4 RESULTS

4.1 Amplification of mtDNA from potential vertebrate hosts of tsetse flies

DNA extracted from various animal species of the family Bovidae was successfully amplified using the *cytb* 1/*cytb* 2 primers described by Kocher et al. (1989). A single PCR amplicon, corresponding in size to the predicted 359 bp fragment was observed (Figure 3).

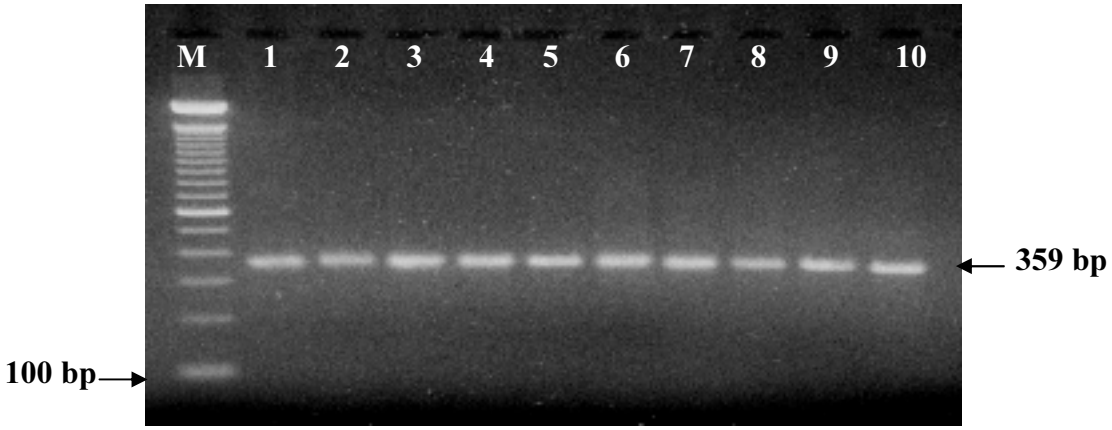

Figure 3. Amplification of a 359 bp fragment of the Cytochrome b gene from different vertebrate species of the family Bovidae. M; 100 bp molecular size marker. Lane **1**; *Bos taurus* (Cattle). Lane **2**; *Bubalus bubalis* (Water buffalo). Lane **3**; *Syncerus caffer nanus* (Red buffalo). Lane **4**; *Kobus leche* (Waterbuck). Lane **5**; *Antidorcas marsupialis* (Springbok). Lane **6**; *Capra hircus* (Goat). Lane **7**; *Ovis aries* (Sheep). Lane **8**; *Hippotragus niger* (Sable antelope). Lane **9**; *Oryx gazella* (Oryx). Lane **10**; *Madoqua kirkii* (Dik-dik).

4.2 The detection limit and stability of host DNA in the guts of tsetse

DNA extracts derived from the guts of non-fed tsetse did not produce a PCR signal when subjected to universal cytochrome b primers (*cytb* 1/*cytb* 2) (Figure 4). Figures 5, 7 and 9 show that the universal primers react with the mitochondrial *cytb* gene of sheep blood 24, 48, 72, 96 and 120 h after feeding by tsetse and consequently yielding a 359 bp fragment.

The results in Figure 5 clearly show that the PCR products were detected in all tsetse fly samples tested 24 h after feeding (100%), while four of them were detectable after 48 h (80%). Figure 7 shows that 4 out of the 5 (80%) samples were positive 72 h after feeding, one of which showed a weak band (lane 2). However, only 3 PCR products were present 96 h after feeding (60%), one of the products resulted in a very weak band (lane 8). Figure 9 reveals that 120 h after feeding; in contrast, there were finally 2 but very weak PCR signals out of 5 samples (40%).

Figures 6 and 8 show the restriction profiles of sheep DNA after digestion with restriction enzyme *Nde*II, yielding species-specific restriction fragments (244 and 115 bp). Figure 6 shows that all samples collected and tested 24 h after feeding were positive (5 out of 5, 100%), while only 4 out of 5 (80%) were positive 48 h after feeding. Figure 8 shows that 3 out of 5 samples (60%) were positive 72 h after feeding, while only 2 out of 5 (40%) samples were positive 96 h after feeding.

A comparison between the sensitivity of PCR-RFLP used in this study and ELISA (Rurangirwa et al., 1986) shows that ELISA was able to detect 100% and 87.5 % of tsetse bloodmeal samples collected after 40 and 74 h, respectively. On the other hand, PCR-RFLP was able to detect 80% and 60% of tsetse bloodmeals at 48 h and 72 h post-feeding, respectively. In addition, PCR-

RFLP detected tsetse bloodmeals up to 96 h after feeding in 40% of the tested samples (Table 5 and Figures 6 and 8).

Figures 10 and 12 show that the PCR products generated from goat DNA were detected in all tsetse fly samples tested 24, 48 and 72 h after feeding. In addition, digestion of these products with the restriction enzyme *Nde*II yielded species-specific restriction profiles (31 bp, 115 bp and 213 bp) up to 72 h post feeding (Figure 11; 13).

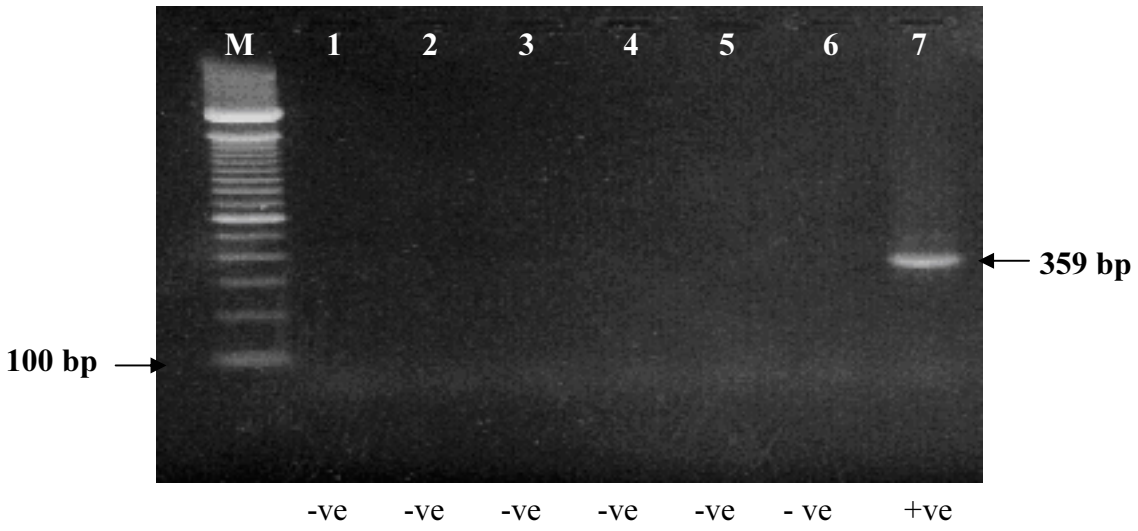

Figure 4. PCR conducted on DNA of unfed tsetse flies. M; 100 bp molecular size marker. Lanes **1-6**; unfed tsetse flies. Lane **7**; positive control DNA (sheep DNA).

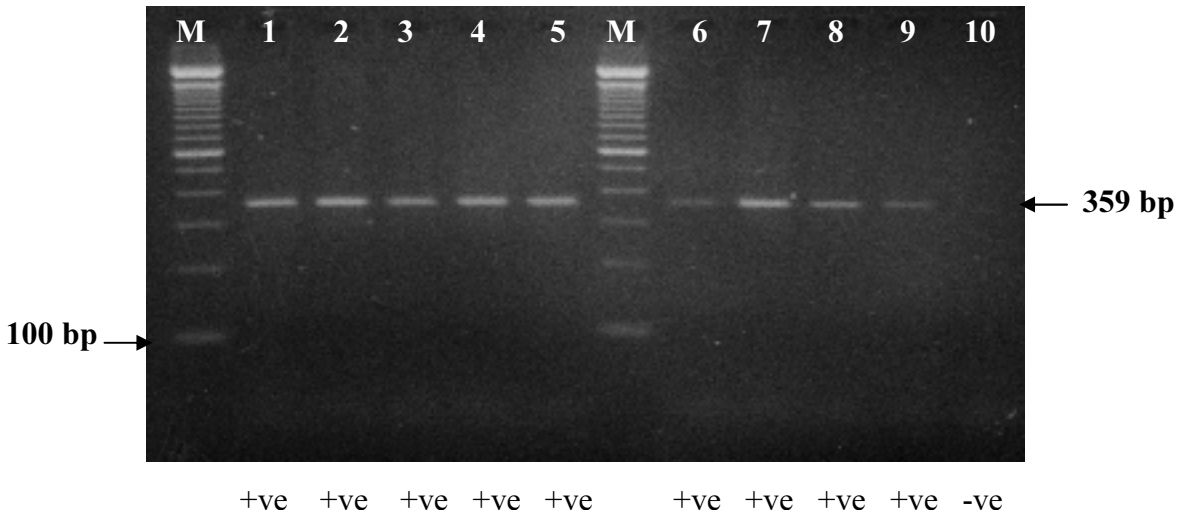

Figure 5. PCR products from tsetse flies fed on sheep blood. M; 100 bp molecular size marker. Lanes **1-5**; Bloodmeal from tsetse flies 24 h after feeding. Lanes **6-10**; Bloodmeal from tsetse flies 48 h after feeding.

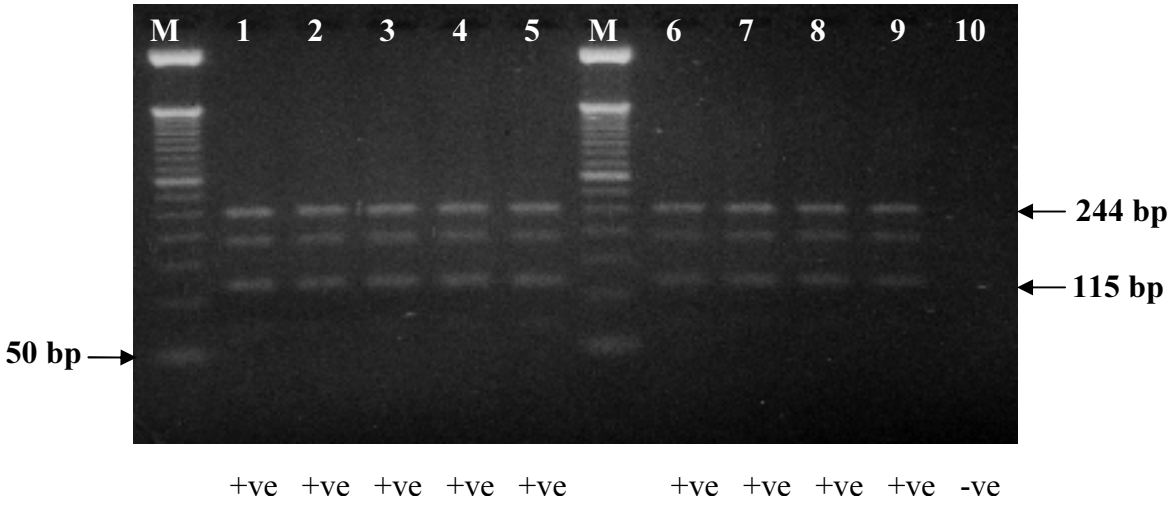

Figure 6. Restriction profiles of *cytb* PCR amplicons (359 bp) generated from sheep and digested with *Nde*II. M; 50 bp molecular size marker. Lanes **1-5**; Bloodmeal from tsetse 24 h after feeding. Lanes **6-10**; Bloodmeal from tsetse 48 h after feeding. *Nde*II restriction profiles showing the typical patterns of sheep DNA (115 bp and 244 bp).

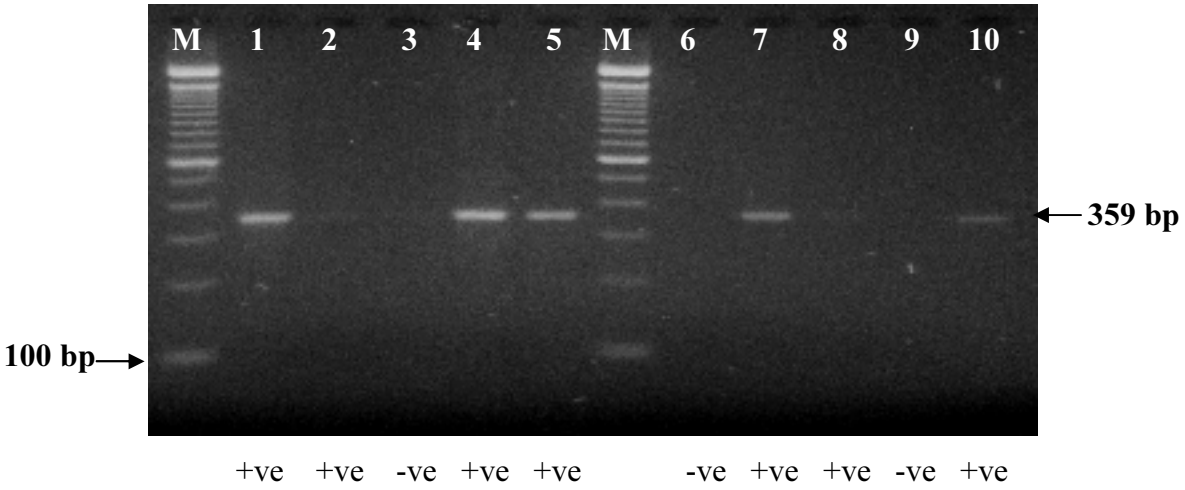

Figure 7. PCR products from tsetse flies fed on sheep blood. M; 100 bp molecular size marker. Lanes **1-5**; Bloodmeal from tsetse flies 72 h after feeding. Lanes **6-10**; Bloodmeal from tsetse flies 96 h after feeding.

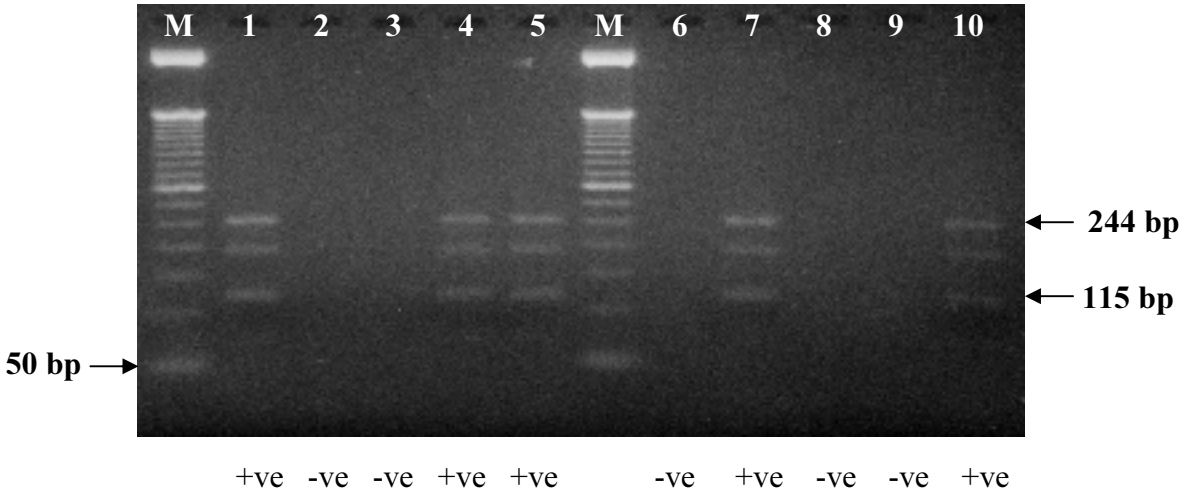

Figure 8. Restriction profiles of *cytb* PCR amplicons (359 bp) digested with *Nde*II. M; 50 bp molecular size marker. Lanes **1-5**; Bloodmeal from tsetse 72 h after feeding. Lanes **6-10**; Bloodmeal from tsetse 96 h after feeding. *Nde*II restriction profiles showing the typical patterns of sheep DNA (115 bp and 244 bp).

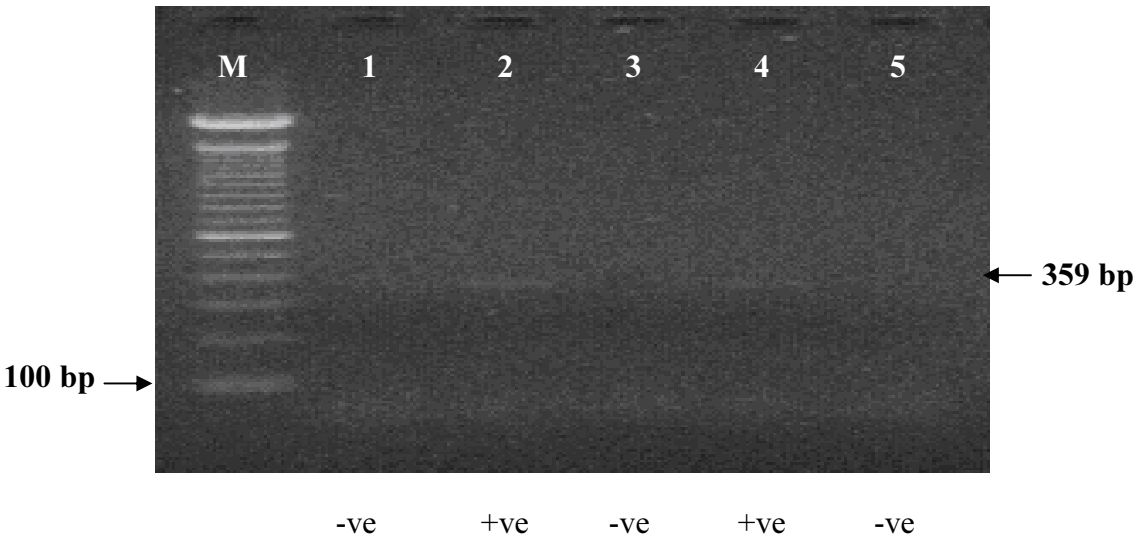

Figure 9. PCR products from tsetse flies fed on sheep blood. M; 100 bp molecular size marker. Lanes **1-5**; Bloodmeal from tsetse flies 120 h after feeding.

Table 5. Comparison of the sensitivity of ELISA and PCR-RFLP in tsetse bloodmeal identification.

ELISA			**PCR-RFLP**	
Hours post feeding	Detection (%)	References	Hours post feeding	Detection (%)
40	100	Rurangirwa et al., 1986	24	100
74	87.5	Rurangirwa et al., 1986	48	80
N/D	-	-	72	60
N/D	-	-	96	40

N/D: Note done

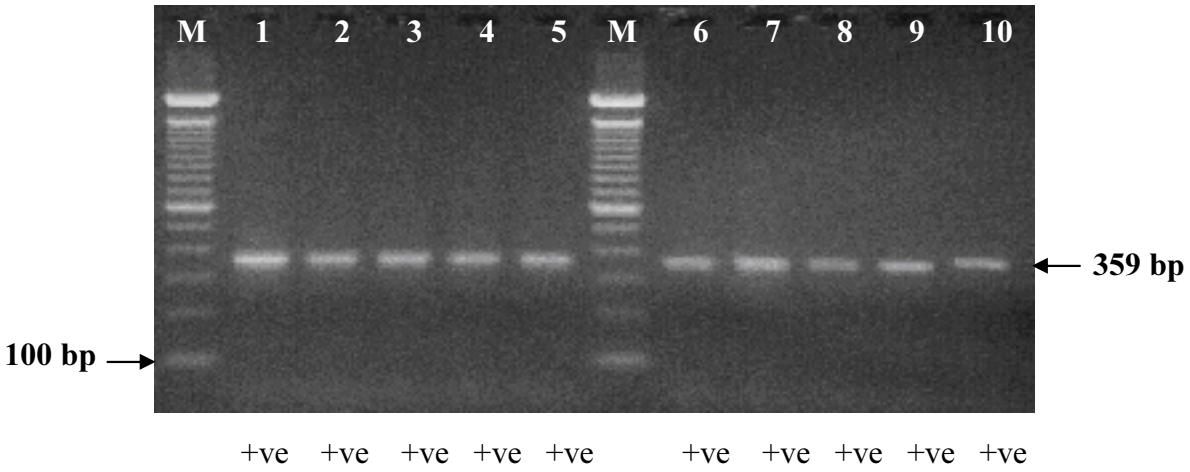

Figure 10. PCR products from tsetse flies fed on goat blood. M; 100 bp molecular size marker. Lanes **1-5**; Bloodmeal from tsetse flies 24 h after feeding. Lanes **6-10**; Bloodmeal from tsetse flies 48 h after feeding.

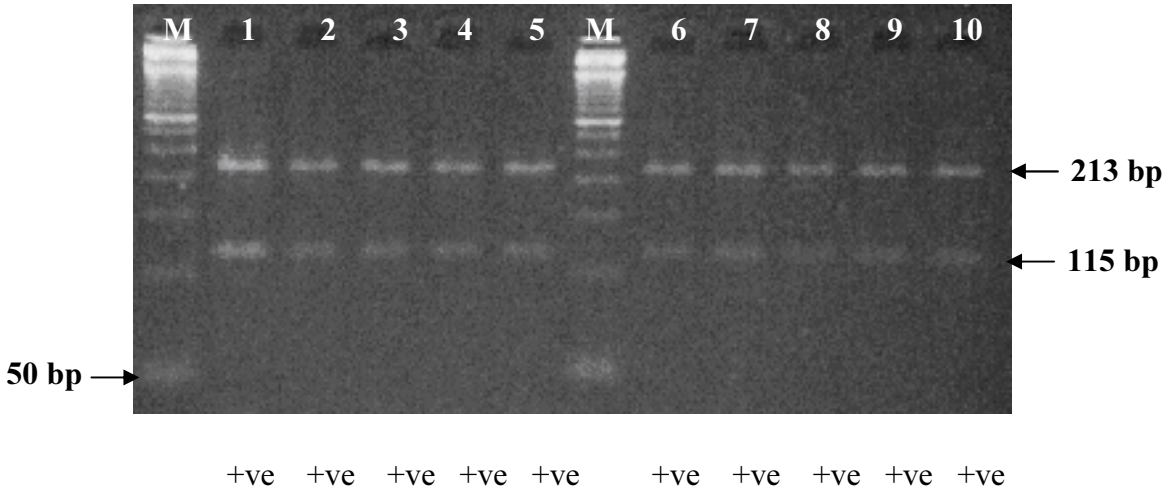

Figure 11. Restriction profiles of *cytb* PCR amplicons (359 bp) generated from goat and digested with *Nde*II. M; 50 bp molecular size marker. Lanes **1-5**; Bloodmeal from tsetse 24 h after feeding. Lanes **6-10**; Bloodmeal from tsetse 48 h after feeding. *Nde*II restriction profiles showing the typical patterns of goat DNA (31 bp, 115 bp and 213 bp). N.B.: the 31 bp restriction fragment described for goats is not visible due to its small size.

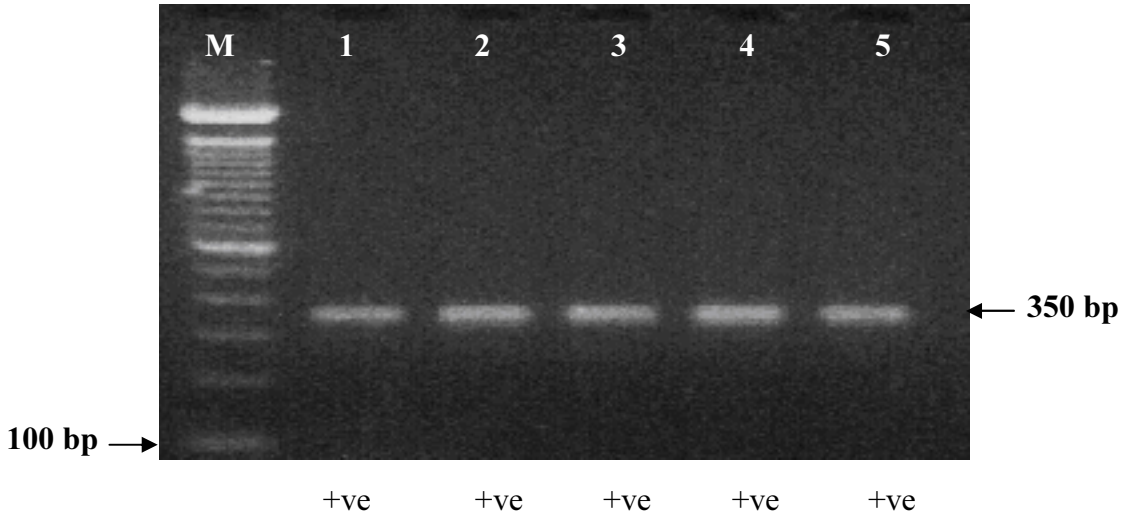

Figure 12. PCR products from tsetse flies fed on goat blood. M; 100 bp molecular size marker. Lanes **1-5**; Bloodmeal from tsetse flies 72 h after feeding.

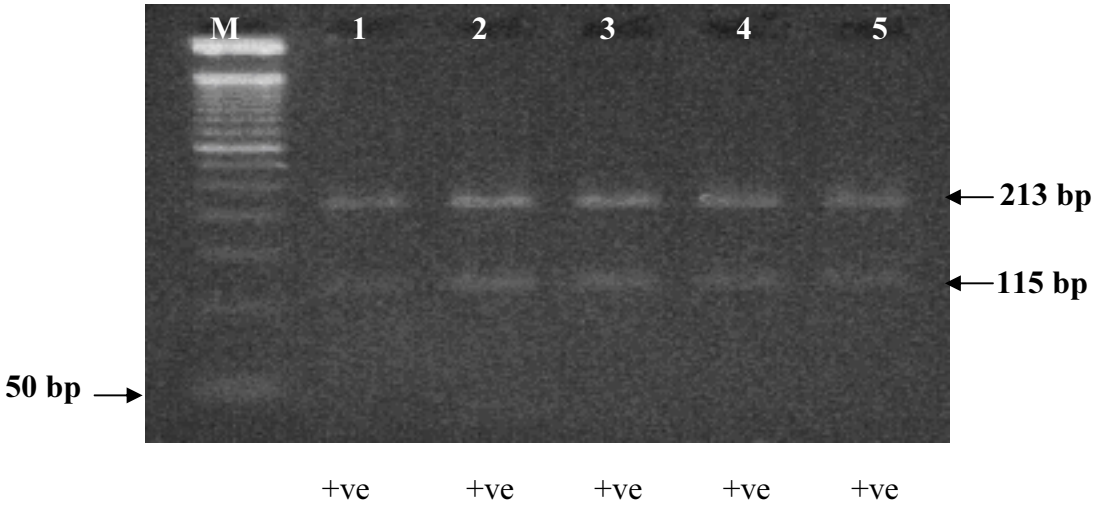

Figure 13. Restriction profiles of *cytb* PCR amplicons (359 bp) digested with *Nde*II. M; 50 bp molecular size marker. Lanes **1-5**; Bloodmeal from tsetse 72 h after feeding. *Nde*II restriction profiles showing the typical patterns of goat DNA (31 bp, 115 bp and 213 bp). N.B.: the 31 bp restriction fragment described for goats is not visible due to its small size.

4.3 Amplification of DNA from filter paper after treatment with antiseptic solution

The DNA extracted from blood smeared onto filter paper treated with acetone, diethyl ether and chloroform for 1 h, was successfully amplified and produced a 359 bp fragment (Figure 14). This treatment process eliminates the potential of spreading infections by contaminated blood samples, without deterioration of the DNA quality.

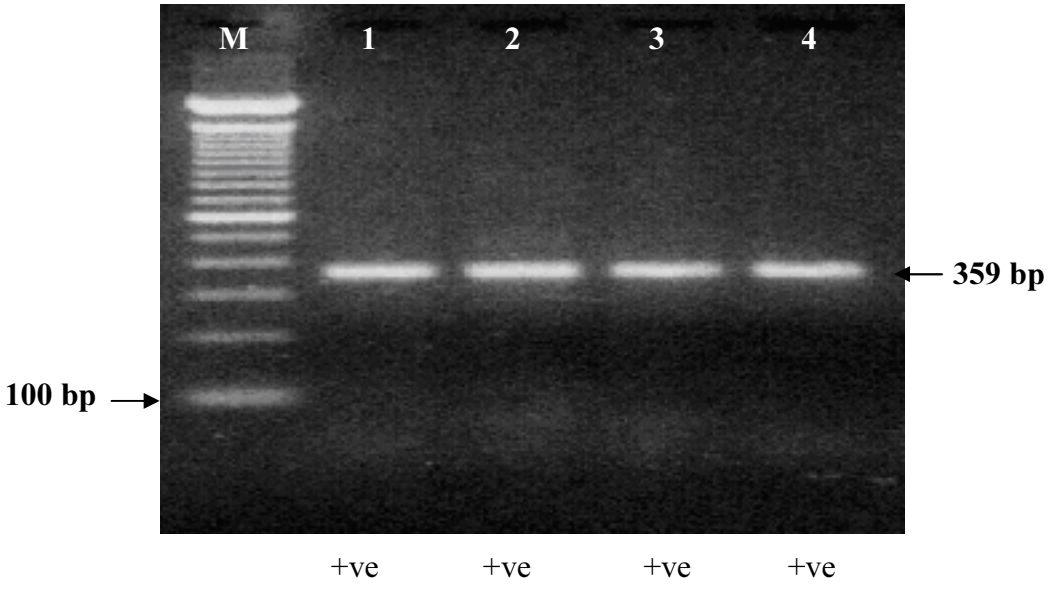

Figure 14. PCR products of DNA extracted from sheep blood smeared onto filter paper. M; 100 bp molecular size marker. Lane **1**; DNA extracted from blood smeared on to filter paper without antiseptic treatment. Lane **2**; Blood smear after treatment with acetone. Lane **3**; Blood smear after treatment with chloroform. Lane **4**; Blood smear after treatment with ether.

4.4 Amplification of mtDNA from hair and skin of vertebrate hosts

A 359 bp region within the *cytb* gene was successfully amplified from DNA extracted from skin and hair samples (Figure 15).

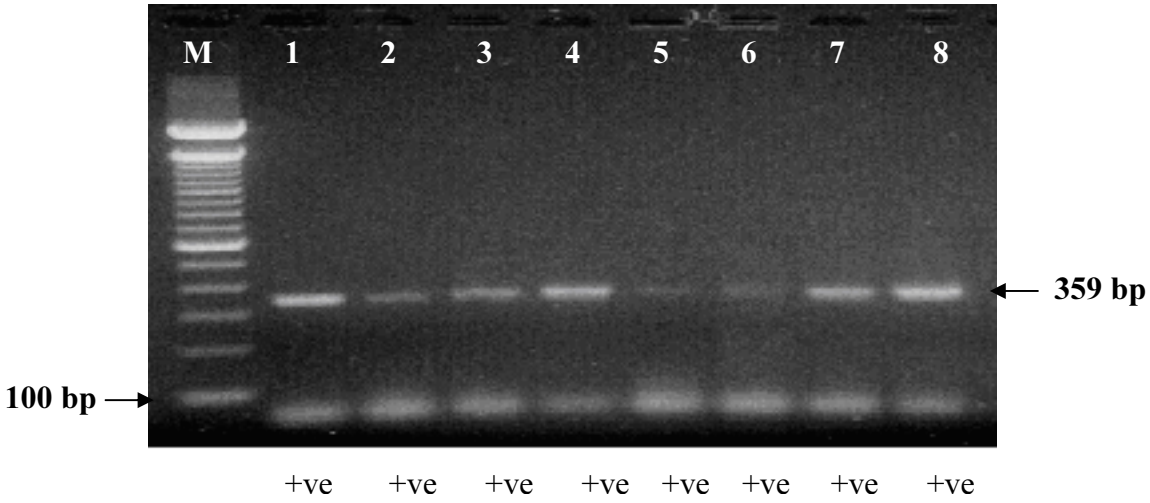

Figure 15. Amplification of a 359 bp fragment of the *cytb* gene from different specimens of vertebrate hosts. M; 100 bp Molecular size marker. Lanes **1-4**; Hair samples from giraffe, lion, rhinoceros and gorilla, respectively. Lanes **5-8**; Skin samples from crocodile, varan, snake and tortoise, respectively.

4.5 DNA sequences of different species of the family Bovidae

Figure 16 shows part of the aligned nucleotide sequence of the *cytb* gene that has been determined for 10 species of the family Bovidae. The differences in the *cytb* sequence in closely related species would help in inter-species differentiation, using restriction enzymes (*Taq*I, *Alu*I and *Hind*II).

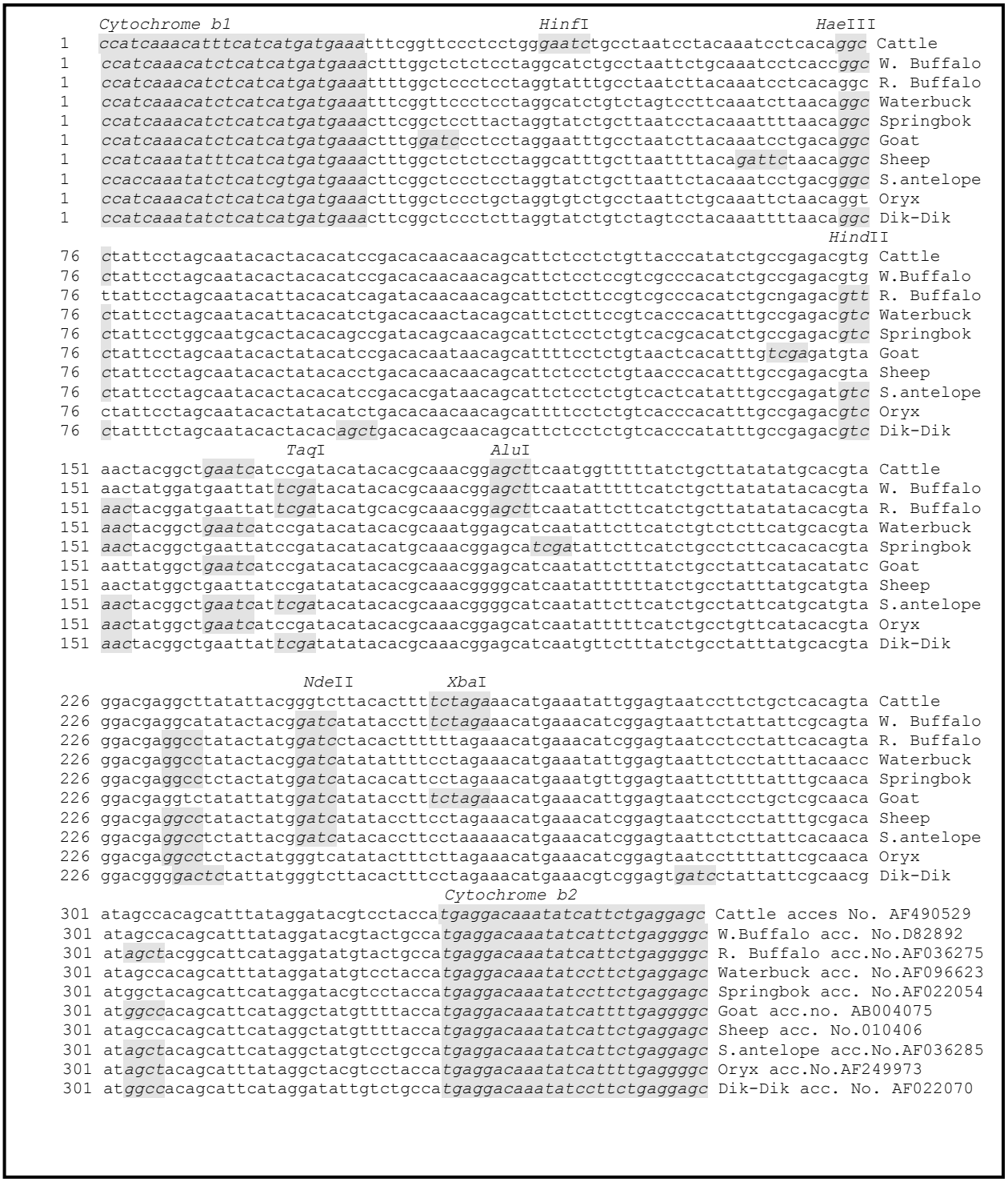

Figure 16. DNA sequences from part of the mitochondrial *cytb* gene (359 bp) of the 10 different animal species aligned together. All sequences were drawn from the NCBI databank (accession numbers). PCR primer sequences and the position of the different restriction enzymes are shown in italics.

4.6 Identification of vertebrate hosts of tsetse by PCR-RFLP analysis

Figure 17 shows a combination of three restriction enzymes (*Taq*I, *Alu*I and *Hind*II), which yielded specific restriction profiles that enabled direct identification of the 10 animal species of the family Bovidae.

*Taq*I restriction sites were found in some but not all tested bovid species (Figures 17; 18; Table 6). All resultant fragment-sizes with *Taq*I were in conformity with the expected sizes according to the gene bank records. The *Taq*I fragments of the *Capra hircus* (goat) amplicons (Figures 17; 18; lane 6) were 218 bp and 141 bp (Pattern 4), the *Bubalus bubalis* (water buffalo), *Syncerus caffer nanus* (red buffalo), *Hippotragus niger* (sable antelope) and *Madoqua kirkii* (dik-dik) amplicons were 191 bp and 168 bp (Pattern 2). The *Taq*I fragments of the *Antidorcas marsupialis* (springbok) amplicons were 193 bp and 166 bp (Pattern 3). No *Taq*I restriction sites were found in fragments 359 bp (Pattern 1) amplified from *Bos taurus* (cattle), *Ovis aries* (sheep), *Kobus leche* (waterbuck) and *Oryx gazella* (oryx).

*Alu*I restriction fragments of the cattle and water buffalo amplicons were 190 bp and 169 bp (Figures 17; 19; Table 7); oryx and sable antelope *Alu*I restriction fragments were 304 bp and 55 bp long. No *Alu*I restriction sites were found in fragments amplified from DNA of waterbuck, springbok, goat and sheep; this was in conformity with the expected sizes according to the gene bank records.

The red buffalo *Alu*I restriction fragments were 190 bp, 114 bp and 55 bp, and the non-specific fragment was 285 bp (Figures 17; 18). The dik-dik amplicons yielded *Alu*I specific fragments 304 bp and 55 bp and also non-specific fragments 259 bp, 190 bp and 100 bp. All

the non-specific resultant fragment-sizes were not in conformity with the expected fragment sizes in the gene bank records.

The *Hind*II helped in the differentiation between the waterbuck and the sheep DNA. The resultant *Hind*II fragments of the waterbuck amplicon were 209 bp and 150 bp and sheep amplicon was not digested with the same enzyme (Figures 17; 20 and Table 8). All fragment-sizes were in conformity with the expected sizes according to the gene bank records. The resultant *Hind*II fragments of the red buffalo, springbok, sable antelope, oryx and dik-dik amplicons were 209 bp and 150 bp (Figure 20; Table 8). No *Hind*II restriction sites were found in the PCR amplicons amplified from mtDNA of cattle, water buffalo and goat.

4.7 Additional restriction enzymes used for species identification

The *Xba*I digested the PCR products from cattle, water buffalo and goat, resulting in 258 bp and 101 bp (Figure 21; Table 9). All resultant fragment-sizes with *Xba*I were in conformity with the expected sizes according to the gene bank records. No *Xba*I restriction sites were found in amplicons amplified from DNA of red buffalo, waterbuck, springbok, sheep, sable antelope, oryx and dik-dik.

The *Hinf*I fragments of the cattle PCR product were 198 bp, 117 bp and 44 bp. The amplified PCR product of waterbuck gave the same *Hinf*I restriction fragments as that of goat, sable antelope, oryx and sheep (Figure 22; Table 10). However, no *Hinf*I restriction fragments were found in amplicons of water buffalo, red buffalo and springbok. According to the gene bank database, there exist differences between the restriction patterns of sheep and waterbuck DNA while, in this study, no difference was established between them.

The *cytb* amplicons of the 10 animal species were also analysed with *Hae*III (Figure 23; Table 11). *Hae*III restriction analysis of cattle and water buffalo amplicons resulted in the expected fragments of 285 bp and 74 bp. In addition, *Hae*III digestion of red buffalo and oryx amplicons gave fragments of 233 bp and 126 bp, which were in conformity with the expected sizes in the gene bank records. Analysis of the waterbuck and springbok amplicons using *Hae*III yielded 159 bp, 126 bp and 74 bp fragments and these were in conformity with the expected sizes. *Hae*III fragments of the goat amplicons resulted in 285 bp, 230 bp, 74 bp and 55 bp fragments. Sheep amplicons restriction fragments 285 bp, 159 bp, 126 bp and 74 bp were not the same as the expected sizes in the gene bank records. However, the dik-dik amplicons resulted in both specific and unexpected *Hae*III restriction fragment-sizes according to the gene bank records as follows: 285 bp, 230 bp, 159 bp, 126 bp and 74 bp.

The *Nde*II fragments of the water buffalo PCR amplicon were 244 and 115 bp, the same restriction sizes were found in red buffalo, waterbuck, springbok and sable antelope amplicons (Figure 24; Table 12). All resultant fragment-sizes were in conformity with the expected sizes in the gene bank records. While *Nde*II fragments of cattle and oryx amplicons resulted in the expected 359 bp, in cattle, in contrast, two additional fragments, 244 bp and 115 bp were detected. In addition, *Nde*II restriction fragments of goat DNA were 213 bp, 115 bp and 31 bp long. Using the same restriction enzyme, the sheep DNA yielded 244, 200, 115 and 74 bp fragments that were not in conformity with expected sizes. In the dik-dik amplicon, the same enzyme yielded a 78 bp specific restriction fragment with 115 bp and 150 bp fragments as additional bands.

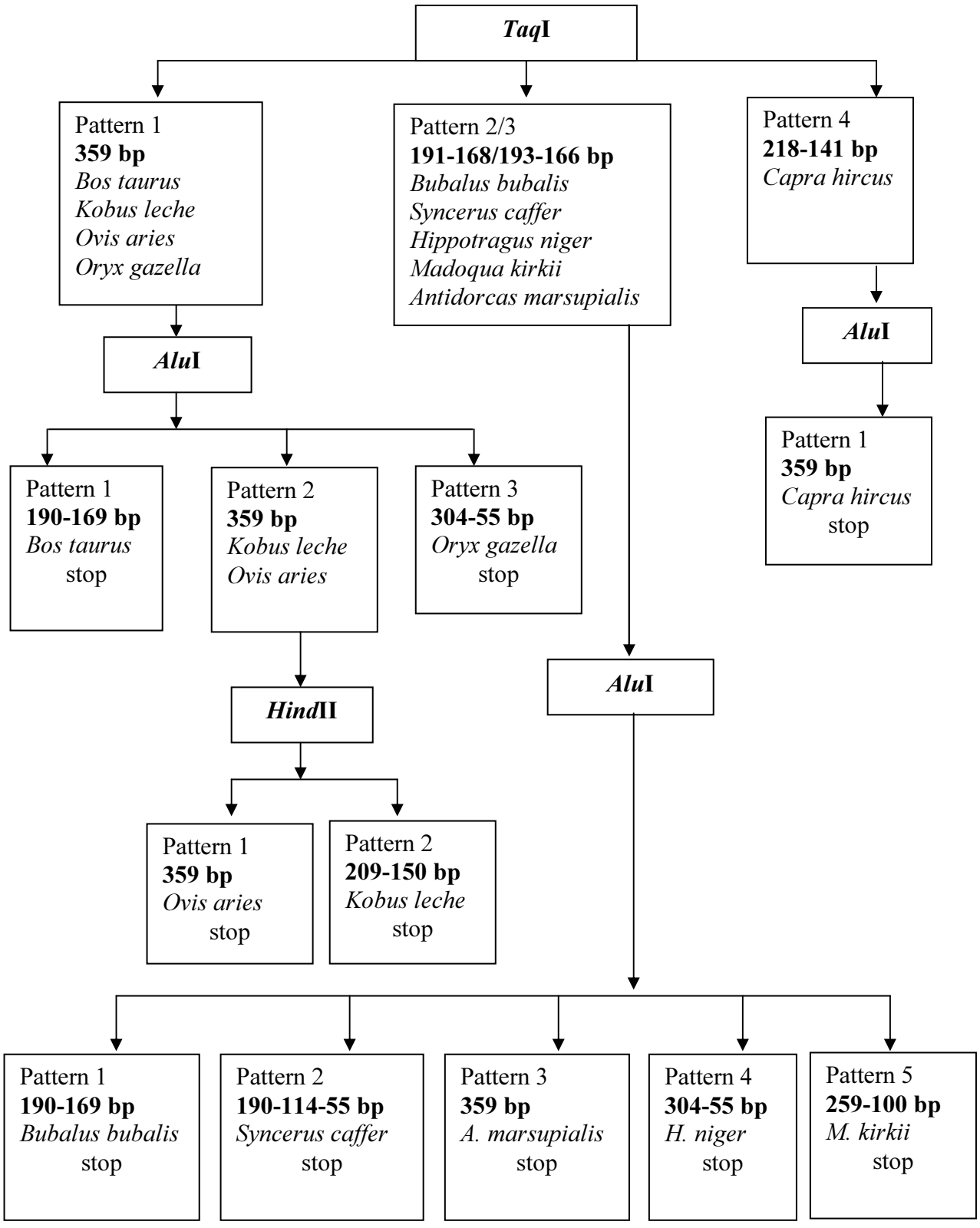

Figure 17. Flowchart of digestion of PCR products of 10 animal species of the family Bovidae with endonucleases

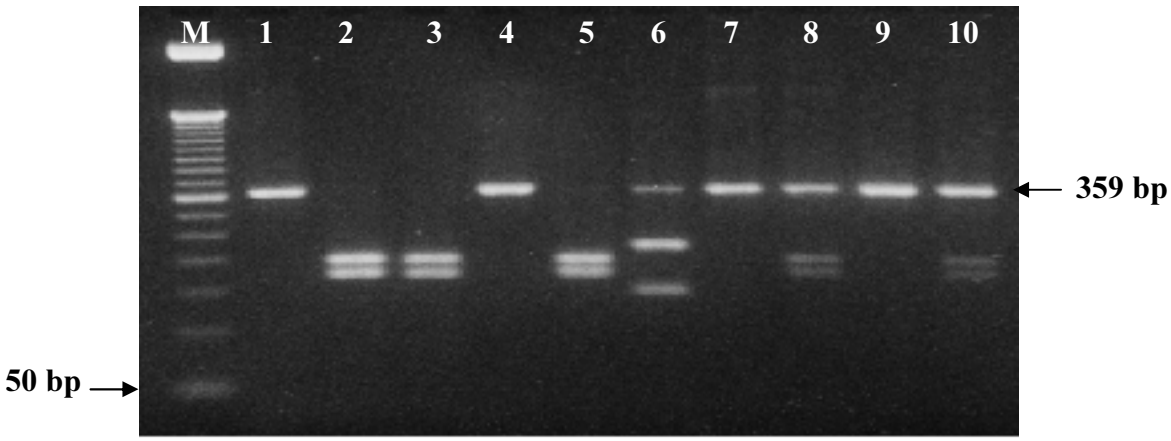

Figure 18. Restriction profiles of *cytb* PCR amplicons (359 bp) digested with *Taq*I. M; 50 bp molecular size marker. Lanes **1-10**; as in Table 6.

Table 6. Expected and resultant restriction fragment-sizes following PCR-RFLP analysis of the *cytb* gene with *Taq*I.

Lane	**Species**	***Taq*I T↓CGA**	
		Expected	**Resultant**
1	*Bos taurus* (Cattle)	359	359
2	*Bubalus bubalis* (Water buffalo)	168-191	168-191
3	*Syncerus caffer nanus* (Red buffalo)	168-191	168-191
4	*Kobus leche* (Waterbuck)	359	359
5	*Antidorcas marsupialis* (Springbok)	166-193	166-193
6	*Capra hircus* (Goat)	141-218	141-218
7	*Ovis aries* (Sheep)	359	359
8	*Hippotragus niger* (Sable antelope)	168-191	168-191
9	*Oryx gazella* (Oryx)	359	359
10	*Madoqua kirkii* (Dik-dik)	168-191	168-191

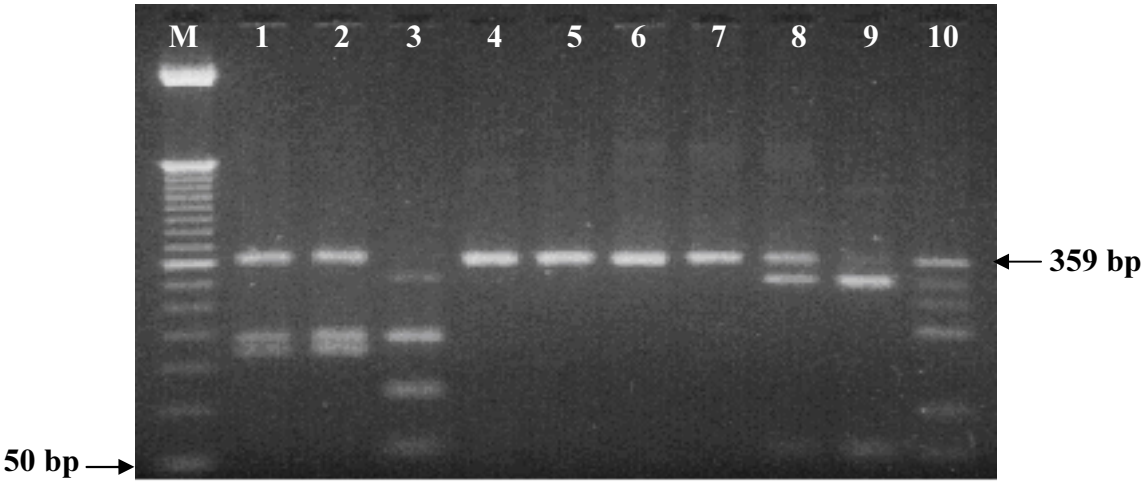

Figure 19. Restriction profiles of *cytb* PCR amplicons (359 bp) digested with *Alu*I. M; 50 bp molecular size marker. Lanes **1-10**; as in Table 7.

Table 7. Expected and resultant restriction fragment-sizes following PCR-RFLP analysis of the *cytb* gene with *Alu*I.

Lane	**Species**	***Alu* I AG↓CT**	
		Expected	**Resultant**
1	*Bos taurus* (Cattle)	169-190	169-190
2	*Bubalus bubalis* (Water buffalo)	169-190	169-190
3	*Syncerus caffer nanus* (Red buffalo)	55-114-190	55-114-190-285
4	*Kobus leche* (Waterbuck)	359	359
5	*Antidorcas marsupialis* (Springbok)	359	359
6	*Capra hircus* (Goat)	359	359
7	*Ovis aries* (Sheep)	359	359
8	*Hippotragus niger* (Sable antelope)	55-304	55-304
9	*Oryx gazella* (Oryx)	55-304	55-304
10	*Madoqua kirkii* (Dik-dik)	100-259	55-100-190-259-304

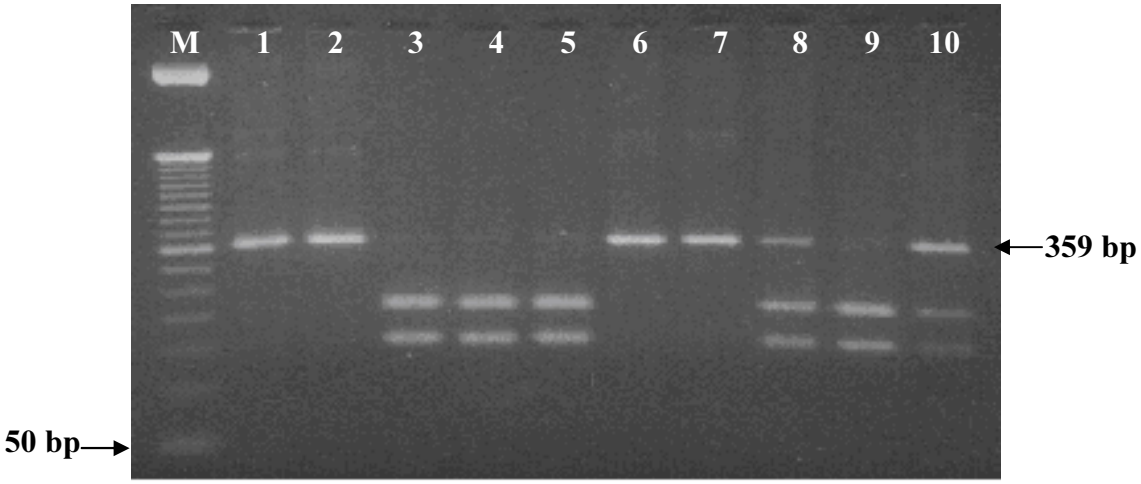

Figure 20. Restriction profiles of *cytb* PCR amplicons (359 bp) digested with *Hind*II. M; 50 bp molecular size marker. Lanes **1-10**; as in Table 8.

Table 8. Expected and resultant restriction fragment-sizes following PCR-RFLP analysis of the *cytb* gene with *Hind*II.

Lane	**Species**	***Hind* II GTY*↓R*AC**	
		Expected	**Resultant**
1	*Bos taurus* (Cattle)	359	359
2	*Bubalus bubalis* (Water buffalo)	359	359
3	*Syncerus caffer nanus* (Red buffalo)	150-209	150-209
4	*Kobus leche* (Waterbuck)	150-209	150-209
5	*Antidorcas marsupialis* (Springbok)	150-209	150-209
6	*Capra hircus* (Goat)	359	359
7	*Ovis aries* (Sheep)	359	359
8	*Hippotragus niger* (Sable antelope)	150-209	150-209
9	*Oryx gazella* (Oryx)	150-209	150-209
10	*Madoqua kirkii* (Dik-dik)	150-209	150-209

*Y = T or C *R = A or G

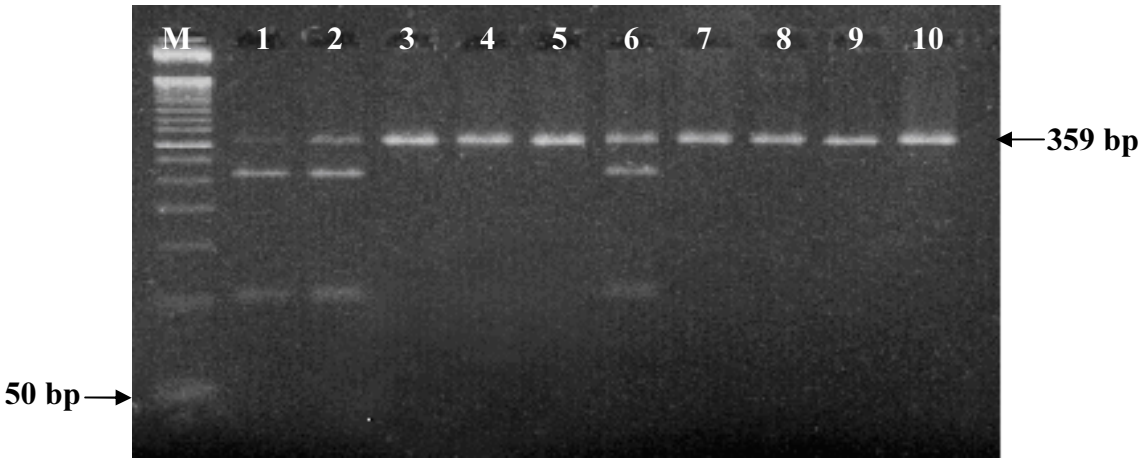

Figure 21. Restriction profiles of *cytb* PCR amplicons (359 bp) digested with *Xba*I. M; 50 bp molecular size marker. Lanes **1-10**; as in Table 9.

Table 9. Expected and resultant restriction fragment-sizes following PCR-RFLP analysis of the *cytb* gene with *Xba*I.

Lane	**Species**	***Xba* I TC↓TAGA**	
		Expected	**Resultant**
1	*Bos taurus* (Cattle)	101-258	101-258
2	*Bubalus bubalis* (Water buffalo)	101-258	101-258
3	*Syncerus caffer nanus* (Red buffalo)	359	359
4	*Kobus leche* (Waterbuck)	359	359
5	*Antidorcas marsupialis* (Springbok)	359	359
6	*Capra hircus* (Goat)	101-258	101-258
7	*Ovis aries* (Sheep)	359	359
8	*Hippotragus niger* (Sable antelope)	359	359
9	*Oryx gazella* (Oryx)	359	359
10	*Madoqua kirkii* (Dik-dik)	359	359

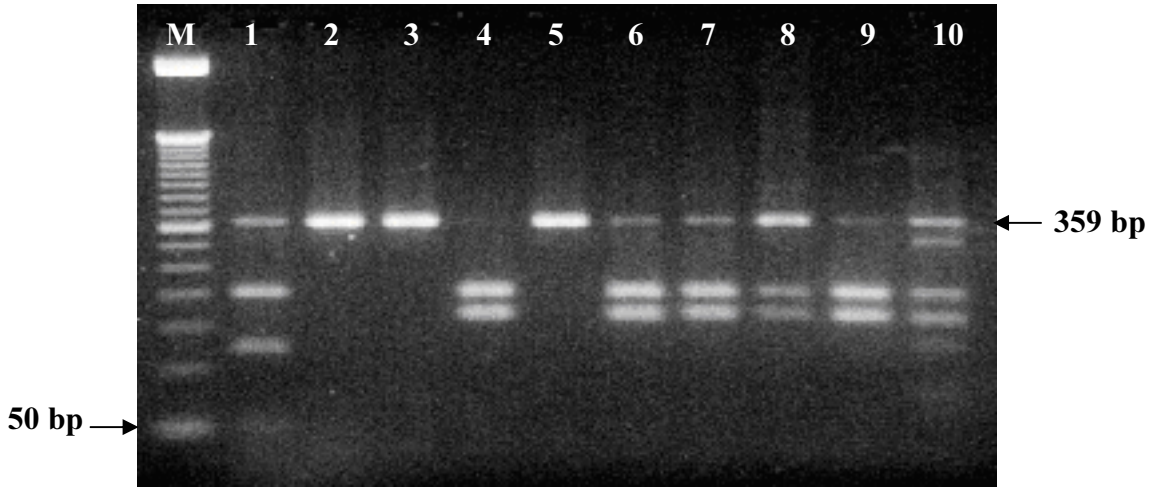

Figure 22. Restriction profiles of *cytb* PCR amplicons (359 bp) digested with *Hinf*I. M; 50 bp molecular size marker. Lanes **1-10**; as in Table 10.

Table 10. Expected and resultant restriction fragment-sizes following PCR-RFLP analysis of the *cytb* gene with *Hinf*I.

Lane	**Species**	***Hinf*I G↓AN*T**	
		Expected	**Resultant**
1	*Bos taurus* (Cattle)	44-117-198	44-117-198
2	*Bubalus bubalis* (Water buffalo)	359	359
3	*Syncerus caffer nanus* (Red buffalo)	359	359
4	*Kobus leche* (Waterbuck)	161-198	161-198
5	*Antidorcas marsupialis* (Springbok)	359	359
6	*Capra hircus* (Goat)	161-198	161-198
7	*Ovis aries* (Sheep)	63-296	161-198
8	*Hippotragus niger* (Sable antelope)	161-198	161-198
9	*Oryx gazella* (Oryx)	161-198	161-198
10	*Madoqua kirkii* (Dik-dik)	126-233	74-126-161-198-300

*N = A, T, G or C

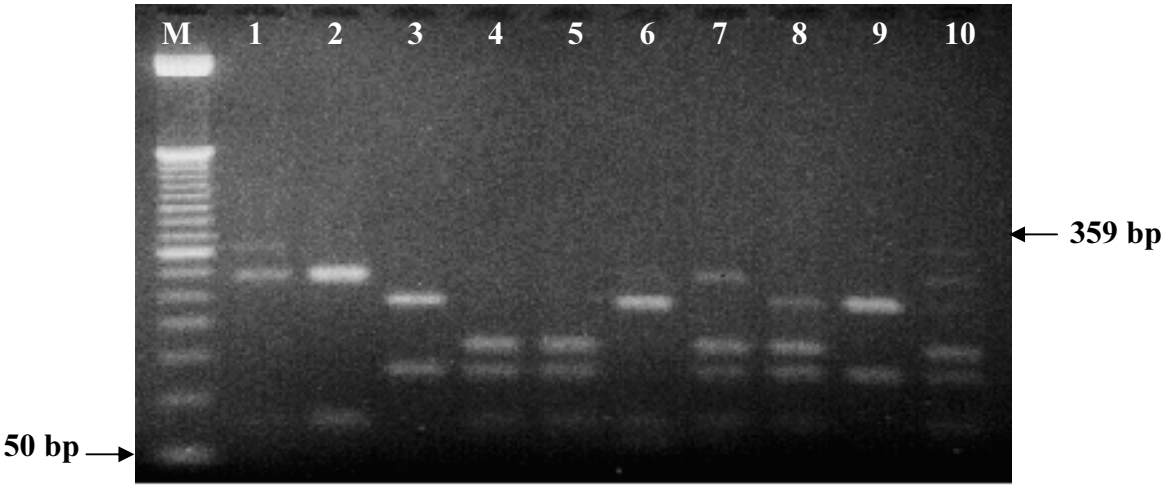

Figure 23. Restriction profiles of *cytb* PCR amplicons (359 bp) digested with *Hae*III. M; 50 bp molecular size marker. Lanes **1-10**; as in Table 11.

Table 11. Expected and resultant restriction fragment-sizes following PCR-RFLP analysis of the *cytb* gene with *Hae*III.

Lane	**Species**	***Hae*III GG↓CC**	
		Expected	**Resultant**
1	*Bos taurus* (Cattle)	74-285	74-285
2	*Bubalus bubalis* (Water buffalo)	74-285	74-285
3	*Syncerus caffer nanus* (Red buffalo)	126-233	126-233
4	*Kobus leche* (Waterbuck)	74-126-159	74-126-159
5	*Antidorcas marsupialis* (Springbok)	74-126-159	74-126-159
6	*Capra hircus* (Goat)	55-74-230	55-74-230-285
7	*Ovis aries* (Sheep)	74-126-159	74-126-159-285
8	*Hippotragus niger* (Sable antelope)	74-126-159	74-126-159-230
9	*Oryx gazella* (Oryx)	126-233	126-233
10	*Madoqua kirkii* (Dik-dik)	55-74-230	74-126-159-230-285

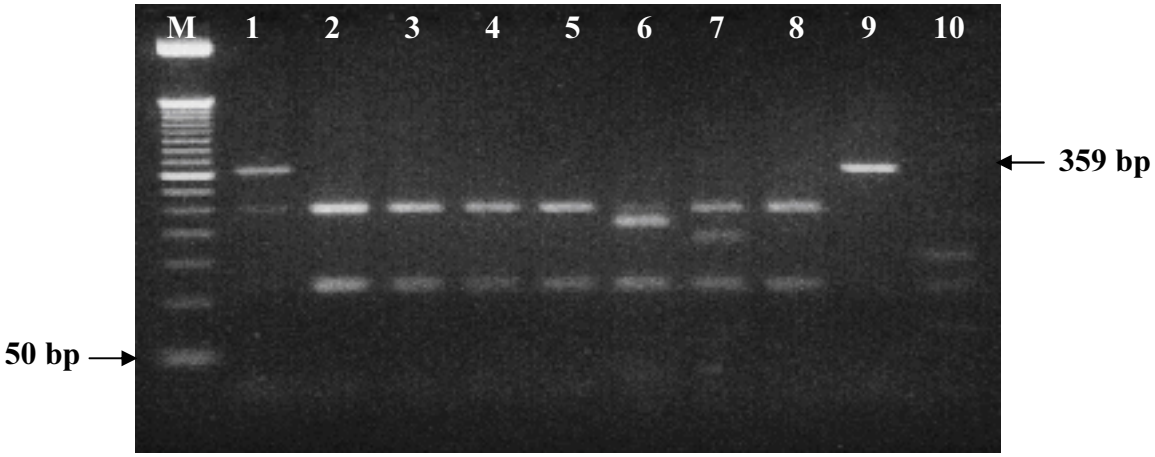

Figure 24. Restriction profiles of *cytb* PCR amplicons (359 bp) digested with *Nde*II. M; 50 bp molecular size marker. Lanes; **1-10**; as in Table 12.

Table 12. Expected and resultant restriction fragment-sizes following PCR-RFLP analysis of the *cytb* gene with *Nde*II.

Lane	**Species**	***Nde*II ↓GATC**	
		Expected	**Resultant**
1	*Bos taurus* (Cattle)	359	115-244-359
2	*Bubalus bubalis* (Water buffalo)	115-244	115-244
3	*Syncerus caffer nanus* (Red buffalo)	115-244	115-244
4	*Kobus leche* (Waterbuck)	115-244	115-244
5	*Antidorcas marsupialis* (Springbok)	115-244	115-244
6	*Capra hircus* (Goat)	31-115-213	31-115-213
7	*Ovis aries* (Sheep)	115-244	115-200-244
8	*Hippotragus niger* (Sable antelope)	115-244	115-244
9	*Oryx gazella* (Oryx)	359	359
10	*Madoqua kirkii* (Dik-dik)	78-281	78-115-150