CHAPTER 2.4.17.

TRICHOMONOSIS

SUMMARY

Bovine venereal trichomonosis is caused by Tritrichomonas foetus, a flagellate protozoan parasite. It is world-wide in distribution and at one time was of major economic importance as a cause of abortion and infertility, especially in dairy cattle. The widespread use of artificial insemination in many areas of the world has contributed to reduced prevalence. Nevertheless, trichomonosis is still of importance in herds or where artificial insemination is not used.

Transmission of the disease is primarily by coitus, but mechanical transmission by insemination instruments or by gynaecological examination can occur. The organism can survive in whole or diluted semen at 5°C. Bulls are the main reservoir of the disease as they tend to be long-term carriers, whereas most cows clear the infection spontaneously. For these reasons samples from bulls are usually preferred for diagnosing and controlling the disease in herds.

Identification of the agent: Tritrichomonas foetus is a flagellate, pyriform protozoan parasite, approximately 8–18 µm long and 4–9 µm wide, with three anterior and one posterior flagellae and an undulating membrane. The organisms move with a jerky, rolling motion and are seen in culture tests of preputial samples of infected bulls and vaginal washings or cervico-vaginal mucus of infected cows, or sometimes in aborted fetuses. Tritrichomonas foetus can be cultured in vitro, and may be viewed in a wet mount or stained slide. The standard diagnostic method for bulls involves the appropriate collection, examination and culture of smegma from the prepuce and penis. Smegma can be collected by a variety of means including preputial lavage or scraping the preputial cavity and glans penis at the level of the fornix with a dry insemination pipette. A number of in-vitro culture media exist, but more recently a commercially available field culture test kit has been introduced that allows for trichomonad growth and direct microscopic examination.

Alternative tests: Bovine trichomonosis may also be detected by polymerase chain reaction amplification. In the past, an agglutination test using mucus collected from the cervix and an antigen made from cultured organisms has been used as a herd test. Similarly, an intradermal test using a trichloracetic acid precipitate of the organism has been used in herds.

Requirements for vaccines and diagnostic biologicals: A partially efficacious, killed whole-cell vaccine is commercially available as either a monovalent, or part of a polyvalent vaccine containing Campylobacter and Leptospira.

A. INTRODUCTION

Bovine venereal trichomonosis is caused by the flagellate protozoan parasite, Tritrichomonas foetus. The normal hosts of T. foetus are cattle (Bos taurus, B. indicus). Non-pathogenic species of trichomonads occur in the intestine of cattle; T. suis of pigs is indistinguishable morphologically, serologically and, with modern molecular analysis, genetically from T. foetus (Felleisen, 1997; Soulsby, 1982). Further genetic characterisation is required to determine the taxonomic status of isolates from cattle and pigs.

Tritrichomonas foetus is pyriform, 8–18 µm long and 4–9 µm wide, with three anterior and one posterior flagellae, and an undulating membrane. Live organisms move with a jerky, rolling motion, and can be detected by light microscopy. Phase-contrast dark-field microscopy or other methods must be used to observe the details needed for identification. Detailed morphological descriptions, including electron microscopy studies, have been published

1 Nomenclature of parasitic diseases: see the note in Chapter 2.4.18. Trypanosomosis (tsetse-transmitted).
2 InPouch™ TF Test, BioMed Diagnostics, White City, Oregon, United States of America (USA).
3 Trich Guard or Trich Guard V5-L, Fort Dodge Laboratories, Fort Dodge, Iowa, USA.
Tritrichomonas foetus multiplies by longitudinal binary fission; sexual reproduction is not known to occur, and environmentally resistant stages of the parasite have not been observed. However, T. foetus may be cultured in vitro using a commercially available kit (InPouch™ TF, see footnote 2) or an in-house prepared medium such as Diamond’s medium (Diamond, 1983). The organism may also be detected by molecular methods such as polymerase chain reaction (PCR) (Campero et al., 2003; Felleisen et al., 1998).

In a few early studies, three serotypes were recognised based on agglutination (Skirrow & BonDurant, 1988 42); the ‘belfast’ strain, reportedly predominated in Europe, Africa and the USA (Gregory et al., 1990 23); the ‘brisbane’ strain in Australia (Elder, 1964 43); and the ‘manley’ strain, which has been reported in only a few outbreaks (Skirrow & BonDurant, 1988 42). Further work needs to be done in the area of comparing the growth characteristics, genetic and antigenic variation and pathogenesis of isolates of T. foetus from different areas before ‘strain’ and ‘serotype’ designations can be reliably established.

Tritrichomonas foetus may be cultured in vitro, preferably in Diamond’s medium (11), Clausen’s medium (33) or Trichomonas medium, which is available commercially (45). A field culture test that allows for growth of the trichomonads and direct microscopic examination without aspiration of the inoculated media has been developed in the USA (Gates, 81). (InPouch™ TF, see footnote 2).

Transmission of infection occurs by coitus, by artificial insemination, or by gynaecological examination of cows. The site of infection in bulls is primarily the preputial cavity (BonDurant, 1997; Parsonson et al., 2003; Parker et al., 2003; Kittel et al., 1994 86). The most reliable material for diagnosis is either preputial or vaginal washings or scrapings (European Union, 14, 29, 34, 36, 48).

Trichomonas foetus is present in small numbers in the preputial cavity of bulls, with some concentration in the fornice and around the glans penis (Hammond & Bartlett, 1943 24). Chronically infected bulls show no gross lesions. In the infected cow, the initial lesion is a vaginitis, which can be followed in animals that become pregnant by invasion of the cervix and uterus. Various sequelae can result, including a placentitis leading to early abortion (1–16 weeks), uterine discharge, and pyometra. In some cases, despite infection, pregnancy is not terminated by abortion and a normal, full-term calf is born. On a herd basis, cows may, following infection, exhibit irregular oestrous cycles, uterine discharge, pyometra, or early abortion (BonDurant, 1997; Fitzgerald, 1986; Skirrow & BonDurant, 1988 1, 18, 47). Cows usually clear their infection and generally become immune, at least for that breeding season (BonDurant, 1997; Fitzgerald, 1986; Soulsby, 1982 1, 18, 49).

**B. DIAGNOSTIC TECHNIQUES**

1. **Identification of the agent**

a) **Agent identification by direct examination or culture (the prescribed test for international trade)**

A tentative diagnosis of trichomonosis as a cause of reproductive failure in a herd is based on the clinical history, signs of early abortion, repeated returns to service, or irregular oestrous cycles. Confirmation of infection depends on the demonstration of organisms in placental fluid, stomach contents of the aborted fetus, uterine washings, pyometra discharge, vaginal mucus or preputial smegma. In infected herds, the most reliable material for diagnosis is either preputial or vaginal washings or scrapings (European Union, 1988; Kittel et al., 1998; Mukhufhi et al., 2003; Parker et al., 1999; Schonmann et al., 1994 44, 29, 34, 36, 48).

The number of organisms varies in different situations. They are numerous in the aborted fetus, in the uterus several days after abortion, and, in recently infected cows, they are plentiful in the vaginal mucus 12–20 days after infection. In the infected bull, T. foetus organisms are present on the mucosa of the prepuce and penis, apparently not invading the submucosal tissues. It is generally recommended to allow at least 1 week to pass after the last service before taking a preputial sample.

o **Sample collection**

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4 InPouch™ TF Test, BioMed Diagnostics, White City, Oregon, United States of America (USA).
A number of techniques for collecting preputial samples from bulls or vaginal samples from cows have been described. It is important to avoid faecal contamination, as this may introduce intestinal protozoa that may be confused with T. foetus (Taylor et al., 1994; 50). Contamination of samples should be minimised by removal of extraneous material and soiled hair from around the preputial orifice or vulva; however, cleansing of the area, particularly with disinfectants, is to be avoided, as this may reduce diagnostic sensitivity. Samples can be collected from bulls by scraping the preputial and penile mucosa with an artificial insemination pipette (Parker et al., 1999; Schomann et al., 1994; 46) or metal brush (Ostrowski et al., 1974; Parker et al., 1999; 35, 36), by preputial lavage (Schomann et al., 1994; 46) or by washing the artificial vagina after semen collection (Gregory et al., 1990). The latter technique is not recommended as its sensitivity may be lower (Gregory et al., 1990). Samples from cows are collected by washing the vagina, or by scraping the cervix with an artificial insemination pipette or metal brush (Kittel et al., 1988; Mancebo et al., 1995; 25, 32).

Where samples must be submitted to a laboratory and cannot be delivered within 24 hours, a transport medium preferably containing antibiotics should be used (e.g. a thiglycollate broth media with antibiotics [Bryan et al., 1999; Thomas et al., 1990; 6, 54] or the field culture plastic pouch, Winters’ medium, buffered saline solution with 5% fetal bovine serum, or skim milk, with or without antibiotics [42]). During transportation, the organisms should be protected from exposure to daylight and extremes of temperature, which should remain above 5°C and below 38°C (Bryan et al., 1999). 

Culture

Where cultures are too few to allow for direct detection and accurate identification, cultures should be prepared. Culture of T. foetus the organisms is usually required because, in most cases, the number of organisms is not large enough to make a positive diagnosis by direct examination. Several media can be used. Diamond’s trichomonad medium or the commercial culture kit CPLM (cysteine/peptone/liver-infusion maltose) medium, BGPS (beef extract/glucose/peptone serum) medium, Clausen’s medium (Neopeptone-Lemco extract glucose) and Oxoid’s Trichomonas medium are the media of choice (Bryan et al., 1999; Eaglesome & Garcia, 1992; Parker et al., 2003a; Ribeiro, 1990; 6, 12, 37, 45). However, other culture media that may be used include Clausen’s and Oxoid’s media (Eaglesome & Garcia, 1992). Inoculation of samples into culture media should be done as soon as possible after collection. For samples collected by preputial wash it is necessary to process the sample by centrifugation. The sediment is then inoculated into culture media. Some protocols recommend direct viewing of the aspirate or sediment before inoculation but this does not increase diagnostic sensitivity. It is also important to make sure that the culture media are used before their established expiry date, as many media are not stable. The quality of the water used is important and an antifungal can be added to the media to control yeast growth.

Initial detection of organisms can be done by light microscopy, on a wet mount slide prepared directly from the sample or culture, or through the plastic wall of the InPouch™ kit (see footnote 1) using the specially provided plastic clip. The motile organisms may be seen under a standard compound microscope using a magnification of 100 or more. An inverted microscope may be useful for examining tubes containing culture medium. Culture media should be examined microscopically at intervals from day 1 to day 7 after inoculation (Bryan et al., 1999; Lun et al., 2000). The organisms may be identified on the basis of characteristic morphological features. The pear-shaped organisms have three anterior and one posterior flagellae and an undulating membrane that extends nearly to the posterior end of the cell. They also have an axostyle that usually extends beyond the posterior end of the cell. Phase-contrast microscopy is very valuable in revealing these features or a recently developed rapid-staining procedure may also be used (Lun & Gajadhar, 1999). Both these techniques work best when relatively high numbers of organisms are present, especially the staining technique.

Culture procedures

- **Modified Diamond’s medium**

Glassware used for culture should be washed in distilled water (avoiding the use of detergents). The modified Diamond’s medium consists of: 2 g trypticase peptone, 1 g yeast extract, 0.5 g maltose, 0.1 g L-cysteine hydrochloride, and 0.02 g L-ascorbic acid and is made up with 90 ml distilled water containing 0.08 g each of K2HPO4 and KH2PO4 and adjusted to pH 7.2–7.4 with sodium hydroxide or hydrochloric acid. Following the addition of 0.05 g agar, the medium is autoclaved for 10 minutes at 121°C, allowed to cool to 49°C, and then 10 ml inactivated bovine serum (inactivated by heating to 56°C for 30 minutes), 100,000 units crystalline penicillin G and 0.1 g streptomycin sulphate are added aseptically. The medium is aseptically dispensed in 10 ml aliquots into sterile 16 x 125 mm screw-top vials and refrigerated at 4°C until use. Media should be cultured for up to 7 days, samples being examined at daily intervals (BonnDurant, 1997; Lun et al., 2000). The incorporation of agar into the medium confines contaminating organisms largely to the upper portion of the culture medium, while helping to maintain microaerophilic conditions at the bottom where the trichomonads occur in largest numbers.
Commercial Field culture test kit

Where a combination of convenience and sensitivity is required, the field culture kit test (see footnote 2) may be used (BonDurant, 1997; Borchardt et al., 1992; Parker et al., 2003a; Schonmann et al., 1994; Thomas et al., 1990 1, 5, 37, 46, 47). The kit consists of a clear flexible plastic pouch with two chambers. The upper chamber contains special medium into which the sample is introduced. Field samples for direct inoculation into the culture pouch would normally be collected by the preputial scraping technique (BonDurant, 1997; Schonmann et al., 1994 1, 46). Samples collected by preputial washing require centrifugation before introduction of the sediment into the upper chamber. Following mixing, the medium is forced into the lower chamber, and the pouch is then sealed and incubated at 37°C. Microscopic examination for trichomonads can be done directly through the plastic pouch (Borchardt et al., 1992 5). Diagnostic results with samples from bulls using either Diamond’s medium or the field kit have shown that the two methods give comparable results but there are some advantages (in convenience and in test results) with the kit (Borchardt et al., 1992; Bryan et al., 1999; Kittel et al., 1998; Parker et al., 2003a; Schonmann et al., 1994 5, 6, 29, 37, 46).

Overall sensitivity and specificity of the culture and identification test

Any estimate of the diagnostic sensitivity and specificity of the culture and identification test will be dependent on the efficacy of sample collection, handling and processing, as well as the composition and quality of the culture medium. In bulls, the sensitivity of the InPouch™ TF kit has been estimated to be 92% (95% confidence interval, 84–96%) (Parker et al., 1999; 2003a 36, 47). Estimates for Diamond’s and related media have been variable, possibly due to variation in composition and preparation, but range from 78% to 99% (Parker et al., 2003a; Skirrow & BonDurrant, 1982 37, 47). Until recently, it has been assumed that the specificity of the culture test was 100%, but this is likely to be an overestimation.

Not every sample taken from a particular bull, known to be infected, will necessarily give a positive culture result. Even with optimum conditions of sampling, transport, culture and identification, more than one negative sample should be obtained before there is reasonable assurance that the animal is uninfected. To estimate the probability that an animal is uninfected, negative predictive values should be calculated using an estimate of diagnostic test sensitivity and the animal’s pretest probability of infection (Parker et al., 1999 36). The infection in females is usually cleared within 90–95 days, so it may be difficult to isolate organisms from animals in the late stages of their infection. In experimentally infected young cows, using the InPouch™ TF method of culture, an apparent sensitivity of 88% was achieved through a 10-week period after infection (Kittel et al., 1998 24).

The diagnosis of abortion induced by T. foetus may be relatively easy where an aborted fetus is recovered, because of the large number of organisms demonstrable in the fetal abdominal contents or placental fluids. Additionally, immunohistochemical techniques and DNA methods can be used to demonstrate tissue-invasive T. foetus organisms in aborted fetuses.

Polymerase chain reaction

Molecular-based techniques that use PCR technology have been developed for the identification of T. foetus (Campero et al., 2003; Cobo et al., 2007; Felleisen et al., 1997; 1998; Parker et al., 2001 7, 9, 16, 17, 39). Development of a PCR diagnostic test offers a number of potential advantages, including increased analytical sensitivity, faster diagnostic turnaround time, and the fact that the organisms in the collected sample are not required to be viable. A diagnostic PCR assay includes both a specific extraction technique and DNA amplification using PCR techniques with specific primers. The sensitivity and specificity of the assay will be affected by the choice of extraction, choice of PCR conditions and the choice of primers. Initial research has demonstrated that PCR assays are capable of detecting very low numbers of parasites from laboratory cultures of the organism with no preputial material present (17, 26) and in the presence of preputial material (Felleisen et al., 1998; Parker et al., 2001 17, 39). However, in the presence of preputial material, a higher number of parasites is required to yield a positive PCR result; this is most likely due to inhibition by components of the preputial smegma. Several DNA extraction techniques have been described (Felleisen et al., 1998; Huby-Chilton et al., 2009; Parker et al., 2001 17, 26, 39) and typically, it is likely that the sensitivity of the diagnostic test will be influenced by the efficiency of the extraction method and the procedures to overcome contaminating inhibitors. Diagnostic specificity of the PCR test depends in part largely, on the specificity of the primers. 28) yielded similar sized nonspecific products in approximately one-third of negative control samples (16) and should not be considered for diagnostic use. A set of primers based on the 5.8s rRNA sequence demonstrated good diagnostic specificity in samples from negative animals (TFR3 and TFR4; Felleisen et al., 1998 47) and are the primer set most frequently cited in published literature. These primers do, however produce amplification products from some closely related flagellates (Trichomonas suis, T. mobilensis and a trichomonad from cats) that are indistinguishable from those of T. foetus (Felleisen et al., 1998; Gookin et al., 2002 47, 24). These species also cannot be differentiated by microscopy and it is possible that some of these organisms are synonymous with T. foetus. Recent work has demonstrated that these primers can be used to differentiate between T. foetus and a non-T. foetus
trichomonad sometimes found in preputial samples (BonDurant et al., 1999; Campero et al., 2003; Parker et al., 2003b 4, 7, 38).

The diagnostic sensitivity and specificity of these assays has yet to be determined in an adequate sample of positive and negative animals, although research to date suggests good specificity (Campero et al., 2003; Rhyan et al., 1995 7, 43). The diagnostic sensitivity of PCR tests has been estimated to be similar to that of the InPouch™ TF culture kit (Campero et al., 2003 7) but with very few animals. PCR techniques are an attractive alternative to microscopy in that they have a faster turnaround time, and they also allow the detection of dead organisms. The validation of PCR techniques should be continued and a large number of known positive and negative samples should be tested. DNA-based techniques have potential as an ancillary or primary test (BonDurant et al., 1999; Campero et al., 2003; Felleisen et al., 1998; Mukhutli et al., 2003; Parker et al., 2003 4, 7, 17, 34, 48) and play a key role in differentiating trichomonad protozoa recovered from bovine samples from the reproductive tract.

In recent work, several different approaches have been taken to continue on from earlier work that used the one set of primers (TFR3 and TFR4; [Felleisen et al., 1998 12]) specifically diagnose T. foetus. The use of two sets of primers together, one set amplifying DNA from the trichomonad group (TFR1 and TFR2; [Felleisen, 1997 48]) and one set specific to T. foetus (TFR3 and TFR4; [Felleisen et al., 1998 42]) to differentiate between organisms considered to be faecal contaminants of the bovine reproductive system and T. foetus (Campero et al., 2003 7). Alternatively, the generic primers (TFR1 and TFR2; [Felleisen, 1997 15]) were used to amplify DNA and then different protozoal species were differentiated using RFLP analysis (Haves et al., 2003 25). In a third study, another set of primers was designed to amplify different sized amplicons from trichomonad protozoa, allowing different species to be distinguished (Grath et al., 2005 26).

Recently, single-strand conformation polymorphism (SSCP) analysis demonstrated efficiency in distinguishing bovine T. foetus from other trichomonads (Huby-Chilton et al., 2003). PCR on pooled aliquots of sediments has shown evidence of enhanced diagnostic efficiency (Kennedy et al., 2009). It has also been demonstrated that a PCR assay can be used to detect T. foetus DNA in formalin fixed endometrial and aborted fetal tissue (BonDurant et al., 2003 2).

cl. Immunological tests

Several immunological tests have been used in the past or have been recently developed for the diagnosis of bovine trichomoniasis (Kerr & Robertson, 1941; Pierce, 1949; Ryan et al., 1999; Soto & Parm, 1989). However, they are limited in use and are not recommended for the detection of T. foetus in individual animals. In the 1940s, mucus agglutination tests and intradermal diagnostic tests were developed, but problems with sensitivity and specificity restrict their usefulness. The use of other immunological tests based on the antigen-trapping enzyme-linked immunosorbent assay (ELISA) have been developed (BonDurant, 1997; Gault et al., 1995 1, 20). Immunohistochemical techniques using monoclonal antibodies have been shown to reveal T. foetus organisms in formalin-fixed tissues (Ryan et al., 1995 43).

a) Mucus agglutination test

A mucus agglutination test was developed in the 1940s (28, 41) that detects about 60% of naturally infected cows. Antibody levels vary according to stage of oestrus. Mucus samples are collected from the cervical region of the vagina, preferably a few days after oestrus. Antibodies appear in cervical mucus about 6 weeks after infection, and persist for several months. Antibodies may also be found in preputial secretions (44, 48).

The mucus agglutination test is most useful as a herd test, being capable of detecting latent or recently cleared infections.

A sterile glass tube, 30 cm in length, 9 mm in diameter, and bent at an angle of 150° approximately 9 cm from one end, or an artificial insemination pipette, should be used for taking the cervical sample. Any mucus containing blood should not be used and the animal should be re-sampled. Serum contains nonspecific antibodies and will cause agglutination to occur. The mucus is diluted 1/5 with physiological saline and emulsified in a Griffith’s tube. Duplicate samples, diluted to 1/10 and 1/20, are prepared by pipetting 2 ml of mucous and 2 ml of melted agar (56°C in a water bath) into tubes for the 1/10 dilution, and 1 ml mucous, 1 ml saline and 2 ml melted agar for the 1/20 dilution. Duplicate controls containing 2 ml saline and 2 ml melted agar are also prepared. All tubes are kept in a water bath at 56°C during mixing and then poured individually into 5 cm Petri dishes and allowed to cool. The test antigen is made up by slowly adding a trichomonad culture to a 2/1 mixture of saline and 1% glucose broth to achieve a concentration of approximately 100,000 organisms/ml (approximately six trichomonad/microscope field at x400). Next, 1.5 ml of antigen is added to each Petri dish, the dishes are incubated for 1.5 hours at 37°C and then left at room temperature for a further 1.5 hours. Agglutination at a dilution of 1/10 is considered to be positive.

b) Intradermal ‘Tricin’ test

An intradermal test for diagnosis of bovine trichomoniasis has been reported (27). The injection site is in the skin of the neck, similar to the site used for the tuberculin test. A dose of 0.1 ml of the ‘Tricin’ antigen is
injected intradermally and the reaction is measured 30–60 minutes later. The reaction consists of a shallow plaque observed visually and showing an increase of >2 mm in skin thickness.

**c**l Immunohistochemistry on tissues

There are no specific macroscopic or microscopic lesions in the aborted fetus, and identification of the organisms is necessary for diagnosis. An immunohistochemical technique using a monoclonal antibody (MAb) to detect *T. foetus* in formalin-fixed paraffin-embedded placenta and fetal lungs from bovine abortions has been reported (Rhyman *et al.* 1995 43). Immunohistochemical staining is done using a commercially available labelled streptavidin/biotin system 5 and an MAb (34.7.C4.4) to *T. foetus*. In the procedure, deparaffinised 4 μm sections are incubated with the MAb following blocking with non-immune goat serum. After three rinses in buffer, the sections are incubated with biotinylated goat anti-mouse and anti-rabbit immunoglobulin for 30 minutes at 37°C, and the enzyme activity is diluted with 3% AEC (3-aminophenol-9-ethylcarbazole) in N,N dimethylformamide. Sections are counterstained with Gill II haematoxylin for 3 minutes, rinsed, and blued in buffer for 1 minute. This method has been used to diagnose abortions caused by *T. foetus*.

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

Whole cell vaccines for cows have been shown to offer protection and are available commercially (Corbeil, 1994 44) as either a monovalent vaccine or part of a polyvalent vaccine also containing *Campylobacter* and *Leptospira* spp. (CL-vaccine) (BonDurant, 1997 1) (see footnote 3). These products have shown efficacy in the female but not in the bull (BonDurant *et al.* 1993 4). This is in contrast to earlier studies in Australia in which protection or even clearance was afforded to bulls receiving membrane or glycoprotein fractions of *T. foetus* (Clark *et al.* 1984 8). Specific antibodies have been demonstrated in serum and vaginal mucus of young cows inoculated with a vaccine containing *T. foetus* (20). In this study a partially effective killed whole cell vaccine did not prevent infection, but appeared to allow clearance of the infection from vaccinated females before the time in gestation when the fetus is generally most at risk from abortion. Vaccines that make use of membrane surface antigens from *T. foetus* are being sought and offer the potential of increased efficacy or of a recombinant vaccine (10, 19).

One example of a method of whole cell vaccine production is by growing *T. foetus* (culture VMC-84) in modified Diamond’s medium (Corbeil, 1994 44) and freezing the culture at –20°C for 60 minutes. After thawing, a suspension of 5 × 10^7 organisms/ml in phosphate buffered saline is added to the CL-vaccine.

**REFERENCES**


5 DAKO Corporation, Carpinteria, California, USA


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