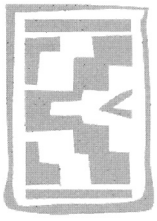

The rise and fall of tuberculosis in a free-ranging chacma baboon troop in the Kruger National Park

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ABSTRACT

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A single troop of free-ranging chacma baboons (*Papio ursinus*) was found to be infected with tuberculosis caused by *Mycobacterium bovis*. It is assumed that some members of the troop originally became infected when feeding on a tuberculous carcass in the veld or on tuberculous material scavenged at a nearby post mortem facility. Subsequently, apparent aerosol transmission took place while sleeping in an unused room. Oral transmission probably also occurred due to continuous contamination of the floor of this room and the common, narrow access (a train bridge crossing the Sabi River) to it with faeces and urine. A macroscopic prevalence of 50 % was found and the disease was noted to progress rapidly in infected baboons. A variety of organs had typical tuberculous lesions, of which the spleen, lungs and mesenteric lymph nodes were consistently, grossly affected. Using Restriction Fragment Length Polymorphism analysis, all but one of the baboon isolates were found to be identical to the most common African buffalo (*Syncerus caffer*) isolate (genotype 1) in this Park. The opportunistic sleeping facility was made inaccessible to the troop, which was forced to revert to sleeping in trees. A follow-up survey six months after closure, demonstrated that the disease had disappeared from the troop, and that no spillover infection had occurred into neighbouring troops.

Keywords: Chacma baboon, free-ranging, indoor sleeping facility, Kruger National Park, *Mycobacterium bovis, Papio ursinus*, tuberculosis

INTRODUCTION

Tuberculosis caused by *Mycobacterium bovis* or *Mycobacterium tuberculosis* in free-ranging troops of non-human primates is a rare event (Thoen, Beluhan, Himes, Capek & Bennet 1977; O' Reilly & Daborn 1995). This disease usually takes on epidemic proportions in captive primate populations where it shows a rapid progression in individual animals (Francis1958; McLaughlin1978). Bovine tuberculosis (*M. bovis*) is widespread in African buffaloes (*Syncerus caffer*) in the southern region of the Kruger National Park (KNP) (Keet, Kriek, Huchzermeyer & Bengis 1994; Bengis, Kriek, Keet, Raath, De Vos & Huchzermeyer 1996), it's prevalence in different herds varying between two and 85 % (unpublished results). It would appear that high tuberculosis prevalence in these buffalo herds lead to the spillover of the disease into other species as all cases of crossing the species barrier were recorded in the southern part of the KNP where the tuberculosis prevalence in buffalo is the highest (unpublished data). Spillover into cheetah (*Acinonyx jubatus*), lion (*Panthera leo*) chacma baboon (*Papio ursinus)* (Keet, Kriek, Penrith, Michel & Huchzermeyer 1996) and kudu (*Tragelaphus strepsiceros*) (Bengis & Keet

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1998) has been documented. Thereafter each of these "spillover" species was monitored to determine their potential of becoming maintenance hosts under free-ranging conditions. The particular troop of chacma baboons, the so-called Train Bridge Troop, from which the first case was described (Keet *et al.* 1996), showed a high prevalence of infection and a large proportion of advanced cases. The Train Bridge Troop slept in an old unused pumphouse room on the train bridge near Skukuza Rest Camp (Fig. 1A). The size of the room is 4,1 m by 4,2 m and is 4 m high with a sliding door of 1,5 m wide and 2 m high on the railway side of the room. In the middle of the floor is an elongated opening 2 m by 0,4 m over the well. Various pipes are still present in the room that were being used by the baboons to sit on. The roof has been removed and only the rafters remain. A 100 mm layer of faeces was covering the floor. Not all the members of the troop could sleep in this room and it is assumed that the more dominant individuals and their relatives had preference. The home range of this baboon troop included the Skukuza Rest Camp (31° 35,9'S, 24° 59,6'E), part of the staff village and the area to the east and south east of Skukuza.

The investigation on this troop commenced in April 1996 after a second emaciated and depressed baboon was found. This second case was presented 4 months after the first case (Keet *et al.* 1996) from this troop was described. Clinical signs in advanced cases were non-specific and included weakness and reluctance to move, emaciation, depression, areas of non-pruritic alopecia, dyspnoea and coughing.

This publication reports on the fulminating nature of this disease in free-ranging chacma baboons living in a near natural environment, the wide variety of lesions present in these animals, and the disappearance of the disease when one environmental influence was corrected. It must be borne in mind that, at any time, individual free-ranging baboons in the KNP may become infected when feeding on tuberculous carcasses. However, under natural free-ranging conditions they do not appear to easily infect other members of that troop through the aerosol route of infection.

MATERIALS AND METHODS

Animals

Certain unusual looking baboons or baboons bearing distinctive scars were used to distinguish the Train Bridge Troop from its three neighbouring troops and to determine their home ranges. Subsequently, baboons from the Train Bridge Troop were captured by means of cage traps set in the centre of their territory, thus ensuring that only members of this infected troop were captured. The Train Bridge Troop occasionally raided the Skukuza Rest Camp and a sector of the staff village and officials destroyed some of these problem individuals during the raids. All of these *ad hoc* cases were also submitted for necropsy.

The pumphouse room, opportunistically used as sleeping quarters by this troop, was locked up on 1 August 1996, and thereafter regularly checked to ascertain whether the baboons still attempted to sleep on the train bridge. During January 1997, 20 more baboons from this troop were sampled. After the sampling of the Train Bridge Troop was completed, 32 baboons from the three neighbouring troops were also sampled in a similar fashion.

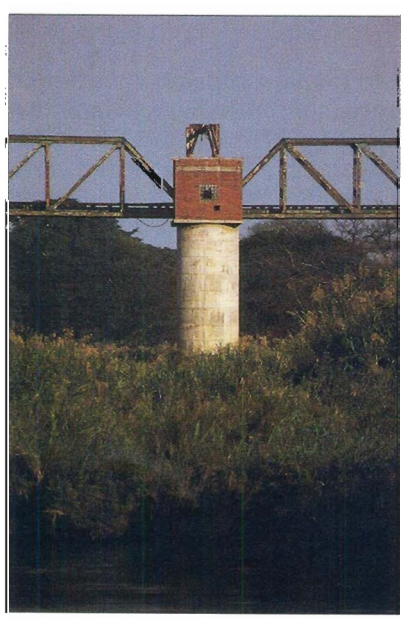

FIG. 1A The pump house room on the train bridge crossing the Sabi River

FIG. 1B The interior of the pump house-room. Note the build up of faeces on the floor

Immobilization

The first three free-ranging diseased baboons were immobilized in the Skukuza Rest Camp by darting with a combination of zolazepam (2 mg/kg) and tiletamine (2 mg/kg) (Zoletil®, trademark: Logos Agvet, Private Bag X3, Halfway House 1685) (Burroughs 1993). Cage-trapped baboons on the other hand were injected with a pole syringe containing ketamine hydrochloride at 8–10 mg/kg (Kyron Laboratories, P.O. Box 27329, Benrose 2011 (Burroughs 1993) as described by De Wet (1993). The majority of the cage-trapped baboons did not appear to be emaciated or obviously sick.

Tuberculin testing

An intradermal palpebral tuberculin test was done on each captured individual. Bovine tuberculin (0,1 mℓ, 3 000 CTU) (Central Veterinary Institute, Lelystad, Netherlands) was injected into the right upper eyelid using a 25-gauge needle. The baboons were then transported to the primate holding facilities in Skukuza where they were placed under observation for 48 h. During this period they were fed with a fruit of the season, such as oranges or mangoes, and leftover food from the Skukuza Rest Camp restaurant kitchen. After the 48 h period, they were re-immobilized with ketamine hydrochloride and their eyelids examined for any swelling or skin reaction. Thereafter, all the tested baboons were euthanazed by administration of an overdose of sodium pentobarbitone (EuthaNase®, trademark: Centaur, P.O. Box 912–686, Silverton 0127) injected into the saphenous vein.

Post mortem examination

All euthanazed animals were subjected to a complete post mortem examination during which specimens of selected organs and lesions were taken for histopathological and bacteriological examination and smears of exudates, if present, were made.

Histopathological examination

The organ specimens for histopathological examination were preserved in 10 % buffered formalin after which sections for light microscopic examination were cut using routine procedures. The sections were stained with haematoxylin and eosin. Selected tissue sections and smears of exudates were stained by the Ziehl-Neelsen method for the detection of acid-fast bacteria.

Bacterial isolation

Specimens of the spleen, lungs, kidneys, heart and various affected lymph nodes of the baboons were processed and cultured, and any organisms isolated were identified as previously described by Bengis *et al.* (1996).

Sample preparation and Polymerase Chain Reaction amplification

Heat-killed mycobacterial suspensions were prepared and amplified as described by Keet *et al.* 1996.

Restriction Fragment Length Polymorphism analysis

Genomic typing of *M. bovis* isolates was carried out according to the recommendations of Van Embden, Cave, Crawford, Dale, Eisenach, Gicquel, Herman, Martin, McAdam, Shinnick & Small (1993). The entire sequence of IS*6110*, IS*1081* and the polymorphic GC-rich repetitive sequence PGRS were used as probes. The National Institute of Public Health and the Environment, Department of Mycobacteria, Bilthoven, The Netherlands kindly performed Spoligotyping of all the isolates.

RESULTS

Tuberculin testing

Results of the intradermal palpebral tests correlated significantly with the presence of disease and suggested a high specificity and sensitivity. Reactions were considered positive when swelling and drooping of the injected upper eyelid was both visible and palpable. No sex or age predilection was present. The disease prevalence amongst the animals sampled during the outbreak, was approximately 50 % (14 out of 30). The original troop size was approximately 80 animals.

Macro- and histopathology

All infected animals had visible granulomas in the spleen, lungs and mesenteric lymph nodes. Granulomas in the spleen varied in size but were well circumscribed. They had yellowish-white granular caseous-necrotic centres. The lungs manifested multifocal to confluent granulomatous lesions which varied in consistency from fibrous to soft with liquefied centres containing a greyish-white exudate. In some cases, smaller granulomas were calcified. The lymph nodes of the head were only affected in certain individuals, and the kidneys were only affected in advanced cases (Fig. 2C). Other incidental sites were the inguinal, mammary and axiliary lymph nodes. Tuberculous granulomas were also seen on the atrio-ventricular valves of one individual. One 6-months-old individual had a lesion in a lumbar vertebral body as well as liver lesions. Loss of body condition was not apparent until the disease had progressed to an advanced stage.

Histologically all lesions were typical tuberculous granulomas that were highly proliferative in lymph nodes but extensively necrotic in the lungs and

spleen. These granulomas were characterized by the presence of large numbers of epithelioid cells and Langhans' giant cells. In the areas of caseous necrosis, large numbers of neutrophils were present and the giant cells contained large numbers of acid fast bacteria. Lung lesions, manifesting as a pronounced tuberculous bronchitis with large quantities of necrotic exudate in the lumens of bronchi and bronchioles, and associated with extensive fibrosis, were also present (Fig. 2B). Large numbers of acid-fast bacilli were seen in all smears prepared from the exudate of various lesions.

The 20 baboons from the Train Bridge Troop that were sampled 6 months after the closure of the room were all found to be negative for tuberculosis. None of the 32 baboons from the three neighbouring troops that were sampled, was found to be tuberculous.

Bacterial isolation and Polymerase Chain Reaction analysis

Mycobacterium bovis was cultured from 14 specimens submitted from the Train Bridge Troop baboons, and was identified by standard biochemical tests (Bengis *et al.* 1996). PCR amplification of all the mycobacterial isolates obtained from all the infected baboons produced a single, 372-bp DNA band, specific for the *M. tuberculosis* complex. In each case specificity of the amplification was confirmed by digestion with *Sac* 1, rendering two bands, 220 bp and 152 bp in size, respectively.

Restriction Fragment Length Polymorphism analysis

Fingerprints generated by IS*1081* RFLP, PGRS and spoligotyping indicated that all 14 *M. bovis* isolates belonged to the same genotype (genotype 1). IS*6110* RFLP, however, revealed a different genotype in one baboon that could be characterized as a replicative transposition of two IS copies in the common genotype 1.

DISCUSSION

An outbreak of tuberculosis in a free-ranging troop of chacma baboon is described. The impression was gained that this disease had a rapid progression. A

FIG. 2A An emaciated baboon

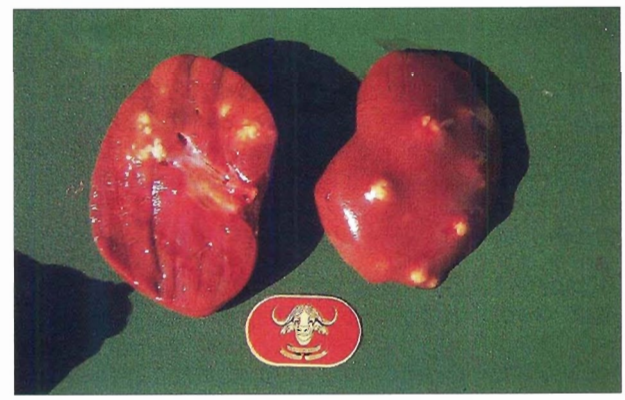

FIG. 2C Kidney lesions in an advanced case

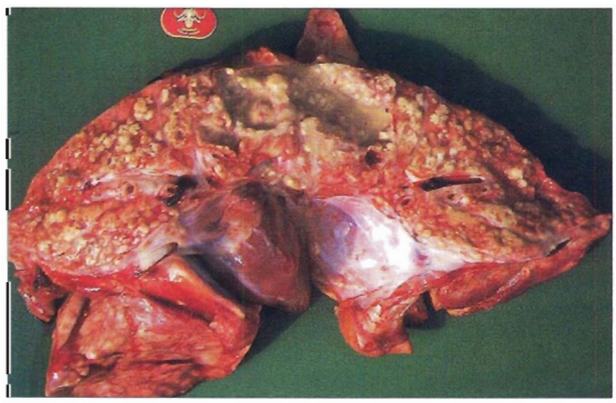

FIG. 2B The severely affected tuberculous lungs are completely adherent to the pericardium

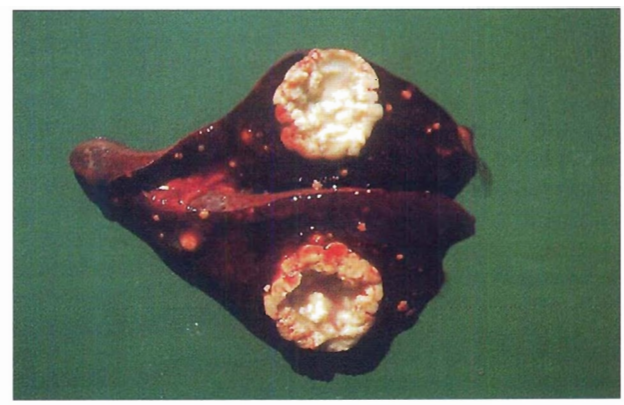

FIG. 2D A spleen containing a typical and massive necrotic tuberculous granuloma

TABLE 1 Distribution of lesions in the 14 cases of tuberculosis in chacma baboons from the Train Bridge Troop

All cases were sampled in an around Skukuza Rest Camp

No.	Date of necropsy	Sex	Age	Troop	Test	Lesions
1.	5/12/95	M	Adult	TBT	N.D.	H, L, S, K, M
2.	1/04/96	F	Adult	TBT	N.D.	H, L, S, K, M, atrio-ventricular valves
3.	11/04/96	F	Adult	TBT	Pos.	H, L, S, M
4.	11/04/96	M	Sub adult	TBT	Pos.	L, S, M
5.	15/04/96	M	Adult	TBT	N.D.	H, L, S, K, M
6.	21/05/96	F	Sub adult	TBT	Pos.	L, S, K, M
7.	21/05/96	F	Sub adult	TBT	Pos.	L, S, M
8.	23/05/96	M	2 years	TBT	Pos.	L, S, M
9.	24/05/96	M	6 months	TBT	Pos.	L, lLiver, S, sacral vertebra, M, linguinal lymph node
10.	31/05/96	F	Adult	TBT	Pos.	L, liver, S, K, M, inguinal lymph node
11.	4/06/96	F	Adult	TBT	Pos.	L, S, K, M, pregnant
12.	4/06/96	F	Adult	TBT	Pos.	H, L, S, M
13.	4/06/96	F	Adult	TBT	Pos.	L, S, M, mammary gland, axiliary lymph node, biceps
14.	4/06/96	F	Adult	TBT	Pos.	L, S, K, M

H Lymph nodes of the head
L Lungs and associated lymph nodes
S Spleen
M Mesenteric lymph nodes
K Kidney
N.D. Not done
Test Intradermal palpebral test
TBT Train Bridge Troop

wide range of organs was affected in infected individuals. The outbreak dissipated when the troop was no longer able to use their opportunistic indoor sleeping facility on the train bridge, and, as an alternative, once again slept in trees on the banks of the Sabi River.

The only other tuberculosis outbreak in free-ranging baboons was described in Kenya (Suleman, Tarara, Kamunyi & Runyenje 1983; Tarara, Suleman, Sapolsky, Wabomba & Else 1985; Sapolsky & Else 1987). This outbreak occurred in olive baboons (*Papio cynocephalus anubis*) that were feeding on village slaughterhouse offal of *M. bovis*-infected cattle. This population had declined from approximately 85 to 55 subjects over a period of 9 months (Sapolsky & Else 1987). A similar rapid infection rate has been described in captive chacma baboons infected with *M. tuberculosis* (Fourie & Odendaal 1983) and in rhesus monkeys (*Macaca mulatta*) infected with *M. bovis* (McLaughlin 1978). Under these enclosed captive conditions, aerosol transmission appears to be the more important mode of transmission (Kaufmann & Anderson 1978). Experimental aerosol transmission in rhesus monkeys has been demonstrated but natural airborne transmission has never been confirmed (Renquist & Whitney 1978). Thoen *et al.* (1977) suggested that the infection of two captive baboons (*Papio papio*) with *M. bovis* occurred by aerosol transmission as pulmonary lesions were seen. They did, however, mention that lesions were also found in the liver, spleen and mediastinal lymph nodes. Renquist & Whitney (1978) reviewed the pathology in non-human primates according to primary foci of infection. They described primary pulmonary lesions with and without secondary alimentary involvement as well as primary alimentary lesions with and without pulmonary involvement. In the 14 cases encountered in the outbreak described here, pulmonary, alimentary and splenic lesions were present in all cases suggesting a combination of routes of infection.

Fourie & Kleeberg (1978) stated that there can be little doubt that the monkey in captivity is the most susceptible of all animals to tuberculosis. Various other authors (Kaufmann & Anderson 1978; Fourie & Odendaal 1983) have confirmed this. Old World monkeys in general appear to be extremely susceptible to infection by any route under intensive captive conditions, and fulminating, rapidly fatal disease develops (Sapolsky & Else 1987). Chacma baboons appear to be as susceptible and within a period of months, infected baboons have the potential of excreting large numbers of organisms via the aerosol route, as well as in faeces and urine. This can be concluded because of the large numbers of acid-fast bacteria seen on impression smears made from lesions. With such disease disseminating potential, all the other members in the confines of an infected primate house are at risk of becoming infected.

The original source of infection of the Train Bridge Troop remains unknown, but it is speculated that one or more troop members initially became infected either when raiding a nearby post mortem facility and ingesting tuberculous material of buffalo origin or possibly when scavenging on a tuberculous buffalo carcass in the veld. It must, however, be stated that both possibilities were unlikely to be a continuous source of tuberculous material because predators and scavengers are more likely to consume carcasses before baboons do. The impression was

gained that mortality due to tuberculosis appears to be rare as predators usually kill emaciated animals before they actually die from the disease. Since a large number of necropsies on tuberculous buffaloes was performed at the facility near Skukuza, exposure of the baboons to genotype 1 was a likely event. Biosecurity was maintained through incineration of the carcasses but a breach may have occurred. All but one *M. bovis* isolate were found to be identical to genotype 1 which has been shown to be the predominant genotype in the KNP, particularly in the southern part (Michel & Maré 1999). The fact that one baboon was infected with a different genotype with a similarity value to genotype 1 of 91 % could be an indication of genetic rearrangements within genotype1 either after ingestion by the baboon or while being circulated in the buffalo population. Coincidentally this was the last baboon found to be positive in this outbreak. However, this genotype has not as yet, been isolated from buffalo.

Before being prevented from doing so, the Train Bridge Troop opportunistically slept at night in the deserted pump-house room on the disused railway bridge over the Sabi River for security from predators. This enclosed room probably precipitated rapid aerosol transmission between individuals, a situation that can be compared to that of many primates kept captive. Furthermore, the build up of faeces and urine in this room was significant, and faeces and urine of cases suffering from advanced tuberculosis were potentially infectious (Fig. 1B). These baboons when walking through this material undoubtedly soiled their hands. Two potential routes of infection were thus possible.

In 1970, McConnel, Basson, Myers & Kuntz (1974) sampled one hundred baboons in and around Skukuza and found them to be free of tuberculosis. The non-specific clinical signs and necropsy findings described in this paper were so conspicuous and disease progression in infected individuals so rapid, that infection of the troop must have taken place relatively shortly before the detection of the first case in 1995. Furthermore, this suggests that if the disease had been present during the McConnel *et al.* (1974) survey, they would have discovered it. The monitoring of this troop was relatively easy as almost their entire foraging range was in close proximity to human activities, and they always were frequent scavengers and easy to trap.

The fact that all the infected baboons but one had the same strain of *M. bovis* suggests a single initial exposure. Thereafter horizontal baboon-to-baboon transmission must have taken place to maintain infection and increase prevalence in the Train Bridge Troop. Sapolsky & Else (1987) ascribed the prevalence of 40 % in the Garbage Dump Troop in Kenya due to the continuous oral exposure to tuberculous refuse. In our study, no known continual source of tuberculous material was available to the Train Bridge Troop. In spite of this, the prevalence in the Train Bridge Troop reached about 50 % and had the potential of increasing further had the pump house-room not been made inaccessible to them. The other possibility, however, was that tuberculosis disappeared from this troop following the removal of the original source of infection.

Transfer of olive baboon males between troops has been described as a common phenomenon in the East African study (Sapolsky & Else 1987) and must also take place in the KNP chacma baboon population (Skinner & Smithers 1995). In spite of this male transfer, tuberculosis was never found in any of the neighbouring troops in the East African study. Similarly, in the KNP study, neighbouring troops may have been exposed to infected males from the Train Bridge Troop, but tuberculosis was never diagnosed in any of these troops, indicating that aerosol transmission under free-ranging conditions appears to be a rare event.

The epidemiological pattern seen in the KNP outbreak is similar in many aspects to that described by Sapolsky & Else in 1987 in that they recorded a 40 % prevalence, a decline in troop size and no transmission to neighbouring troops. They did come to the conclusion, however, that because of the movement of males between troops, the risk of spread to neighbouring troops does exist. The results of our study indicate that the spread of infection between free-ranging troops is unlikely because:

- The disease is rapidly progressive and fatal.
- Transmission via the aerosol route is less efficient if large numbers of animals do not congregate in close contact.
- Contamination of the environment is not as concentrated as it would be in an enclosed area and organisms are disseminated more diffusely through the large home range.

The rate of survival of *M. bovis* exposed to harsh environmental factors is decreased (Tanner & Michel 1998). The possibility of other infected troops being present in the tuberculosis-infected south of the Kruger National Park was also investigated through surveillance and opportunistic necropsies but no evidence of infection could be found.

An important and consistent feature (previously described by Fox in 1926) that became apparent in this study is the presence of tuberculous granulomas in the spleen (Fig. 2D). Macroscopic lesions were also consistently found in the lungs and mesenteric lymph nodes. Coughing and wheezing were common clinical signs seen in advanced cases in this study although Fourie & Odendaal (1983) found that coughing was not a conspicuous clinical sign in an infected captive colony of chacma baboons.

In conclusion, it is reasoned that tuberculosis persistently smouldered in the Train Bridge Troop because they slept in an unnatural enclosed space which facilitated aerosol transmission, and they all had to follow the same narrow faecal and urine contaminated access route (i.e. the narrow train bridge) to this room. It is our opinion that individual baboons in a troop may occasionally become infected when feeding on carcasses of animals that have died of tuberculosis but, because of the reduced potential for aerosol transmission in a totally free-ranging situation, the disease is unlikely to reach a high prevalence in a troop. It appears that free-ranging chacma baboons do not have maintenance host potential and can be considered to be an incidental spillover species.

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