenga et al., 1999a
B. bennetti	Kenya, South Africa	Natural	Horse (Equine)	Bwangamoi, 1972; Van Heerden et al., 1993
Besnoitia sp.	Kenya, Nigeria, South Africa	Natural	Cattle, goats, rabbit (*Oryctolagus* spp), warthog (*Phacochoerus africanus*)	Keep and Basson, 1973; Heydorn et al., 1984; Mbuthia et al., 1993; Igbokwe et al., 2000
B. darlingi-like	Namibia	Natural	Cheetah (*Acinonyx jubatus*)	Schares et al., 2021

infections by unidentified species (*Besnoitia* spp.) were documented in cattle in Nigeria (Igbokwe et al., 2009), goats (Bwangamoi, 1969; Heydorn et al., 1984) and rabbit (Mbuthia et al., 1993) in Kenya, and in wildebeest (Basson et al., 1965) and warthog (Keep and Basson, 1973) in South Africa (Table 1).

3.2. Prevalence of Besnoitia infection in livestock in sub-Saharan Africa

Prevalence of *B. besnoiti* infection ranged from 2% based on histopathology (Zango et al., 2016) to 80.3% based on immunofluorescence antibody test (IFAT) (Sambo et al., 2014) in Nigeria (Table 2). The highest prevalence of *B. besnoiti* infections were documented in cattle from Nigeria (80.3%) (Sambo et al., 2014), followed by 39.4% in Mozambique using IFAT (Alsacia et al., 2017). The prevalence of *B. besnoiti* in wildlife ranged from 3.4 to 20% in African lion and blue wildebeest in Namibia using ELISA and confirmed by immunoblot (Seltmann et al., 2020). The overall prevalence of *B. caprae* in goats ranged from 0.7 to 46.53% in Nigeria (Sambo et al., 2007) and Kenya (Bwangamoi et al., 1989). Prevalence of *B. caprae* infections in sheep in Kenya was 5.45%. In Nigeria, the prevalence was higher in males (84.0%) and in adults (81.7%,) (Sambo et al., 2014; Zango et al., 2016).

3.3. Clinical manifestations of besnoitiosis in livestock from sub-Saharan Africa

Results showed that clinical manifestation of besnoitiosis in sub-Saharan Africa were recorded for *B. besnoiti* in cattle, *B. caprae* in goats and *B. bennetti* in horses. Acute phase of *B. besnoiti* was documented in three studies, where Afrikander cattle presented with fever and swelling of the prepuce (Bigalke and Schoeman, 1967), Friesian bulls had anorexia and fever (106 °C) (Sekoni et al., 1992), and shortness of breath (Chatikobo et al., 2013). Fever was one of the early signs of *B. caprae* infection documented in Kenya where goats were 40–40.5 °C (Njenga et al., 1993), and South African horses presented with bilateral purulent nasal discharge with severe stridor nasalis, laryngealitis and inspiratory dyspnoea (Van Heerden et al., 1993) (Table 3). When the disease progressed to the chronic phase, the animals showed generalized thickening and wrinkling of the skin around the neck, eyes, muzzles and the abdomen, anasarca, hyperkeratosis and alopecia (Table 3). Other observed clinical signs of besnoitiosis included the presence of sand-like cysts on the sclera conjunctiva and nodules/lumps on the skin. The Kenyan horse examined during this stage was emaciated and weak, alopecic, hyperkeratosis and lesions all over the body before death (Bwangamoi, 1972). There was an observed lesion on the vulva in females (Chatikobo et al., 2013). Bulls showed inflammation, wrinkling, and thickening of the scrotum (Kumi-Diaka et al., 1981; Sannusi, 1991; Sekoni et al., 1992; Dubey et al., 2013). There were lesions on the scrotum which deteriorated progressively and became generalized over time despite weekly spraying with Pfizona^{R} against ectoparasites and treatment with long-acting Terramycin for secondary bacterial infection (Sekoni et al., 1992). Results further highlighted that 13 of the 36 examined bulls were severely affected, and as a result were culled from the herd (Kumi-Diaka et al., 1981). Post-mortem examination of the bulls showed the presence of small defuse nodules under the skin in the tunica albuginea and vaginalis, and in the parenchyma of the testes and epididymis (Kumi-Diaka et al., 1981).

4. Discussion

Ten species of *Besnoitia* have been documented globally, and known to parasitize a wide range of animals (Dubey et al., 2003,

Table 2
Prevalence of *Besnoitia* species in livestock and wildlife from sub-Saharan Africa between 1960 and 2021.

Country	Species	Host	Total examined	Total positive	Prevalence (%)	Diagnostic method	References
Namibia	*B. besnoiti*	African lion	59	2	3.4	ELISA and immunoblot	Seltmann et al., 2020
Namibia	*B. besnoiti*	Blue wildebeest	20	4	20.0	ELISA and immunoblot	Seltmann et al., 2020
Mozambique	*B. besnoiti*	Cattle	94	37	34.9	IFAT	Alsacia et al., 2017
Nigeria	*B. besnoiti*	Cattle	100	2	2.0	Histopathology	Zango et al., 2016
Nigeria	*B. besnoiti*	Cattle	400	321	80.3	IFAT	Sambo et al., 2014
Nigeria	*B. besnoiti*	Cattle	400	126	31.5	Clinical examination	Sambo et al., 2011
Nigeria	*B. besnoiti*	Cattle	400	321	80.3	IFAT	Sambo et al., 2011
Nigeria	*Besnoitia* sp.	Cattle	1780	60	3.4	Histopathology	Igbokwe et al., 2009
Nigeria	*B. caprae*	Goats	145	1	0.07	Histopathology	Sambo et al., 2007
Kenya	*B. caprae*	Goat	38477	4909	12.76	Histology	Njenga et al., 1999a
Kenya	*B. besnoiti*	Cattle	2340	260	11.0	Histology	Njenga et al., 1999a
Kenya	*B. caprae*	Goat	533	248	46.53	Clinical examination	Bwangamoi et al., 1989
Kenya	*B. caprae*	Sheep	55	3	5.45	Clinical examination	Bwangamoi et al., 1989
South Africa	*B. besnoiti*	Cattle	303	165	54.5	ELISA	Janitschke et al., 1984
South Africa	*B. besnoiti*	Cattle	303	187	61.7	IFAT	Janitschke et al., 1984
Uganda	*B. besnoiti*	Cattle	15	6	40	Histopathology	Bwangamoi, 1968
South Africa	*B. besnoiti*	Cattle	333	36	10.8	Clinical examination	Bigalke and Schoeman, 1967
South Africa	*B. besnoiti*	Cattle	1777	176	10.47	Histology	Bigalke and Naude, 1962

Table 3
Checklist of clinical signs of besnoitiosis in ungulates from sub-Saharan Africa between 1960 and 2021.

Country	Species	Host	Breed	No. of animals	Diagnostic test	Sex	Clinical signs observed	Reference
Rwanda	*B. besnoiti*	Cattle	Friesian	1	Histopathology& clinical examination	Female	- Hard scaly corrugated skin affecting both sides of the neck. - Multiple pinpoint grayish white foci in the sclera conjunctiva and corneal opacity of the left eye.	Habarugira et al., 2019
Zimbabwe	*B. besnoiti*	Cattle	–	5	Clinical examination	Females	- Dyspnea "magwiriri" (vernacular for snoring or labored breathing), followed by appearance of skin nodules. - Skin developed extensive thickening, puckering, and wrinkling, giving an "elephant skin" appearance with time. - Cyst-like structures in the sclera, and palpebral conjunctiva of some animals with lesions in the vulva.	Chatikobo et al., 2013
South Africa	*B. besnoiti*	Cattle	*Bos taurus*	1	Necropsy, histopathology, PCR	Male	- Gross external lesions included thin body condition, unilateral epistaxis, conjunctival edema, diffuse subcutaneous edema of ventral head and neck, and areas of dry, cracked, hard skin, in some places sloughing-off to expose underlying irregular erosions or ulcers affected the ventral sternum and trunk, the limbs from the elbow and stifle joints distally, and the entire scrotum	Dubey et al., 2013
Kenya	*B. caprae*	Goats	–	10	Histopathology, necropsy & clinical examination	Female & male	- Bucks had acute orchitis, characterised by swollen testes, would not allow palpation. - Fever of 41.8 °C. - Bucks afflicted with subacute and chronic orchitis had atrophied testes that were firm on palpation, and normal body temperature. - Chronically affected does and bucks had alopecia and hyperkeratosis of the face and the carpal, metacarpal, tarsal and metatarsal joints, and cysts in the conjunctiva.	Njenga et al., 1999b
Kenya	*B. besnoiti*	Cattle	Orma Boran	17	Histopathology & clinical examination	–	- Animals had sand-like cysts on the sclero-conjunctiva - There was mucus membrane on the nasal cavity - Mucus catarrhal to oozing conjunctivitis with crusts of dried exudates that attracted flies - The skin was thick and nodular, non-elastic and thrown in fold and some sections were alopecic - Generalized lymphadenomegaly of the superficial lymph nodes - Large and benign swellings exuding pus were occasionally encountered on the skin.	Njagi et al., 1998
South Africa	*B. bennetti*	Horse	Shetland pony stallion	1	Histopathology & clinical examination	Male	- Bilateral purulent nasal discharge with severe stridor nasalis and laryngealitis - Inspiratory dyspnoea - Occurrence of generalized thickening and diffuse alopecia of the skin over the ventral abdomen, antero-medial aspect of the hind legs, sub-cervical and presternal areas and the scrotum. - Development of prominent folds of thickened skin between the front legs	Van Heerden et al., 1993

(*continued on next page*)

Table 3 (*continued*)

Country	Species	Host	Breed	No. of animals	Diagnostic test	Sex	Clinical signs observed	Reference
							and on the anteromedial aspects of the hind legs - Enlarged and oedematous prepuce - Anarsaca.	
Nigeria	*B. besnoiti*	Cattle	Freisian	2	Histopathology & clinical examination	Male	- Anorexia and fever - Thickening and wrinkling of the skin around the neck, eyes, muzzles, and abdominal regions. - Alopecia. - Inflammation, thickening and wrinkling of the scrotum. - The lesions on the skin and scrotum deteriorated progressively and became generalized in spite of treatment.	Sekoni et al., 1992
Nigeria	*B. besnoiti*	Cattle	Friesian	1	Histopathology& clinical examination	Male	- Infected bull had alopecia, severely thickened and wrinkled skin, often thrown into folds around the neck, shoulder and rump regions. - The skin was very thick, with small seed-like lumps of various sizes lying deep in the subcutaneous fascia when palpated. - The scrotum and the eyes were involved, with whitish miliary cysts in the conjunctiva.	Sannusi, 1991
Nigeria	*B. besnoiti*	Cattle	–	60	Histopathology & clinical examination	Male & females	- Gross lesions in the skin in 60 affected animals and scrotal skin of 36 bulls showed varying degrees of alopecia, thickening and encrustations. - All of the bulls which were examined at post-mortem had small diffuse nodules underneath the skin in the subcutaneous tissues, in the tunica vaginalis and albuginea and in the parenchyma of the testes and epididymis. - Nodules, consisted of aggregates of small, somewhat white, gritty granules, were also felt underlying the subcutaneous tissues of the clinically affected animals. - The devitalized gangrenous testes were brown in colour and soft in consistency.	Kumi-Diaka et al., 1981
Sudan	*B. besnoiti*	Cattle	Western Baggara	1	Histopathology& clinical examination	Male	- The ox had lesions in the form of alopecia, underlying subcutaneous tissue and muscles. - Observed nodules averaged 5 mm in diameter and each consisted of an aggregate of smaller, but somewhat uniform, raised, whitish nodules ranging between 0.5 mm and 0.7 mm in size.	Hussein and Haroun, 1975
Kenya	*B. bennetti*	Horse	–	3	Pathological examination	Male	- All geldings were emaciated and weak - Horses had alopecia in some areas, and hyperkeratosis measuring between 2 and 6 cm in diameter. - Lesions were spread all over the body but were very severe in the legs, shoulders and head. - In areas where the adjacent lesions coalesced, the foci were much larger in size and had an irregular shape. - Haemorrhagic erosions and ulcers covering the skin from the coronets	Bwangamoi, 1972

(*continued on next page*)

Table 3 (*continued*)

Country	Species	Host	Breed	No. of animals	Diagnostic test	Sex	Clinical signs observed	Reference
South Africa	*B. besnoiti*	Cattle	Afrikander	5	Clinical examination	Female & males	to both joints - Haemorrhagic bed sores were at point of both ilium bones - The prepuce was swollen by oedema fluid and resembled a tumorous growth. - Severe illness accompanied by fever and swelling of the prepuce, i.e. symptoms of the acute anasarcatous stage of besnoitiosis. - Severe sclerodermatitis and alopecia. - Presence of cysts in the scleral conjunctiva - The bull became sterile	Bigalke and Schoeman, 1967

2013). Reviewed studies from sub-Saharan Africa documented four species namely *B. besnoiti*, *B. bennetti*, *B. caprae* and *B. darlingi*-like, and unidentified *Besnoitia* spp. which have been found to naturally infect a wide range of animals. Amongst these species, *B. besnoiti* was the most widely distributed species, with natural infections reported in Kenya, Mozambique, Namibia, Nigeria, Rwanda, South Africa, Sudan, Uganda and Zimbabwe. Outside Africa, outbreaks of *B. besnoiti* have been reported in endemic and non-endemic countries such as Israel, Kazakhstan, the People's Republic of China, India, Venezuela, Spain, Portugal, Italy, France, Germany, Switzerland, Croatia, Hungary, Ireland, and Belgium (Mehlhorn et al., 2009; Mutinelli et al., 2010; Gentile et al., 2012; Lesser et al., 2012; Álvarez-García et al., 2013; Cortes et al., 2014; Hornok et al., 2014; Duvallet and Boireau, 2015; Vanhoudt et al., 2015, Ryan et al., 2016; Garrido-Castañé et al., 2019; Villa et al., 2019; Delooz et al., 2021; Napoli et al., 2021; Rhodes et al., 2022). According to Bianchini et al. (2019) and Neve et al. (2022), the current dispersion scenario and outbreaks of *B. besnoiti* in non-endemic areas may be attributed to animal trade, management practices, and climate change which has modified vector survival and activity. In endemic areas, this species has been reported to cause economic loses in cattle (Dubey et al., 2013), which have also been recently associated with high milk somatic cell count (Neve et al., 2022).

Besnoitia besnoiti has shown to be infective/pathogenic to a wide range of domestic and wildlife vertebrates such as cattle (Bigalke and Naude, 1962; Bwangamoi, 1968; Hussein and Haroun, 1975; Kumi-Diaka et al., 1981; Janitschke et al., 1984; Sekoni et al., 1992; Njagi et al., 1998; Njenga et al., 1999a; Dubey et al., 2013; Chatikobo et al., 2013; Sambo et al., 2014; Zango et al., 2016; Alsacia et al., 2017; Habarugira et al., 2019), blue wildebeest (McCully et al., 1966; Ellis et al., 2000; Seltmann et al., 2020), impala (McCully et al., 1966), kudu (McCully et al., 1966) and African lion (Seltmann et al., 2020). However, the results further showed that infections in sub-Saharan Africa were predominantly reported in cattle. These vertebrate host species correspond with those listed by Dubey et al. (2003) with an exception of the African lion, which natural infections were reported in Namibia (2/59, 3.4 %) (Seltmann et al., 2020). Previous research has also showed that experimental infections of *B. besnoiti* were successfully attempted in mice, rabbits, hamsters, gerbils, sheep and goats (Dubey et al., 2003), and in sub-Saharan Africa experimental infections of *B. besnoiti* were successful in mice, rabbit and cattle through inoculations of suspension tissues harboring numerous cysts from chronically infected cattle (Bigalke, 1962; Bigalke, 1967; Basson et al., 1970; Bigalke, 1960; Bigalke et al., 1974a; Shkap et al., 1985; Njenga et al., 1995; Njagi et al., 2004).

Results showed that *B. caprae* infections in the reviewed countries was intensively studied in Kenya and this species mainly infected goats, and to a lesser extent sheep. Natural infections of *B. caprae* were documented in goats and sheep through observation of cysts in the sclera-conjunctiva (Bwangamoi et al., 1989) and histology (Njenga et al., 1999a) in Kenya, and through microscopic examination of stained sections of the Haematoxylin and Eosin in Nigeria (Sambo et al., 2007). Infection of *B. caprae* in goats have also been documented in goats with clinical manifestations in Iran, and confirmed using histopathological techniques (Oryan and Azizi, 2008) and PCR based on the ITS region (Namazi et al., 2011). According to Elsheikha et al. (2020), *Besnoitia* infection in donkeys went from being hardly detected to being reported in various countries including some African countries (Van Heerden et al., 1993; Bennett, 1933), Spain (Zafra Leva et al., 2013), Belgium (Liénard et al., 2018), Italy (Villa et al., 2018), Portugal (Waap et al., 2020), Mexico (Terrell and Stookey, 1973); and USA (Foley et al., 1990; Davis et al., 1997; Dubey et al., 2005; Elsheikha et al., 2005; Ness et al., 2012). In sub-Saharan Africa, natural infections have been reported in Kenya (Bwangamoi, 1972) and South Africa (Van Heerden et al., 1993). According to Elsheikha et al. (2020), the increasing number of reports in infections of *B. bennetti* in donkeys and other equids may be attributed to the improved awareness of the disease. Although infections in Africa has only been detected based on clinical signs, histological and pathological examination, exposure or infection of *B. bennetti* in equids in Europe were detected based on clinical signs, serological test (IFAT and western blot) (Gutiérrez-Expósito et al., 2017) and partial rDNA sequencing (Liénard et al., 2018), which have not been able to conclusively distinguish between species (Villa et al., 2021). Schares et al. (2021) isolated and identified *B. darlingi*-type naturally occurring in cheetah in Botswana based on conventional and real-time PCR. Although reviewed studies showed that this is the only report of *B. darlingi*-type in sub-Saharan Africa, natural infections of *B. darlingi*-like have been documented in opossum (*Didelphis marsupialis*) in Panama and Brazil, and *D. virginiana* in North America) and lizard species (*Basiliscus basiliscus*, *Ameiva ameiva*, *A. festiva*, *A. leptophrys*) from Panama, (*Basiliscus vittatus*) from Belize, (*Ameiva ameiva*) from Brazil, and in

rodents (*Akodon montensis*) from Brazil (Dubey et al., 2003; Elsheikha et al., 2003; Elsheikha et al., 2004; Kiehl et al., 2010). Other studies have shown that domestic cats serve as definitive hosts (Olias et al., 2011). Smith and Frenkel (1977) reported cats as experimental definitive hosts, whilst Verma et al. (2017) reported that bobcats (*Lynx rufus*) were identified as natural definitive hosts of *B. darlingi*. Natural infections of *Besnoitia* spp. have also been documented in cattle, goats, rabbit and warthog in Kenya, Nigeria, South Africa (Keep and Basson, 1973; Heydorn et al., 1984; Mbuthia et al., 1993; Igbokwe et al., 2009).

Reviewed results showed that early onset or acute signs of besnoitiosis were associated with fever and anorexia in naturally infected cattle and goats (Njenga et al., 1993; Sekoni et al., 1992). Acutely infected animals also presented with vasculitis and thrombosis in small to medium diameter vessels, and several authors suggested that the replication of tachyzoites in the epithelial cells occurring during this phase may be the cause of the vascular damages (McCully et al., 1966; Dubey et al., 2013; González-Barrio et al., 2020). This stage is short and rarely diagnosed, and the disease progress to the chronic phase in less than one month, but severely affected animals may die due to respiratory dysfunction and nephrotic syndrome (Dubey et al., 2013; Alvarez-García et al., 2014; González-Barrio et al., 2020). The presence of cysts in the sclera-conjunctiva was one of the most common signs, and these are typically used to diagnose besnoitiosis. Results also show that chronically infected animals had thickened and wrinkled skin around the legs, neck, muzzles, and abdomen, which often resulted in alopecia (Van Heerden et al., 1993; Njagi et al., 1998; Chatikobo et al., 2013). Few female cattle were reported to have lesions around the vulva (Chatikobo et al., 2013).

Although majority of the bulls were in the chronic stage of the disease, some bulls and bucks were found anorexic and feverish, and with swollen testes and would not allow palpitation, which is a characteristic of the acute orchitis (Sekoni et al., 1992; Njenga et al., 1999b). Dubey et al. (2013) and González-Barrio et al. (2020) also stated that bulls may develop orchitis, and alveolar and interstitial oedemas in the lungs, causing respiratory disorders. Furthermore, development of sterility may also occur during this phase as a result of injury of the vascular in the pampiniform plexus and scrotal skin lesions that hamper testicular thermoregulation (González-Barrio et al., 2020, 2021). Results further showed that other males presented typical chronic besnoitiosis such as wrinkling, thickening and inflammation of the scrotum and in some cases the scrotum became encrusted resulting in sterility of the animals, and in other cases, the animals were bilaterally castrated or often culled (Bigalke and Schoeman, 1967; Kumi-Diaka et al., 1981; Sekoni et al., 1992). Additionally, numerous cysts were present in the scrotal skin, the epididymis and ampullae, and in the wall of the blood vessels of the pampiniform plexus which many contribute to thermoregulation failures by the intense fibrosis and thickening of scrotal skin that could interfere with normal spermatogenesis (González-Barrio et al., 2020, 2021). According to authors, these transient or definitive sterility or infertility in bulls are one of the relevant economic consequences of *B. besnoiti* infections (Kumi-Diaka et al., 1981; Jacquiet et al., 2010; Dubey et al., 2013; Gazzonis et al., 2017; González-Barrio et al., 2021).

Results showed that reviewed studies diagnosed *Besnoitia* infections using mainly histopathology/histology, examination of clinical signs, and to a lesser extent gross pathological examination/necropsy, serological tests (ELISA, IFAT, immunoblot), and PCR based on the ITS-1 primers. While these tests provide evidence of parasite exposure in terms of serological techniques and/or parasite detection, however, they cannot distinguish between *Besnoitia* species (Dubey et al., 2003; Elsheikha et al., 2020). Furthermore, the existence of cross-reactions between *Besnoitia* species and other related tissue cyst-forming coccidian parasites, especially *Neospora caninum* have been reported (Dubey et al., 2013; Gutiérrez-Expósito et al., 2017), and hence several authors have recommended the use of at IFAT or western blot as a confirmation test (Cortes et al., 2006c; Jacquiet et al., 2010; Schares et al., 2011; García-Lunar et al., 2013). Although the ITS-1 region has been considered the most effectively used molecular marker to discriminate between closely related Sarcocystidae species (Tenter et al., 2002; Olias et al., 2011), discriminatory power between *Besnoitia* species in small mammalian hosts is significantly lower and totally absent for large mammalian *Besnoitia* species (Olias et al., 2011). Therefore, microsatellite genotyping has been developed and is useful in identifying and distinguishing between closely related *Besnoitia* species, especially *B. besnoiti* and *B. tarandi* in cattle and reindeer, respectively (Madubata et al., 2012; Arnal et al., 2017; Schares et al., 2019).

Prevalence of *B. caprae* ranged from 0.07% to 46.53% in Kenyan goats, based on histological technique and clinical signs (detection of cysts in the sclera-conjunctiva), respectively (Bwangamoi et al., 1989). Although histological examinations are commonly used to detect infection, the EFSA (2010) stated that this diagnostic method alone is not recommended for routine diagnosis since chronically infected but inapparent animals may remain undetected due to the presence of low number of tissue cysts. Furthermore, relying only on clinical signs for detection can also lead to misdiagnosis, as bovine besnoitiosis can be confused with other infectious diseases with similar clinical signs (Neve et al., 2022). Our results showed that prevalence of *Besnoitia* infections were higher based on antibody tests compared to clinical signs. Relative to the sample size, the lowest prevalence of *B. besnoiti* was recorded in cattle in Nigerian (2/100, 2.0%) and African lion in Namibian (2/59, 3.4%) based on histopathology and serology (ELISA and immunoblot) (Seltmann et al., 2020; Zango et al., 2016), whilst the highest prevalence rate was documented in cattle in Nigeria (321/400, 80.3%) (Sambo et al., 2011, 2014) and South Africa (187/303, 61.7%) based on IFAT (Janitschke et al., 1984). Several authors have reported that bovine besnoitiosis infect cattle of all sex, age and breed (Cortes et al., 2014). Although the reviewed studies did not compare infection between age, previous studies have shown that both adult and young animals get infected with bovine besnoitiosis. Studies have shown high infection rate in older animals as compared to young animals (Ashmawy, 2014; Fernández-García et al., 2010), and the risk of bovine besnoitiosis infection, prevalence and clinical cases increase with age (Fernández-García et al., 2010; Gazzonis et al., 2017). However, acute and chronic besnoitiosis rarely occur in calves under 6 months, as these are unusual (Bigalke, 1968; Alzieu et al., 2007; Cortes et al., 2014; Diezma-Díaz et al., 2017).

Reviewed results showed that males were more susceptible to infections than females (Sambo et al., 2014; Zango et al., 2016). High susceptibility of males to besnoitiosis as compared to females have been reported by several authors (Jacquiet et al., 2010; Gazzonis et al., 2017), and Gazzonis et al. (2017) showed that infection incidence rate increased with time. However, other studies reported contrasting results where higher seroprevalence of *B. besnoiti* was observed in females than males in Egypt (Ashmawy, 2014) and Spain (Fernández-García et al., 2010), and the authors suggested that the low prevalence reported in males may have been due to sex bias

during survey. The high seropositivity of besnoitiosis in bulls in the herd pose a high risk for the rest of the herd as the bull in service may spread the infection through natural mating and mucosal contact (Gazzonis et al., 2017). The males showed to present severe clinical signs as compared to cows, and those severely affected often develop irreversible intratesticular lesions of vasculitis, focal necrosis, sclerosis and atrophy, which often result in transient infertility or sterility (Jacquiet et al., 2010; Álvarez-García et al., 2013; Cortes et al., 2014). Although live attenuated vaccines have been developed in South Africa and Israel, these vaccines do not completely prevent clinical infections (Bigalke and Prozesky, 1994). Furthermore, these vaccines poses a risk of introducing besnoitiosis into uninfected herds and possibly inducing carriers amongst vaccinated animals (Cortes et al., 2014).

5. Conclusion

Results from this review has shown that natural infections with *B. besnoiti*, *B. bennetti*, *B. caprae*, *B. darlingi*-like, and unidentified *Besnoitia* spp. have been reported from nine sub-Saharan African countries in the past six decades. *Besnoitia darlingi*-like was only documented in Namibia, and there are still uncertainties as to whether *B. darlingi*-like occur in other African countries. *Besnoitia besnoiti* was the most common species documented in all nine countries. High prevalences of besnoitiosis were recorded using serological methods compared to clinical signs. Although there are several scientific literature on besnoitiosis, there are still gaps in literature elucidating the life cycle of several species including the range of intermediate hosts and the identity of the definitive host. The review also showed paucity of epidemiological studies of the different species of *Besnoitia* and the risk factors associated with transmission in different livestock husbandry systems in sub-Saharan Africa. Furthermore, most studies used histopathology, serological tests and to lesser extent PCR to confirm infection, however, these diagnostic tools cannot differentiate between species. Hence, future research needs to focus on detecting and identifying all species of *Besnoitia*, and scoping their natural intermediate and definitive hosts including arthropod vectors, as well as assessing the socio-economic burden of the disease. For routine diagnosis, we recommend the use of standardized and reliable diagnostic procedures which involve the use of combined clinical inspection, histopathology, and serological tests to confirm diagnosis, and use of genotyping tools to differentiate between species. We also recommend the refinements of currently available genotyping tools and development of novel genotyping tools for *Besnoitia* spp. infections in the intermediate and definitive hosts.

Author contributions

Samson Mukaratirwa, Mokgadi Pulane Malatji: Conceptualized the study. Mokgadi Pulane Malatji, Danisile Tembe: Literature search, selection and data extraction. Mokgadi Pulane Malatji wrote the first draft of the manuscript. All authors read and approved the final version of the article.

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Declaration of Competing Interest

Authors declares no conflict of interest.

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Appendix A. Supplementary data

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